Hepatoprotective and Anti Parasitic Effect of Dromedary Female Camel Urine

By

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A Thesis Submitted in Accordance with the Requirements of the University of Khartoum for the Degree of Doctor of Philosophy (Ph.D)

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October 2004
قالوا سبحانه لا علم لنا إلا ما علمتنا إنك أنت العلي الحكيم

(31)
Dedication

To my Family and The Soul of my Father

in recognition to their care

To the Soul of my husband

To my daughters

for their encouragement and support

To those whom I learned from

In appreciation of their kindness

To those who made it easier by their

Valuable assistance

To all

I dedicate this work which is a

Fruit of a seed they have sown
ACKNOWLEDGEMENT

My thanks and prayers, to Alla, who blessed upon this work and enabled me to make it possible.

I am indebted to Professor Abdulla Mohm. ElHassan for his council guidance and kind supervision. Thanks are extended to Dr. Osama Yousif Mohmed and Professor Ali M. A./ Majid for their criticism and sustained co supervision throughout the preparation of this study.

Thanks also go to Dr. Hassan ElSubki, Dr. Salah Mukhtar; and Dr. Kamal Salih and Dr. Faiza Ahmed Omer for their valuable advice. I deeply appreciate Dr. Shiayuob for his critical and sustained contributions.

I am indebted to my brother Hafez Khogali, Al-ElNaiem and Dr. Ali M. Shamat for their outstanding help.

I also wish to thank Mr. Abdulrahman Adam, Madam Rowda Hassan and Mr. Nasir Ibrahim for their technical help.

My daughters, mother, sisters, and brothers have cheerfully allow this thesis to become a member of the family, I am deeply grateful to them for their patience and bovyonancy, they have truly shared in its prevalence.

Thanks are due everyone who offered me help during my studies. Special thanks are to the Director of the Cent. Vet. Res.Lab., Soba and Animal Resource Research Corporation for the financial support and facilitions.
Thanks for all.
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ABSTRACT

Arabian camel urine is reputed in our honourable sunna and flake medicine for the treatment of a wide variety of health problems in particular liver ailments. Pharmacological and therapeutic effects of camel urine were performed through in vivo and in vitro experiments, with a comparative physical, biochemical and analytical analysis to know the constituents of livestock urine (Camelus dromedarius).

The physical and biochemical investigation depicted that camel urine has an alkaline pH 9.5± 0.5, with a pronounced amount of potassium 7.25 ± 0.05 mmol/L, urea 14.2 ±3.3 mg/L, and total protein 8.87± 3.3 g/L, sodium, uric acid and creatinine were slightly low when compared to other species. Pharmacological preliminary screening effect of camel urine on isolated tissue strips of differed species (rats/rabbits and chick) revealed in an stimulant effect on rat fundus which was blocked by cyprohyptadine. Rabbit intestine showed a dose dependant stimulant response to diluted urine, crude urine has a marked (relaxant) effect followed by transient contraction on first washing, it was blocked by atropine. Rat duodenum showed relaxant response. Camel urine and Camel urine chlorofomic extract has a significant antihepatotoxic effect at P<0.05, it was proven experimentally by using hepatotoxic agents (CCL₄ and paracetamol) and compared to Silymarin (natural hepatoprotective drug). Camel urine protein precipitate and camle urine (diluted) has a significant cellular changes on Bovine kidney and liver cell culture.

Clinical trails were also conducted for the treatment of naturally infected calves with (F. gigantic) early moring female camel urine and compared to the liver fluke commercial drug (Rafoxanide).
Livestock urine particularly that of *Camelus dromedarius* were subjected to chemical and chromatographic investigations. Extraction, separation and fractionation of camel urine revealed the presence of alkaloid substances, purine bases and essential amino acids. IR techniques revealed the presence of identical compounds but not similar in 4 different aged female camel urine.
کهربا یعنی یافته‌گر سریعی یکی برای شکر یافته‌گر بی‌خوایی و انتقال بین‌النگین و انتقال ماده‌های غیرقابل انتقال در فضای فرستاده شده.

(۳۵۰۰ میلی‌گرمی یافته‌گر یافته‌گر)

\[
\begin{align*}
\text{PH} &= 9.5 \pm 0.5 \\
\text{Uric acid} &= 7.25 \pm 0.5 \text{ mmol/L} \\
\text{Bovine cell culture} &= \\
\text{Stimulant} &= \text{Cyproheptadine and Atropine respectively.}
\end{align*}
\]
Fresh urine (Fasciola gigantica) was used for the preparation of the samples for analysis. The samples were divided into two groups: one group received Rafoxanide, and the other group received placebos. The results showed a significant difference in the efficacy of the treatment between the two groups. The analysis was performed using various methods, including IR, U.V, Amines, and Acids. Alkaloids, Bicarbonates, and Sulphates were also analyzed to determine their concentration in the urine samples.
افَلَا ينظرونُ الي الأبل بكيفٍ خلقته\nالآية 17 سورة الفاحشة

أولم يروا أنّا خلقنا لهم معايِمتين أينما فهم لها ما يكون وزللناها لهم فمنها ركوبهم ومنها ياخلون ولهن فيما منافع ومشاربٓ أفلاميشاكرنو
الأية 69 - 71 سورة يس

البيان:
"الأبل عز لإهلها والغنم بركة والخيل محقوِّك بناحيتها الخير الي يوم القيامة "

عليكم بالبُكاء الأبل وأبوالها فإنها تنفع الخربة "بطونهم"
CHAPTER ONE
INTRODUCTION AND LITERATURE REVIEW

1.1. Camel encyclopedia

**Order: Artiodactyla** The even toed hoofed mammals in which two or four of the toes touch the ground forming the “cloven hoof” many domestic animals belong to this order.

**Sub-order:** *Tylopodia*

**Family:** *Camelidae*

**Genus:** *Camelus*

**Species:** *Dromedary* and *Bactrian.*

1.1.1. General introduction

Camels and *llamas* are quite distinct from other ruminants and sometimes placed in a separate sub-order *Tylopodia.* They have no horns, stomach with three (not four) compartments and peculiar feet. These end in two toes, but the animals weight rests on two joints of the toes instead of only on the end joint, so that there is no true hoof present in the two species of camel.

The *Bactrian* and the Arabian camel or *Dromedary,* only the former now exist in the wild states. The llama, alpaca, and the wild guanaco are domesticated forms. They live in mountainous regions of South America.
1.1.2. Origin and Geographical distribution.

Although camels are found in Africa, Asia and the Arabian peninsula, the family *camelidae* probably originated in north America during the Eocene period (about 50 million years ago), before spreading towards either south America, where the family evolved as llamas, alpacas, quanacos and vicunas, or across the bearing strait into Asia, the near east Arabia and Africa via north Africa (Higgins, 1984). The one-humped camel was probably first domesticated about 3000BC in southern Arabia. From there it spreads throughout its present range in the desert and semi-desert of Africa and other near east, most notably the Sahara desert.

The species has also been introduced into dry and arid region of central Australia (Nowak, 1991).

1.1.3. Physical characteristics:

The dromedary camel is characterized by a long curved neck, deep – narrow chest and a single hump. The hump is composed of fat bound together by fibrous tissue (acting as food storage in times of need). The size of the hump varies with nutritional status of the camel, becoming smaller to non-existent during times of starvation. The lips of dromedary camel are thickened and the upper lip is split to allow consumption of thorny plants. Dromedary are typically caramel brown or sandy brown in colour, however, shades can range from almost black to nearly white, hair length is longer on the throat, shoulder and hump area. The feet of dromedary are pad-shaped and adapted for traveling on sand (Ency.Bull. 1974). Male dromedary in comparison to female, are about 10% heavier, weighing 400-600kg, and are about 10cm taller at shoulder height, measuring 1.8-2.0m, additionally male dromedary have an inflatable soft palate which is used to attract females during the rutting season. The one-humped camel have a total of 34 teeth, with a dental formula 1/3;
1/1; 3/2’ 3/3/ (Kohlen-Rolefson, 1991). The camel eyes are protected from blowing sand and dust by a double row of eyelashes. Additionally, on the onset of sandstorm the camel has the ability to close its nostrils to prevent sand from entering (Phoenix Zoo, 1995). Water is conserved by the camel’s ability to fluctuate its body temperature throughout the day from 34°C to 41°C. This fluctuation in body temperature allows the camel to conserve water by not sweating as the external temperature rises (Schwarts and Dioli, 1992). Groups of camels also avoid the excess heat from the environment by pressing against each other. The dromedary camel may drink every 8 to 10 days and can tolerate greater than 30% water loss of its body mass (Yagil, 1982, 1985). This condition is lethal for most other mammals, since water is transferred from interstitial and intracellular body fluids. When camels come across water, they are capable of consuming enormous quantities, 100 litre in 10 minutes. (Schmidt-Nielsen, 1979; Wilson, 1984) camels are unique among mammals in their oval shape of the Red Blood Cell (Ibrahim and Mona 1989). Behaviouraly, the females have characteristic patterns when they are in oestrus. They exhibit frequent urination (Ibrahim, 1989). Camels become static and dance at the Arab songs (Sa'adi, 1928).

1.1.4. Naming:

There are many terms that describe the camel, this is clearly seen in the early Arabic poetry and the “Seven odes” in particular the word “Ibil” indicating the dromedary and Bactrain one and two humped respectively. “Dhamel” is sometimes used for male, and “Naga” is the female “Ho war” is a sucking young camel, once weaned, it is called “Faseel”, “Bakar” and “Bakra” are two years old male and female respectively.
The male is ready to mount at this age. Also named “Galoud”. A 5 years old is named “Nageib”.

1.1.5. Locomotion:

When the camel runs it moves both legs on one side in a parallel manner (Phoenix Zoo, 1995).

1.1.6. Life-span:

The normal life span of a camel is around 30-45 years but the working camels are generally retired at 20-25 years (Yagil, 1992).

The camel is returning as an animal of leisure and hobby. In large areas of arid tracts of Africa, the camel is still a condition for human survival.

1.1.7. Camel in Holly Books:

Camel was mentioned approximately 1800 B.C. before the time of the prophet Abraham. In sura VI (Al Anaam) is used for Gud chewing animals and such further details are given in verses (143, 144, 146). Sura VII (AL ARAF) verse 73 & 77. Sura XXI “ALHAJ” verse 36, verse 17 sura “ALGashia” LXXXIII reads don’t they look at the camel how are they created). Verse 160 (Al Dusougi, 1988).

1.1.8. Urine as medicine in Suna:

Narrated Anas: the climate of Medina did not suit some people, so the prophet ordered them to follow his shepherd, i.e his camels, and drink their milk and urine (as a medicine). So they followed the shepherd and drank the camel milk and urine till their bodies became healthy. Then they killed the shepherd and drove away the camels. When the news reached the prophet, he sent-off some people in their pursuit, when they were brought, he cut their hands and feet and their eyes were branded with heated pieces of iron [Sahih Al-Bukhari Vol (7 and 8)] Sahih Muslim, 1987). Therefore camel urine and milk were used for enteric
1.2. The liver:

It is a large organ with many regulatory and storage functions. The liver is situated in the upper abdomen, and weighs about 2kg (4.5lb) in humans. It is divided into four lobes. The liver receives the products of digestion, converts glucose to glycogen (a long chain carbohydrate used for storage) and break down fats. It removes excess amino acids from the blood, converting them to urea, which is excreted by the kidneys. The liver also synthesizes vitamins, produces bile and blood-clotting factors, and eliminates damaged red cells and toxins such as alcohol from the blood.

1.2.1. The liver as a tool for nutritional healing:

The liver is the largest gland of the body and will regenerate itself when part of it is damaged (Gabriel, 1986). Up to 25 percent of the liver can be removed, and within a short period of time, it will grow back to its original shape and size (Robbin, 1967). The liver has many functions, perhaps the more important is its secretion of bile, this fluid is stored in the gallbladder for release when needed for digestion. (unlike mammalians, camels and horses have no gallbladder). Bile is necessary for the digestion of fats; it breaks fat down into small globules, bile also assists in the absorption of fat-soluble vitamins (A, D, E and K), and helps to assimilate calcium. In addition, bile converts beta – Carotene to vitamin A. It promotes intestinal peristalsis as well, which helps and prevents constipation.

The absorbed food into the blood stream from the intestinal wall is transported via the hepatic portal system to the liver. In the liver nutrients such as iron and vitamins, A, B₁₂ and D are removed from blood stream and stored for further use. These stored substances are utilized for every
day activities and in time of physical stress. In addition, the liver plays an important role in fat metabolism, in the synthesis of fatty acids from amino acids and sugars, in production of lipoproteins, cholesterol, and phospholipids, and in the oxidation of fats to produce energy. Finally excess food is converted to fat in the liver, which is then transported to fatty tissues of the body for storage. The liver also acts as a detoxifier. Protein metabolism and bacterial fermentation of food in the intestine produces ammonia as a by-product, which is detoxified by the liver (Wayne, 1996). In addition to detoxifying ammonia, the liver also combines toxic substances including metabolic waste, insecticide residues, drugs, alcohol, and chemical with other substance that are less toxic. The substances are then excreted though the kidneys. Thus in order to have proper liver function there must be proper kidney function.

In addition to its many functions, the liver is responsible for regulating blood sugar levels by converting thyroxine, a thyroid hormone, into its more blood active form. Inadequate conversion by the liver may lead to hypothyroidism. The liver synthesized glucose tolerance factor (GTF) from chromium and glutathione. GTF is required for insulin to regulate blood sugar levels properly. The liver also breaks down hormones like adrenaline, aldosterone, estrogen, and insulin after they have performed their needed functions (John Maclead, 1987).

1.2.2. Liver Diseases:

Inflammation of the liver cell (hepatitis or hepatitis)
The cause of liver disease may be:
- Toxical
- Viral
- Bacterial
- Protozoal
- Fungal
- Parasitic
- Nutritional
- Chemical

1.2.3. (Hepatitis)

1.2.3.1. Toxic hepatitis may be caused by

a) Inorganic and organic poisons
b) Poisonous plants

1.2.3.2. Toxemia perfusion hepatitis.

This occurs in many bacterial infections regardless of their location. By bacterial toxins or by shock, anoxia or vascular insufficiency. The same position in case of burns, injury and in fraction.

1.2.3.3. Infectious hepatitis:

It is a diffuse hepatic lesion, in animals rarely caused by infectious agents, but most significant are:

a) The virus of Rift Valley Fever causing local liver necrosis.
b) Systemic mycosis. Histoplasmosis may be accompanied by multiple granulomatous lesions of the liver.
c) In case of salmonellosis, listeriosis, liptospirosis, hepatotuberculosis and in case of infectious necrotic hepatitis due to Clostridium novyi.

1.2.3.4. Parasitichepatitis

The main parasitic infestation of the liver include

- Acute and chronic liver fluke infestation
- Migrating larvae of ascaries species
- Malarial parasite
- Lishmanial parasite
- Fibrosing granuloma of liver in case of schistosomaisis

1.2.3.5. **Nutritional hepatitis:**

Selenium and Vit. E deficiency in a diet, Multiple dietary deficiency has been suggested to cause massive hepatic necrosis in lambs. Also other mineral deficiency leads to fatty change and liver necrosis. Metabolic changes involving protein, energy, nutrition and malnutrition.

1.2.3.6. **Bacterial hepatitis:**

Bacterial diseases of the liver in sheep and cattle. Hepatic abscess in cattle caused by *Corynebacterium pyogenes, Streptococcus and Staphylococcus*.

Microscopic lesion in the liver fine coagulation, necrosis with zone of leucocytes dominate with neutrophils, e.g. infectious necrotic hepatitis caused by *Clostridium novyi* (Fanconi syndrome).

1.2.3.7. **Chemical hepatitis:**

Chemical inflammation of hepatocytes due to toxic and/or irritant substances such as CCL₄, pracetamol, chloroform etc. Carageenan inhibit the regression of carbon tetrachloride induced collagen accumulation in the liver of rats (Szend *et al.* 1992).

1.3. **Liver Cirrhosis:**

Liver disorder may be classified as acute or chronic hepatitis, inflammatory liver disease and hepatitis (non-inflammatory disease of the liver). An actual curative therapeutic agent has not yet been found. In fact most of the available remedies, rather support and promote the process of healing or regeneration of the liver cells.
Liver Cirrhosis is a morphologic alteration of the liver that has received a great amount of alterations. Histologically cirrhosis is characterized by presence of separate of collagen distributed throughout the liver cells (Schinella and Becken 1975; Gabriel, 1986). The development of fibrous separate within the liver lobules together with disorganized regeneration of liver cells following death (Brown et al., 1989). Cirrhosis is associated with alcohol abuse, (Cotran, et al 1989; Hunt and Mccosker 1992). Dietary derangement can induce fatty change in the liver. Billiary cirrhosis following chronic obstruction of bile flow (Cholestasis).

A greater exposure to hepatotoxic drugs and chemicals cause diffuse liver toxicity leads to cirrhosis (Wayne, 1986). In the later stages of cirrhosis many complications may develop, such as ascitis, gastrointestinal bleeding, and mental deterioration encephalopathy (Fracer and Ariell, 1985; Sax and Fischer, 1986). Hepatocellular carcinoma develops in as many as 10% of human with long standing cirrhosis.

**1.3.1. Epidemiology**

It is difficult to site an incidence of cirrhosis since patients do not exhibit any signs of symptoms. A frequency ranging from 3 to 15% have been shown from various hospitals. Cirrhosis is a leading cause of death in the United States (Anon, 1983). World wide the annual death rate from cirrhosis of all causes is as high as 15 to 40 persons per 100,000 populations, (World health statistics annual, 1985). In the third world countries, children are frequently affected following maternally acquired hepatitis (Cotran, et al 1989; Anon, 1983). In 1155 patients with cirrhosis from a variety of causes, the over all 5 year survival was about 40% (D”Amico, et al 1986) the cause of death was liver failure in 49%, hepatocellular carcinoma in
22%, bleeding in 14%, hepatic renal syndrome in 8%, and other causes in the remainder. Cirrhosis has become one of the 5 most frequent causes of death in persons over the age of 40 years (Anon, 1983).

1.3.2. Clinical findings and diagnosis:

Cirrhosis is insidious in its development and often produces no clinical manifestations. Up to 50% of all cases discovered only at the time of post mortem examination. Many patients seek medical help complaining of vague, non-specific symptoms such as weight loss, loss of appetite, nausea, vomiting, and ill-defined digestive disturbances. Others were acutely ill with the full syndrome of acute alcoholic hepatitis (Precursor to cirrhosis). They have jaundice, mildly elevated serum aminotransferases (ALT and AST), and alkaline phosphates levels, a low serum albumin level, evidence of impaired coagulation (Prolonged prothrombin time) and might be quadual pain. Despite extensive investigation of liver function and pathologies, there is no effective therapy for many liver diseases. At base only symptomatic management (rather support or promote the process of healing or regeneration of the liver). Jaundice and ascetic are signs of advanced liver damage and are late signs of cirrhosis (Chrestopher, et al 1995).

1.3.3. Treatment of cirrhosis

The drugs available in the modern system of medicine are the corticosteroids and/or immunosuppressive agents, which bring about only symptomatic relief (Handa, et al. 1986).

Sudanese traditional camel owners used camel urine with or without milk for the treatment of jaundice, hepatomeagerly, spleenomeagerly, ascites, and many internal disorders. Management of cirrhosis is largely symptomatic.

- Fluid and electrolytes balance should be maintained
- Anti-ascitics.
- Analgesics may be administrated to relive gastric pain.
- Dietary supplements rich in branched chain amino acids and low in aromatic amino acids e.g. (Hepatic Aid).
- Vitamin replacement.
- Thiamin (B1).
- Vitamin K.
- Diuresis is the cornerstone of drug therapy of ascetic, but the diuresis may be slow (Pockros, 1986).

Some of the interesting drugs that used for liver cirrhosis are:

1- Thiamine.
2- Vitamin K.
3- Spironolactone.
4- Vasopressin.
5- Sodium tetradecyl sulfate or ethanolamine oleate
6- Dopamine.
7- BCAA: AAA ratio
# 1.3.3.1. drugs used in cirrhotic patients

<table>
<thead>
<tr>
<th>drug</th>
<th>Reason</th>
<th>Dose</th>
<th>Monitoring parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiamin</td>
<td>Reverse mental confusion secondary to thiamin efficiency (Wernick’s syndrome) and decrease peripheral neuropathies. Reason: prevent GI bleeding</td>
<td>dose: 40-320 mg/day</td>
<td>Mental status Decrease in nystagmus, peripheral neuropathies; more than 10 days of therapy is unwarranted</td>
</tr>
<tr>
<td>Vitamin K (phytonadi one)(AquaM ethyton preferred)</td>
<td>Prevent bleeding secondary to decreased production of factors 11, VII, IX and X (vitamin k-dependent factors)</td>
<td>Dose: 10-15 mg/day, not to exceed 3 doses</td>
<td>Hypersensitivity-fever chills, anaphylaxis, flushing, sweating prothrombin time.</td>
</tr>
<tr>
<td>Spironolactone</td>
<td>Diuresis in ascites; specific for antagonism of preexisting hyperaldosteronism.</td>
<td>200-400 mg/day, occasionall y higher, may be given as a single daily dose</td>
<td>Weight (avoid more than 1-kg weight loss per day Mental status Serum K* Urine Na+ (Na+ should exceed K* at therapeutic doses) Abdominal girth Bun (increase in dehydration) Cynecomastia prolonged use blood pressure</td>
</tr>
<tr>
<td>Lactulose</td>
<td>Prevent GI bleeding</td>
<td>40-320 mg/day,20-30mg q.i.d or to 3-4 soft stools per day</td>
<td>Hepatic encephalopathy; converted to lactic acid to lower bowel Ph and prevent absorption of NH₃</td>
</tr>
<tr>
<td><strong>Hepatamine and hepatic aid</strong></td>
<td>hepatic encepalopathy; replace branched-chain amino acids.</td>
<td>titrate to caloric and nitrogen needs</td>
<td>Mental status, Serum ammonia, CSF glutamine Serum amino acid levels (BCAA:AAA ratio) Electrolyte balance.</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>-------------------------------------------------------------</td>
<td>-------------------------------------</td>
<td>--------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td><strong>Loop diuretics</strong></td>
<td>Diuresis in ascites after failure of high-dose spironolactone.</td>
<td>Start at 40 mg. Titrate to 1-k weight loss per day, occasionally very high doses (200-60 mg/day) required</td>
<td>Same as spironolactone except urineelectrolytes of no value possible bearing loss with rapid IV bolus.</td>
</tr>
<tr>
<td><strong>Vasopressin</strong></td>
<td>vasoconstrictor for esophageal bleeding</td>
<td>0.2-0.4 u/min IV infusion</td>
<td>Rate of GI bleeding Signs of ischemia – chest pain, elevated blood pressure, bradycardia GI cramping Serum Na+</td>
</tr>
<tr>
<td><strong>Sodium tetradeceyl sulfate or ethanolamine oleate</strong></td>
<td>sclerosing agent for esophageal bleeding</td>
<td>0.5-2ml of 1 to 1.5% tetradecyl or 5% ethanolamine solution into each varix about 2cm apart</td>
<td>Signs of GI bleeding Chest pain, fever local ulceration</td>
</tr>
<tr>
<td><strong>Dopamine”</strong></td>
<td>hepatorenal syndrome</td>
<td>1-4 ug/kg/min.</td>
<td>Mental status, liver flap Urine output Blood pressure</td>
</tr>
<tr>
<td><strong>Colchicine Investigational use only: efficacy unclear</strong></td>
<td>anti-inflammatory and antifibrotic effects</td>
<td>0.6 mg p.o.b.i.d. or 1mg p.o.q.d. 5 days/week.</td>
<td>Nausea abdominal pain, diarrhea</td>
</tr>
</tbody>
</table>
**Signs of GI bleeding**

Mental changes, pulse > 60; Bp > 100/70 signs of congestive heart failure, bradycardia signs of bronchospasm renal function.

<table>
<thead>
<tr>
<th><strong>Propranolol</strong></th>
<th>Prevent GI bleeding</th>
<th>40–320mg/ per day</th>
<th>Signs of GI bleeding Mental changes, pulse &gt; 60; Bp &gt; 100/70 signs of congestive heart failure, bradycardia signs of bronchospasm renal function.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Neomycin</strong></td>
<td>Hepatic encephalopathy; sterilizes gut to prevent bacterial break down of protein and thus decreases serum NH₃ levels.</td>
<td>2-6 gm/per day, orally or rectally</td>
<td>Mental status, liver flap diarrhea, Bacterial overgrowth renal function auto toxicity</td>
</tr>
</tbody>
</table>

*Not recommended for all patients.

However, lesions of advanced cirrhosis are irreversible, it is estimated that 70% or more of liver tissue must be destroyed before the body is unable to eliminate drug and toxins via the liver (Bass and Williams, 1988).

1.3.4. Other associated disorders:

1.3.4.1. Ascites

Characterized by the accumulation of protein rich fluid in the peritoneal cavity. There are two types of factors contributing to the formation of ascitis: systemic and local factors (Schrier et al 1988). Circulatory albumin concentration and the retention of excess water associated with secondary aldosteronism (Wayne, 1996). Local factors include portal hypertension, impaired hepatic lymph drainage and ascitic peritonitis due to Candida Albicans (Suarez et al, 1994).

Less common causes of cirrhosis are related to chronic viral hepatitis and various metabolic disorders (Wayne and Jan, 1989).
Pneumonia and other haematologic disorder due to chronic alcohol abuse affect folic acid absorption, as well as iron. Fanconi syndrome causes severe liver cirrhosis (Deeinhofer, 1996). Endocrine disorders are seen in advanced cirrhosis because of the inability of the liver to metabolize the steroid hormone of the adrenals and gonads.

1.3.4.2. Hepatic renal syndrome

The concurrent impairment of renal function with hepatic failure is termed the hepatic renal syndrome. There is functional change in the kidney, caused by fluid and electrolyte disturbances, diuretic-induced volume depletion, shock, or accumulation of unmetabolized toxic substances.

1.4. Parasitic cirrhosis

Due to infestation with schistosoma and/or fluke, parasitic cirrhosis could occur. The liver fluke may produce biliary type of cirrhosis due to lodgement in biliary channel. Also there is thickening and dilatations of bile ducts, fibrous and cellular infiltration in the portal spaces, the flukes on their remaindants may be found (Radostis, et al. 2000).

1.4.1 Hepatic fascioliosis (Liver fluke disease)

It is a disease caused by *Fasciola hepatica*, mainly in sheep but also in cattle and other ruminants, *Fasciola gigantica*, mainly infects cattle and it also affects other ruminants. Both of these are large flukes inhabiting the bile duct or intestines, causing damage to the liver resulting in emaciation, jaundice and oedema (Solusby, 1982).

1.4.1.1. Etiology:

*Fasciola hepatica* is the most common and important liver fluke and has a cosmopolitan distribution. Lymnial snails are intermediate
host, and release the infective forms, the metacericaria, onto the herbage. *Hepatic fasciolosis* is an economically important disease of animals and man, (Radostits, *et al* 2000). Mainly sheep and cattle, but other species may provide a reservoir of infection. *Fasciola hepatica* may infest all domestic animal including equidae and many wild life species(Owen, 1977). Chronically infested sheep are the most important source of pasture contamination (Boray 1985). Human cases are usually associated with the ingestion of a harch plants such as water cress or uncooked vegetables which are contaminated with encysted larvae. A similar but large fluke *F. gigantica* larvae is restricted to warmer regions including parts of Africa and Asia (Solusby, 1982; Leather, 1982). Fascioliosis is endemic in 61 countries and has become a food borne infection of public health importance in parts of the world such as the Andean- highlands of Bolivia, Ecuador, Peru, the Nile delta of Egypt and Northern Iran.

It is estimated that 2.4 million people are infected world –wide and more than 180 million are at risk of infection. More than 60% of the population is infected in high land of Bolivia. An outbreak along the shores of the Caspian sea in Northern Iran between 1989-1991 infected more than 10,000 people. Also outbreaks occurred in Algeria, Cuba and France. The infection may be wide spread in humans than is appreciated, as it is present in domestic animals in almost all countries where cattle and sheep are reared (L. Savoili, 1998).

1.4.1.2. Prevalence:

One or two species of the genus fasciola occur in almost every tropical country. In some areas it is enzootic and is a serious hazard in regions where the conditions exist for the survival and multiplication of the snail intermediate host.
The incidence and economic loss from fascioliasis is generally very high. *Fasciola gigantic* occurs most commonly in Africa and Indian subcontinent, Hawaii and the Philippines. Elsewhere *F. hepatica* is most often found.
1.4.1.3. Life cycle:-

When the immature eggs reach fresh water, they take up to two weeks to mature, at which time they hatch, releasing miracidia (hatched larvae). The miracidia swim around until they encounter suitable snail intermediate hosts to infect, or they die within 24 hours (fig1).

Source redrawn from Diseases and Parasitics of livestock in the Tropics. (Hall, 1977)
1.4.1.4. Treatment of Fasciolosis
Rafoxanide is salicylanide compound
3’5 dintro-3 chloro-4(P-chlorophenoxy) –salyclanide

1.4.1.5 Pharmacology of Fasciolocidal Drug (Rafoxanide)
Rafoxanide is well absorbed by cattle and sheep with peak plasma levels occurring between 24-48 hrs. after dosing. Rafoxanide is not metabolized by cattle and sheep to any detectable degree, the half-life varies from 5 to 10 days in sheep.

1.4.1.6. Action and uses
Rafoxanide is active against 99% of adult and immature *fasciola hepatica*, more than 99% adult and up to 91% immature *fasciola gigantica*. The mechanism of action is due to paralysis of the parasite.

1.5. Health and prevention of illness
1.5.1. Health:
It is an equilibrium between the mind, the body, and the external world (environment), where disease is a disruption of this harmony.

Prevention and treatment involve creating the condition in which the body could maintain and cure itself through its internal healing mechanism. When disease did manifest itself -specific intervention would be applied, but natural cures such as dietary changes were preferred over drugs so people generally treated their illness with prayer and such common sense approaches as good food, rest and whatever substances they found in nature that were traditionally known to have medical qualities such as simple herbs, plants, minerals, urine etc.

1.5.2. Urine:
Urine is not a waste product, but a purified, sterile watery solution, it is an extra ordinary valuable physiological substance. It has been shown through out the history of medical science right-up, until to day to have
profound medical uses. It is composed of many non toxic substances, the toxic one’s are being removed from the body through the liver, intestine, through the skin, and through out breath. The main function of the kidney is to keep the composition of the blood in optimal balance. When there is too much water, the kidney will remove it. Camel kidneys unlike other mammalian species, play an important role in water release. It has been clear for centuries that the camel has a degree of independence of water greater than other domestic animals (Yagil, 1985 and 1993).

The composition of urine depends on the life style of humans, and on the type of grazing pasture of animals. Natural urine contains a measurable amount of substances which has been used medically, even in extremely large quantities without causing side effects.

We never think of urine as a nutrient, but a 200 constituents have been reported in urine of healthy human (Free& Free, 1975). There are numerous elements of nutritional value in urine, along with hormones, steroids, and other critical elements that regulate and control key processes of the body. Hence the following list considered and identify only compound of interest in human urine.

**Compound of interest in human urine(cited from your own perfect medicine Martha,2000).**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine, total</td>
<td>38 mg/day</td>
</tr>
<tr>
<td>Arginine, total</td>
<td>32mg/day</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>30mg/day</td>
</tr>
<tr>
<td>Allantoin</td>
<td>12mg/day</td>
</tr>
<tr>
<td>Amino acid, total</td>
<td>2.1 g/day</td>
</tr>
<tr>
<td>Substance</td>
<td>Amount</td>
</tr>
<tr>
<td>---------------------------</td>
<td>----------</td>
</tr>
<tr>
<td>Bicarbonate</td>
<td>140 mg/day</td>
</tr>
<tr>
<td>Biotin</td>
<td>3.5 mg/day</td>
</tr>
<tr>
<td>Calcium</td>
<td>23 mg/day</td>
</tr>
<tr>
<td>Creatinine</td>
<td>1.4 mg/day</td>
</tr>
<tr>
<td>Cystiene</td>
<td>120 mg/day</td>
</tr>
<tr>
<td>Dopamine</td>
<td>0.40 mg/day</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>0.01 mg/day</td>
</tr>
<tr>
<td>Folic acid</td>
<td>4 mg/day</td>
</tr>
<tr>
<td>Glucose</td>
<td>100 mg/day</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>308 mg/day</td>
</tr>
<tr>
<td>Glycine</td>
<td>455 mg/day</td>
</tr>
<tr>
<td>Inositol</td>
<td>14 mg/day</td>
</tr>
<tr>
<td>Iodine</td>
<td>0.25 mg/day</td>
</tr>
<tr>
<td>Iron</td>
<td>0.5 mg/day</td>
</tr>
<tr>
<td>Lysine, total</td>
<td>56 mg/day</td>
</tr>
<tr>
<td>Magnesium</td>
<td>0.5 mg/day</td>
</tr>
<tr>
<td>Manganese</td>
<td>10 mg/day</td>
</tr>
<tr>
<td>Methionine, total</td>
<td>15 mg/day</td>
</tr>
<tr>
<td>Nitrogen, total</td>
<td>10 mg/day</td>
</tr>
<tr>
<td>Ornithine</td>
<td>3 mg/day</td>
</tr>
<tr>
<td>Pantothenic acid</td>
<td>21 mg/day</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td></td>
</tr>
<tr>
<td><strong>Hormonal substances</strong></td>
<td></td>
</tr>
<tr>
<td>Aldoesterone, male</td>
<td>3.5 mg/day</td>
</tr>
<tr>
<td>Female</td>
<td>4.2 mg/day</td>
</tr>
<tr>
<td>Compound</td>
<td>Dosage</td>
</tr>
<tr>
<td>-------------------------------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>Androge, female (20-40yrs)</td>
<td>14mg/day</td>
</tr>
<tr>
<td>Male (20-40yrs)</td>
<td>18.2mg/day</td>
</tr>
<tr>
<td>Estradiol, female luteal phase</td>
<td>7mg/day</td>
</tr>
<tr>
<td>Estriol, luteal phase</td>
<td>28mg/day</td>
</tr>
<tr>
<td>Estrone, luted phase</td>
<td>14mg/day</td>
</tr>
<tr>
<td>17—ketogenic adrenocorticoids</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>12-6mg/day</td>
</tr>
<tr>
<td>Male</td>
<td>14.7mg/day</td>
</tr>
<tr>
<td>Ketosteroids</td>
<td>18.2mg/day</td>
</tr>
</tbody>
</table>
1.5.2.1. Camel urine:

The camel excretes about 2-4 litres of urine/day and this decreases with dehydration to about 0.5 litre of highly concentrated urine, especially with sodium and potassium salts, (Schmidt and Nelsen 1964). Urinary Na drops significantly after three days of food deprivation (Dahlborn et al 1992). The values of Cl in hydrated camel in literature vary greatly, 492-902 Mequ/L (Wilson 1984); 322-429 Mequ/L (Manifield and Tinson 1996).

<table>
<thead>
<tr>
<th>Animal</th>
<th>Fat</th>
<th>No fatty Solids</th>
<th>Protein</th>
<th>Casein</th>
<th>Lact.</th>
<th>Salts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baffalow Indian</td>
<td>7.45</td>
<td>9.32</td>
<td>3.78</td>
<td>3.2</td>
<td>4.9</td>
<td>0.78</td>
</tr>
<tr>
<td>Camel</td>
<td>4.2</td>
<td>8.7</td>
<td>3.7</td>
<td>-</td>
<td>4.1</td>
<td>0.75</td>
</tr>
<tr>
<td>Sheep</td>
<td>7.5</td>
<td>10.8</td>
<td>5.6</td>
<td>4.2</td>
<td>4.4</td>
<td>0.87</td>
</tr>
<tr>
<td>Goat</td>
<td>4.5</td>
<td>8.7</td>
<td>3.3</td>
<td>2.5</td>
<td>4.4</td>
<td>0.8</td>
</tr>
<tr>
<td>Reindeer</td>
<td>22.5</td>
<td>14.2</td>
<td>10.3</td>
<td>-</td>
<td>2.4</td>
<td>-</td>
</tr>
<tr>
<td>Cow (Friion)</td>
<td>3.49</td>
<td>8.59</td>
<td>3.28</td>
<td>2.47</td>
<td>4.46</td>
<td>0.7</td>
</tr>
</tbody>
</table>

1.6. Urine Therapy:

Different types of medicines, with plant, animals or even earth origin, have been discovered and tried throughout history. Some of them are no longer used while other have been modified and developed along with the development of technology.

Historically the medical usage of urine was quite well known throughout the world. There are many reports that dated back thousands of years which extol the virtues of urine both as a diagnostic tool and as a medical treatment for a wide variety of diseases, wounds, and skin
disorders. Urine as a medicine is used externally or internally to promote or to maintain mammalian health. This is based on the principle of “Natural cycles”, it has been known throughout the centuries both in the West and in the East. Urine therapy has proven helpful in a great number of various diseases ranging from simple cold and throatache, to tuberculosis and asthma (Kroon, 1996). Also in minor skin problems such as itching to more serious skin disease such as eczema, psoriasis, and even skin cancer. Urine therapy can be combined with any other natural medicine to yield good results. Nowadays cancer is treated in different parts of the world by urine therapy (Rostan, 1992).

1.6.1. Urine therapy in Eastern countries:

Urine therapy in Eastern countries has been practiced for thousands of years, especially within Yoga and Tantra tradition where the use of urine has been kept alive. It served particularly as a real “therapy”, a method to clean the physical body of impurities, but also as a way to further spiritual growth. The way of the Yoga is the practice of ingesting one's own urine (Martha 2000; Shankandevanda, 1978) in Rome, India, and Egypt (Raojibhia, 1973 and Burzynsky, 1986).

Since 5000 years ago, old document has been found that describes the practice of urine in different respects. This document consists of 107 verses (Slokas) it is called shivambu kalpavidhi.

1.6.2. Urine therapy in Western Countries:-

The urine therapy practice has been known on many cultures. German encyclopedia stated that the Greek and Romans were acquainted with the use of urine as a medicine (Plesch, 1947). The English man who was the urine therapy pioneer, cured himself of tuberculosis, which had been declared “incurable”(Armstrong, 1944). Also an Australian scientist
found a hormone in morning urine called melanin.

1.6.3. Medicinal use of human urine:

- Urea has an anti septic and inhibitory effect on the growth of microorganisms. (Wilson, 1906; Duncon 1918).


- Auto-urine vaccine therapy for nephritis. (Tiberi, 1934).

- Wounds and burns were treated by urine. (Leon, 1938)

- Urine extract was used for peptic ulcer, (Sandweiss, et al, 1941).

- A natural urine injection was used in medical practice extensively and with excellent success on large a variety of disease conditions (Plesch, 1947).


- Urea is one of the most useful non-metabolized non-electrolyte diuretics. In comparing the effect of urea with Diamox on intraocular pressure, urea is found to be more effective. (The physician’s Desk Reference 1992).

- Natural anti bodies were found in the urine. (Martin, et al 1962).


- It is used as miracle drug for AIDs, obesity, cancer, ageing (Kent, 1982) AIDs treatment (Forber and Lederer 1989). In cancer, AIDS and Autoimmune diagnosis (Burzynski, 1993).

- Anti-allergic effect (Dunne, 1981; William, 1982)


- The urokinase, a urine constituent that used is in dissolving blood clots in veins, arteries heart and lung. (Mannucci and Angelo 1982).
Urea has been used during the last two decades in the treatment of dry skin, both clinically and in cosmetic products. (Gunnar, 1992; Serup, 1992).

There are a few more examples of commercial medical applications of urine and urea in use today.

- Urea Phil: diuretic made from urea
- Urofollitropin: urine extract fertility drug.
- Puneaskin: urea cream for skin problems
- Amino-cerv: urea cream used for cervical treatments.
- Premarmin: urine extract estrogen supplement
- Panafil: urea papain ointment for skin ulcers, burns and infected wounds.

1.6.3. Medicinal use of animals urine:-

A sick person will try the prescribed remedy regardless of the cost, taste or odour. Therapeutic uses of animal’s urine have along history as well as that of human. (Ibn-Albitar and Alrazi, 1925). Cow’s urine is well known in India (Raojibhai 1973; News week 1977). Such a therapy of cow’s urine is known in different parts of southern Sudan (Ohaj, 1993). Goat urine with some medicinal plants used for treatment of jaundice and ascites, (Ibn Sina, 1037). (Al Nasimi 1992) described the general properties of all animal urine and mentioned the effect of camel urine in treatment of ascetics. (Beaton, 1971), isolated anorexiginic mobilizing substances from animal urine. While (Veerangevank 1992) investigated trypsin inhibition substance from the urine of pregnant mare, but few researches had been done on is the camel urine. Recently camel urine is used in Arab Desert for the treatment of Leukemia and digestive cancer(Kabariti et al., 1988; Muddathir, 1995 personal communication). Also it was used as hair detergent in Morocco, such therapy is known in some parts of Sudan, Saudi Arabia, and in Somalia (Ibrahim and Mona
1989; Ohaj, 1993), also used in healing of bad burns and injuries. It is more extensively used for splenomegaly, hepatomegaly, liver disorder and sometimes for fever (Ohaj 1998). The initial concern of using camel’s urine lies in its more concentrated salt contents (almost twice that of sea water. Yagil, 1994).

1.6.3.1.1. Medicinal use of Camel Urine

Camel urine is used by the camel owners, and Beduines as medicine in different ways. The Beduine in the Arab desert used to mix camel urine with milk and give it to patients who were suffering from many enteric disorders and illnesses. Milk was added to urine to overcome its strong odor, also urine must be fresh and excreted from young animals. The duration of the treatment lasts for 2-3 months for ascitic patients (Ohaj, 1998). Kabarity (1988) used camel urine for a few weeks to treat carcinogenic patients after which they were declared to be healthy. Ibrahim, (1989) used camel urine as a hair detergent.

Alhawi AlKabeer used two ounces plus one pound of milk for the treatment of ascites (Al-Razi, 1937). Also Alyahodi used goat urine plus fox apple. Dried and burned stool of camel was topically administered for skin dermatitis (Al-Yahodi 1958). They treated some patients with camel urine after boiling (Ali and Erwa, 1993). People in Yemen would dry camel urine under the sun and compress it in the form of tablets to be used in case of burns and wet body injuries (Al-Zhrawi, Ibn Elbitar, 1009). The percentages of use of camel urine among 5 nomadic tribes in eastern Sudan as follows: 72% use camel urine for internal problems in general, while 52%, 32%, 20%, and 32% used it for malaria, ascitis, dental problems and hair shampoo respectively. Regarding the type of the animal which urine is used, 88% used that of female and 12% of male. 72% drink it pure, whereas the remainder 28% mix it with the milk (Ohaj 1998).

1.7. Aims of the present study

Aims of the present work are to undertake greater in depth research on Camel urine.

1. Physical and Chemical analysis of the normal constituents of camel urine and compare it with that of other animals.
2. To assess camel urine as a remedy clammed in traditional medicine from therapeutic point of view. (Clinical studies on naturally infected calves (Faschiolosis)).

3. Pharmacological investigation by *invivo* and *invitro* methods.
   a) Camel urine hepatoprotective effect against CCl₄ & paracetamol.
   b) Bioscreening response to camel urine and its extracts.
   c) Camel urine effect on cell culture.

CHAPTER TWO

Materials and Methods

2.1. Materials

2.1.1. Experimental Animals

150 male and female Wistar albino rats weighing (90-250) gram were used. The animals were housed in groups and provided with a balanced diet and water ad libitum.

2.1.2. Naturally infected calves

Twenty five calves naturally parasitized with Fasciola gigantica, aged 1-2 years, weighing 105-160kg, were used in this study. Animals were kept in pens at the premises of the Central Veterinary Research Laboratory (C.V.R.L.) at Soba. They are identified by plastic ear tags. Then the animals were allotted into five groups and provided with a balanced diet and water ad libitum.

2.1.3. Sources of urine

Two adult female camel (5-6 years) urine was collected during twenty four hours, from Soba animal house, at the C.V.R.L., Soba, Khartoum, Sudan.

Young female camel urine (6 month up to two years old) urine was brought from different areas, Butana, Gazira (Wadballal).

The camel urine samples were collected by Tashweel technique which was done by touching the abdominal side of the female camel near the hide of the back leg. (Ohaj, 1998). By this technique urine sample could be available at any time. Urine extracts were prepared at the department of Biochemistry, Nutrition and Toxicology at the Central Veterinary Research Laboratory Unit-Soba, Khartoum, Sudan.

2.1.4. Extraction of camel urine components for screening programs:

2.1.4.1. Chloroformic extract:

Equal volumes of urine and chloroform were placed in a volumetric flask V/V, and allowed to shake for 3 hours, at room temperature using circular horizontal shaker. The mixture was poured in a separating funnel, till two layers were clearly separate. The lower chloroformic layer was displaced in a weighed beaker, the emulsified
layer was centrifuged and the clear chloroform layer was aspirated and added to the former one, then left to complete dryness at room temperature.

2.1.4.2. Protein precipitation:-

Native protein precipitate was obtained by the method of salt saturation (Ammonium Sulphate 40% W/V – salting out). The temperature was kept at 0°C. The mixture was allowed to shake for one hour using (KARL Kolb) water bath shaker. Then the mixture was centrifuged for 5 min at 4000 rpm, the supernatant was discarded and the precipitate was weighed and dissolved in 1ml distilled water, some were dissolved in Alkaline phosphate buffer pH 7.2 and kept at –20°C for further analysis.

2.1.4.3. Ultra centrifugation of camel urine

Ultra centrifuge (Class R) was adapted to 10000 round per minute for 20 minutes at (0-10)°C, then the precipitant was collected and dissolved in 0.5ml phosphate buffer and frozen at –20°C for further analysis.

2.1.4.4. Chemicals

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic acid</td>
<td>BDH</td>
</tr>
<tr>
<td>Alanine aminotransferase</td>
<td>Linear-chemical kits</td>
</tr>
<tr>
<td>Alkaline phosphates</td>
<td>Linear-chemical kits</td>
</tr>
<tr>
<td>Aspartate aminotransferase</td>
<td>Linear-chemical kits</td>
</tr>
<tr>
<td>Atropine</td>
<td>Sigama</td>
</tr>
<tr>
<td>Ammonium sulphate</td>
<td>BDH</td>
</tr>
<tr>
<td>Albumin</td>
<td>Linear chemical kits</td>
</tr>
<tr>
<td>Billirubin</td>
<td>Linear chemical kits</td>
</tr>
<tr>
<td>Chemical Name</td>
<td>Manufacturer</td>
</tr>
<tr>
<td>-------------------------------------</td>
<td>-----------------------</td>
</tr>
<tr>
<td>Barium chloride</td>
<td>BDH</td>
</tr>
<tr>
<td>Carbon tetra chloride</td>
<td>BDH</td>
</tr>
<tr>
<td>Calcium chloride</td>
<td>BDH</td>
</tr>
<tr>
<td>Chloroform</td>
<td>BDH</td>
</tr>
<tr>
<td>Cilica Gell</td>
<td>SD fine-Chemical India</td>
</tr>
<tr>
<td>Diethyl acetate</td>
<td>Merck</td>
</tr>
<tr>
<td>Diethylechloro methane</td>
<td>BDH</td>
</tr>
<tr>
<td>Ethylene diamine tetrachloride</td>
<td>BDH</td>
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<tr>
<td>Ethanol</td>
<td>BDH</td>
</tr>
<tr>
<td>Glacial acetic acid</td>
<td>BDH</td>
</tr>
<tr>
<td>Gamma glularyle transferase</td>
<td>Linear chemical kits</td>
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<tr>
<td>Glucose</td>
<td>BDH</td>
</tr>
<tr>
<td>Hydrochloric acid</td>
<td>SD fine-Chemical India</td>
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<td>5-Hydroxytryptamine</td>
<td>Sigama</td>
</tr>
<tr>
<td>Lactate dehydrogenase</td>
<td>Linear chemical kits</td>
</tr>
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<td>n-Butanol</td>
<td>Merek</td>
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<tr>
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<td>Sigama</td>
</tr>
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<td>Picric acid</td>
<td>Riedel DE haenag</td>
</tr>
<tr>
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<td>Hopkin / Will</td>
</tr>
<tr>
<td>Propanol</td>
<td>Merek</td>
</tr>
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<td>Potassium chloride</td>
<td>(BDH)</td>
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<td>sigma</td>
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<tr>
<td>Sodium carboxymethyl cellulose</td>
<td>Merck</td>
</tr>
<tr>
<td>Sodium hydroxide</td>
<td>Met lab.U.K</td>
</tr>
<tr>
<td>Trichloroacetic acid</td>
<td>Perkin &amp; William</td>
</tr>
</tbody>
</table>
2.2. Methods

2.2.1. Qualitative chemical tests

The pH of each sample was tested with a yellow litmus paper (Advantic Ptyo Roshi Kashi Ltd. Japan). The litmus paper after being dipped in the sample was compared to a colour-scaled paper with whole range of 0 to 14.

Total urinary protein was detected by the method of Varley (1984). This is simply done by placing 5ml of urine sample into a test tube and adding few drops of glacial acetic acid. A cloudiness or turbidity indicates the presence of urinary proteins.

2.2.1.1. Detection of chloride:

Inorganic compounds were detected qualitatively according to Vogel method (1982).

1- Chloride one gram of the sample (freeze dried camel urine) was placed in 100ml volumetric flask, the volume was completed to 100ml, and stirred for 10 minutes then filtered.

2- 10ml of the solution were diluted to 100ml with distilled water.

3- Then 0.5ml + 10ml of buffer (Contain 105g/L CH₃COOH+ 9 gm/L HNO₃) solution were mixed and then read on chloride meter.

2.2.1.2. Detection of Bicarbonate:

10mg of dried camel urine were dissolved in 5ml of distilled water. The solution was then treated with a solution of magnesium sulphate; no precipitate was produced (that means no carbonate). The solution was then boiled; a white precipitate is produced (indicate that the bicarbonate group is existing).

2.2.1.3. Detection of phosphate:

5ml of camel urine was treated with 5ml of silver nitrate solution. A yellow precipitant was produced, the colour of the precipitant was not changed on boiling and which is soluble in ammonia.
2.2.1.4. Detection of Sulphate:

About 50mg of freeze dried camel urine was dissolved in 5ml of distilled water. The solution was then treated with 1ml of 2M Hydrochloric acid and 1ml of barium chloride solution.

2.2.2. Quantitative chemical tests

2.2.2.1. Determination of sodium and potassium.

The sodium and potassium were estimated by flame photometer (Jenway, PEP7). Two separate standard curves were prepared ranging from 100 to 150 m Equ/L for sodium and potassium respectively. 0.2ml of urine sample was completed to 20ml (1 to 100) with distilled water and directly read on the (Flame photometer).

2.2.2.2. Determination of Magnesium, Copper and Zinc:

Mg, Cu and Zn were determined by the use of atomic absorption spectrophotometer (AAS). Stock, working standard, and sample specimens were prepared for each of the above elements as described by Beaty (1978). The instrument was calibrated according to the instruction manual and the absorbance was read.

2.2.2.3. Determination of calcium:

Urinary calcium was determined according to the method of Sulkowitch (1937). Sulkwitch reagent was prepared by mixing 2.5g oxalic acid, 2.5g ammonium sulphate and 5 ml glacial acetic acid completed to 100 ml with distilled water. Equal volumes of the above reagent and urine sample were mixed.

2.2.2.4. Determination of total protein in urine:

Urinary protein was determined by the method of Biuret reaction as discribed by Weichselbaum (1946). The protein was concentrated by precipitation using trichloroacetic acid. It was redissolved in an alkali and measured spectrophotometrically at 420nm.

2.2.2.5. Determination of non-protein nitrogen:
(Urea, uric acid creatinine, and creatine)

The urea was determined by the manual method described by (Evans, 1968; March et al., 1965). The urine sample was diluted 1 to 10 with distilled water. The possible proteins were precipitated with tricholoroacetic acid and centrifuged, the sample absorbance was read against urea standard (10mmol/L) at 520nm.

The creatinine was determined by the method of alkaline picrate of (Bonsnes and Taussky 1945). One ml of urine sample was completed to 100 with distilled water, 1ml picric was added to 3ml diluted urine, followed by 1ml of NaOH. 3ml creatinine standard (0.3 micromol and 3ml distilled water) used as blank were treated the same way. The absorbance was measured after 15 minutes at 500nm.

The creatine in urine was transferred to creatinine by heating urine sample with picric acid before adding NaOH. Then total creatinine was determined according to the above method.

2.2.2.6. Determination of amino acids in urine:

Amino acids in urine samples were determined according to the method of (Goodwin 1968b, 1970) using dinitroflouro-benzene. 1ml of urine sample was diluted to 10 ml with distilled water. Three drops of phenophthaline and 200mmol/L NaOH was added until the mixture turn pink. The mixture was boiled for 15 minutes at 70°C, cooled and 5ml acidified dioxane were added. Stock and working standard of glutamic acid glycine were prepared and treated as directed. The absorbance of the sample was measured against that of standard.

2.2.2.7. Determination of Uric acid in urine:

Uric acid was estimated according to Cavawy (1954). Diluted urine (1:100), 5ml of urine, 1ml of 10% sodium tungestate and 1ml of 2N H$_2$SO$_4$ were mixed and centrifuged. 5ml of the supernatant was put in one of two test tubes, (standard and blank). o each tube, 1ml of 10%
sodium carbonate and 1ml of phosphatungestic acid were added and left to stand at room temp. for 30 minutes, then was read at 680 nm.

2.2.2.8. Determination of urine Albumin:

Urine albumin was determined according to (Kentman et al. 1971).

2.2.29. Determination of serum total Bilirubin:-

Serum total Bilirubin was determined according to (Kind and King 1954).

Principle:

Billirubin in the sample reacts with diazotized sulfanilic acid in the presence of DMSO. The formed coloured azobilirubin is measured photometrically: there is two Bilirubin fractions in serum, bilirubin-glucuronide and free bilimute which is bound to albumin.

2.3. Biochemical methods:

2.3.1. Aspartate amino transferase (AST: EC. 2.6.11.1)

Aspartate amino transferase, formerly known as glutamic oxaloacetic acid transaminase (GOT) catalysis the reversible transfer of the amino group from an amino acid to a keto acid. This enzyme is mainly present in the liver and many other tissues including kidneys, heart, and skeletal muscle. The enzyme activity was measured as described by Reitman and Frankel (1957). The principle of the assay depends on the intermolecular transfer of an amino group from aspartic acid to α-keto glutaric acid without the intermediate formation of ammonia, and measuring the amount of the reaction product (Oxaloacetate).

\[
\text{Glutamic acid} + \text{oxaloacetic acid} \xrightarrow{\text{AST}} \alpha \text{-keto glutaric acid} + \text{aspartic acid.}
\]

Aspartate amino transferase catalysis the transfer of amino group from aspartate to 2-oxoglutarate forming glutamate and oxaloacetate. The oxaloacetate is reduced to malate by dehydrogenase and NADH.
The rate of decrease in concentration of NADH is proportional to concentration of AST present in the sample.

\[
\text{Aspartate} + 2\text{-oxoglutarate} \xrightarrow{\text{AST}} \text{Glutamate} + \text{oxaloacetate}
\]

\[
\text{NADH}^+ + \text{H}^+ + \text{oxaloacetate} \xrightarrow{\text{MDH}} \text{Malate} + \text{NAD}^+
\]

2.3.2. Alanine amino transferase (ALT: EC1.2.6.1.2 formally known as GPT):

Alanine amino transferase, catalyses the transfer of an amino group from alanine to 2-oxoglutarate forming glutamate and pyruvate. The pyruvate produced is reduced to lactate-by-lactate dehydrogenase (LDH). Lactate dehydrogenase catalyses the reduction of pyruvate by NADH is proportional to concentration of ALT present in the sample.

\[
\text{Alanine} + \text{H}^+ + \text{Pyruvate} \xrightarrow{\text{ALT}} \text{Glutamate} + \text{pyruvate}
\]

\[
\text{NADH} + \text{H}^+ + \text{pyruvate} \xrightarrow{\text{LDH}} \text{Lactate} + \text{NAD}^+
\]

2.3.3. Alkaline phosphates (Linear-Chemical)

(ALP; E.C.3.1.3.1):

Alkaline phosphates was determined according to Chemie,(1972)

Principle:

Alkaline phosphates catalyze the hydrolysis of p-nitrophenylphosphate, in the presence of magnesium ions, liberating inorganic phosphate and p-nitrophenol. The rate of p-nitrophenol formation is proportional to the concentration of ALP present in the sample.

\[
\text{4-nitrophenyl phosphate} + \text{H}_2\text{O} \xrightarrow{\text{ALP}} 4\text{-nitrophenyl} + \text{P1}
\]

2.3.4. \( \gamma \) -Glutamyl transferase(Linear-Chemical)

(GGT:E.C.2.3.2.2.):

Principle:
γ-Glutamyl transferase catalysis the transfer of a γ-glutamyl group from γ-glutamyl-carboxy-4-nitroanalide, glutamate is proportional to the concentration of GGT present in the sample.

\[
\begin{align*}
\gamma\text{-glutamyl-3-carboxy-4-nitroanalide} + \text{glycyl glycine} & \rightarrow \\
\downarrow & \\
\gamma\text{-glutamyl glycyl glycine} + 3\text{-carboxy-4-Nitroanaline} & \\
\end{align*}
\]

2.4 Haematological methods:

Blood samples for whole blood examinations were withdrawn from the jugular vein at intervals in vacutainer tubes (5ml) containing ethylene diamine tetrachloroacetic acid (EDTA) as anticoagulant (Becton, Dickinson, France) as described by (Schalm 1965).

2.4.1. Determination of Haemoglobin (Hb)

The concentration of haemoglobin was measured by the cyanomethaemoglobin technique using colorimeter (CIBA CORNING colorimeter model 252). 0.2ml of blood was added to 4ml of Drabkins solution (0.2gm of potassium ferricyanide and 1gm bicarbonate per litre of distilled water). The haemoglobin concentration was measured in g/dl of blood. This method is based on the conversion of haemoglobin by Drabkins solution to cyanomethaemoglobin as described by (Schalm 1965).

2.4.2. Red Blood Cells Count (RBC):

Erythrocytes were counted using Neubauer haemocytometer (Hawksley and sons Ltd., England) using Hayems solution as a diluent consisting of 0.5gm of mercuric chloride and 1 gm of sodium chloride made up to 200ml with distilled water as estimated by (Schalm, 1965).

2.4.3 White Blood cells count (WBC):
Leukocytes were counted according to Schalm (1965) using Neubauer haemocytometer. Turk’s solution (1% glacial acetic acid coloured with gentian violet made up to 200ml with distilled water) was used as a diluent.

2.4.4. Packed Cell Volume (PCV):

Blood samples were drawn into microhaematocrit capillary tubes and sealed at one end with cristaseal (Hawksley). The capillary tubes were centrifuged at 8000 rpm for 5 minutes using a microhaematocrit centrifuge (Hawksley and sons Ltd., England). The PCV percentage was read with Hawskley microhaematocrit reader (Scalm, 1965).

2.4.5. Mean Corpuscular Volume (MCV):

The MCV was calculated from the PCV and RBC values as follows

\[
MCV (fl) = \frac{PCV \times 10}{RBC}
\]

2.4.6. Mean corpuscular haemoglobin concentration (MCHC):

MCHC was calculated from the PCV and Hb values as follows

\[
MCHC(\%) = \frac{Hb (g/dl) \times 100}{PCV}
\]

2.4.7. Thromboplastin test with calcium (TT):

Prothrombin time studies the total extrinsic clotting system. It measures the clotting time of a plasma at 37°C in the presence of excess tissue thromboplastin and calcium. The test is dependent on factors II, V, VII and X.

2.4.8. Activated partial thromboplastin time (APTT):
Activated partial thromboplastin time (APTT) measures the clotting time of plasma or serum at 37°C in the presence of a platelet substitute and an activator.

2.5. Pathological methods

2.5.1. Macroscopic examination

Macroscopic examination was carried out on each necropsied animal, all organs were examined for the presence of any gross changes. The liver was examined carefully for the presence of fasciola worms.

2.5.2. Microscopic techniques:

Representative samples from liver, intestine, kidney, lung, heart, spleen were taken and preserved in normal saline (10%). Tissues were trimmed and dehydrated in serial dilution of alcohol, 70, 85, 95 and 100%, using automatic tissue processor and cleared twice by xylene. Then embedded in paraffin wax and 5µ sections were cut using a microtome. Sections were stained with Haematoxylin and Eosin and special stain may also be used. Processing and staining procedures were those described by Clayden (1971).

2.6. Pharmacological methods

2.6.1. ED_{50} Determination:

Preliminary experiments were carried out for each type of urine collection and from chloromoforic extract of camel urine to determine the approximate dose, which produces 0-100% lethality. Doses chosen based on the honorable Haddith and on the preliminary data, which were administered randomly. The tested agents were given camel urine orally. While CE was injected intraperitoneally to each group of rats. Death on each group was observed over the next 24 hours. Percent mortalities in all groups were then calculated.
2.6.2. Assessment of hepatoprotective activity of camel urine and its chloroformic extract against CCL4 induced hepatic toxicity in rats:

CCL4 was dissolved in 2% Na carboxy methyl cellulose CMC (15% v/v) and administered orally at a dose of 10ml/kg and IP at a dose of 1ml/kg to Wistar Ablino rats (CCL4 control group). The rise in ALT (GPT) was taken as evidence for impaired liver function. The peak of increase in serum ALT activity was reached 24 hours after CCL4 administration (Mansour, 2000; Handa et al. 1986).

2.6.3. Assessment of Camel urine hepatoprotective activity against paracetamol induced hepato toxicity:

Paracetamol was dissolved in distilled water (1g in 100 ml). A dose of 1 g/kg body weight was orally administrated to male and female Wistar albino rats (Control group) to damage liver cells (Tee et al, 1987) paracetamol was given 1 hour after chloroformic extract of camel urine, 24 hour later samples were collected from treated and control groups, and frozen at –20°C till analyzed for ALT activities.

2.6.4. Evaluation of camel urine and its chloroformic extract (CE) on isolated tissue strips preparations.

Preparation of physiologic salt solution: Kreb's solution is prepared as follows

NaCl, 34.6; KCl, 1.75; CaCl2, 1.4; MgSO4, 1.45; NaHCO3, 10.5; KH2PO4, 1.8; in grams/5 liter (Kitchen, 1984).

The physiologic salt solution should be clear, not milky or cloudy.

Tyrod's solution is prepared of the following concentrations NaCl, 40; glucose, 5; CaCl2, 1; MgCl2, 1; MgCl2 0.5; NaH2PO4, 0.025; NaHCO3, 5; in grams per liters.

2.6.4.1. Rat fundus strip preparation:
A Wistar Albino rat is killed by a blow on the head according to the method of Vane (1957), and the throat was cut, the abdomen was opened, the stomach was removed and placed on a petri dish containing aerated Kreb's solution, cuts were made along the junction of the fundus and pylorus. The fundus was opened and cuts were made alternatively from each side to make a strip about 4 cm long. A thread was attached at each end of the preparation, and it was mounted to the organ bath with aerated Kreb's solution, temp. was maintained at 37°C.

2.6.4.2. Rat dudenum-strip preparation:

The rat (Wistar albino) duodenal strip was prepared according to the method of Kitchen (1984). The duodenal strip (Proximate part of intestine) 2-3 cm long was mounted in an organ bath filled with an aerated Tyrod's solution, temp. was maintained to 37°C.

2.6.4.3. Rabbit jejunum preparation:

Rabbit of local strains weighing 1.5-2kg. were used. The animal was killed by dislocating the neck and exsanguinated. The abdomen was exposed. The first 2-3 cm of the jejunum was taken out and placed in a petri-dish containing tyrod's solution at room temperature. The fat and connective tissues were removed. Then the tissue was tied by thread at the two ends transferred to an organ bath (25ml), containing an aerated Tyrode's solution temp. was maintained at 37°C (Trendelenburg, 1917).

The urine and its extraction were left in contact with tissue for 30 seconds, in a dose cycle of 3 min.

2.6.4.4. Chick rectum strip preparation:

A young chick (7-15 day old) was killed with chloroform. The abdomen was exposed and the last terminal part of the intestine (rectum) attached to anal orifice was cut and placed on a petri dish containing aerated Tyrode's solution. A thread was attached to the strip and then mounted to the organ bath.
2.6.5. Preparation of tissue culture:

2.6.5.1. Primary bovine kidney cells:

Calf kidneys obtained from calves 1-4 weeks old were prepared according to reported method of FAO, (1984). One or both kidneys were separated from the animal under aseptic conditions and carried to the laboratory in sterile beaker. Fats and capsule removed from the organ and the cortex was cut in fragments from the medulla, placed in sterile petri dish and minced with a pair of scissors. The tissue was washed in a balanced salt solution free from calcium and magnesium, as phosphate diluent (PD) containing 200 IU penicillin and 100µg streptomycin. Tissue fragments were transferred to a grooved trypsinization flask agitated by magnetic stirrer bar and washed in Hank's solution until clear. After discarding the last wash pre-warmed 0.25% trypsin in Hanks balanced salt solution (1: 9 dilution) replaced to cover the tissue and repeated the washing. After the pieces of tissue settled to the bottom of the flask, the supernatant was discarded since it contains dead cells and blood. Fresh warmed trypsin was added to the tissue and stirred overnight at 4°C, the digested tissue was allowed to settle and the supernatant filtered through three layers of sterile gauze in a flask containing bovine serum to stop trypsin action. The suspension was centrifuged and the supernatant was discarded, the pellet cells were suspended as 1ml packed cell was added to 250-300ml (GMEM) plus5-10% bovine serum. Cell suspension was dispensed into tissue culture flasks and incubated at 37°C to form monolayers within 4-5 days post-seeding.

2.6.5.2. Secondary cell cultures:

Secondary cell culture was propagated from the primary culture as follows:
After removing the growth medium from the fully-grown culture, the sheet of cells was washed twice with phosphate diluent (PD). Trypsin solution warmed to 37°C added to cover cell sheet and left for 5 minutes. Cells removed by pipetting gently with some (PD) and bovine serum was added to stop trypsin action. Cells were centrifuged at 1000 rpm for 5 minutes and the supernatant was discarded. Then suspended in twice the original volume of growth medium containing antibiotics with 5-10% calf serum, distributed into tissue culture glasses and incubated at 37°C.

2.7. Chromatographic methods:

Chromatography is a method used for purification and identification of chemical substances that consist of many compounds on the basis of physical methods of separation, such as; thin layer chromatography (TLC); gel and ion-exchange method, in addition to gas liquid chromatography (GLC), and high performance liquid chromatography (HPLC). Separations are made on the basis of several differential molecular properties, especially charge, solubility, affinity and adsorption.

2.7.1. Extraction:

As a primary requirement, the substance to be extracted must be soluble, not only in aqueous biological media, but also in a phase which is immiscible (or only slightly miscible) with water e.g. ether, chloroform, carbon tetrachloride etc. If the aqueous phase shaken with the organic solvent, the compound distribute itself between the two phases in accordance with its distribution coefficient. If the aqueous phase is repeatedly extracted with a fresh organic phase, a group of substances can almost be completely separated.

2.7.2. Thin layer chromatography:
The stationary phase is a thin layer [0.1-0.5mm] of the grained silica gel (Kessel Gell G) deposited on a carrier plate [20x20 cm] made of glass, plastic, or aluminum foil. 1-5µl of sample is applied as on a spot of the starting line about [10-15mm] from the bottom edge of the plate. Then placed in a tightly closed tank containing a suitable eluting solvents. The mobile phase diffusing up by capillary action.

Thin-layer chromatography is based on adsorption or partition.

A measurement of the rate of migration of substance is provided by the RF value.

\[ R_f = \frac{\text{distance traveled by the compound}}{\text{distance traveled by solvents}} \]

2.7.3. Preparative thin layer chromatography [PTLC]

Silica gel Kessel Gell G 0.5 mm thickness, were used. Fraction A (chloroformic extract); fraction B (Butanolic extract) fraction C (ethanolic extract), were applied in a form of streeks and the chromatogram was developed using n-butanol-acetic acid and water (BAW) as a solvent system in a ratio of 4:1:1.

On the bases of Ultrviolet light (U.V.) absorption, each streek was composed of more than one band.

Different developing systems were used. Propanol and water in a ratio of 70:30; n-Butanol, acetic acid and water in a ratio 4:5:1.

2.7.4. Gel filtration on sephadex A25-150:

Sephadex R (A25-120 SDEAE) was allowed to swell in (M) methanol, for 24 hours. The gel was then made in a form of thick slurry, degassed and then packed into the column. A 2.5x100 cm glass column was fixed at a vertical stand. The bottom (outlet) was clamped and the
thick slurry was poured gently into the glass column till it was \( \frac{3}{4} \) filled with methanol and the methanol was allowed to flow over until the gel packed and degassed. 5ml of camel urine were added. Then methanol was added to form 20ml eluted layer over the gel. Introduction of air bubble was avoided during the packing and elution process. Samples were collected as 20ml/tube. 60 fractions were obtained. Followed by 20 fractions elution by methanol in 2N HCL in a ratio of (99:1).

2.7.5. Column chromatography

Gel filtration on sephadex (Fluka biochemical DEAE (A25-120) and an ionexchange, were the principle technique employed for the purification of camel urine protein and amino acid classes.

2.7.6. Ion exchange chromatography

60 Mesh or ion-exchange Resin column used for the isolation of camel urine protein, amino acid, amide and polypeptides by a method similar to the procedure employed by Fey et al. (1976).

Microgranules mesh 60 was first soaked with n-butanol solution (BDH), which it self-constituting the starting mobile phase solution. The resin was then packed in 1.5x30 cm glass column, fixed in a vertical position. The outlet was closed and one third of he column was filed with starting solution, 10 ml of camel urine were applied to the 60-mesh resin column. The elution was performed from top downwards at speed of 1ml/min. Samples were collected as 3ml/tube. The elution profile of each preparation was chromatographed by TIC and PTLC. Protein fractions were more identified by Ultra violet spectrophotometer (UV), infra red spectra and gas chromatography (GC).

2.7.7. Electrophoresis:
This technique was performed according to Ouchterlony and Nilson (1979). It was employed for purity testing as well as identification of isolated camel urine fractions.

The technique was carried out in commercial sheets of agarose gel in 0.1 M barbitone buffer, pH 8.6, were noticed.

Five hundred ml of 0.1 M barbitone buffer (pH 8.6) were poured into electrophoresis chamber (Ciba).

0.5µl of camel urine protein, milk protein, and that of other species were applied onto the agarose gel membrane. The membrane was then stretched across the two chambers, with its edges submerged in the barbital buffer. The anode was specified, and the electrophoresis was run at 70 volt, 12 mA for half an hour.

It was then removed and placed in a fixative dye solution, for 5 min. the strips were then destained and cleaned with ethyl alcohol.

The resulting peaks were designated, according to their speed of migration towards the anode as albumin, A₁, A₂, B₁, B₂ and γ globulins.

2.7.8. **Gas liquid chromatography (GLC):**

2.7.8.1. **Protein hydrolysate of biological materials**

One gram of sample was refluxed with 10ml constant boiling HCl for 20 hours. The hydrolysate was filtered through glass fiber (Reve Angel 934A₄ filter paper), and the sample was taken to dryness at room temperature on a rotary evaporator, the dry residue was redissolved in 1 litre of 0.1 N HCl in a 25ml flat bottom flask, lyophilized, then dried and derivatized for gas chromatography.

2.7.8.2. **Derivatization method:**

1- Removal of water to give dry amino acids

2- Esterification of the amino acids to form methyl ester hydrochlorides.

3- Interesterification of the methyl ester to form n-butyl ester hydrochlorides.
4- Acylation of n-butanol ester hydrochlorides to form n-trifluoroacetyl-butytl esters.

2.8.Confirmatory chemical test of chloroformic extract:

2.8.1. Biuret test:

The biuret stock reagent was prepared following the method described by (Henry, et al. 1974). Forty five grams of sodium potassium tarterate were transferred to a 1 litre flask containing 400ml 0.2 N NaOH. Two hundred ml of this stock solution were diluted to 1 litre with alkaline iodide solution to about 4 ml of the Biuret working solution were added to a soluble amount of chloroformic extract crude urine and mixed thoroughly. The mixture was allowed to stand for 10 minutes and the change in colour was observed.

2.8.2. Acid hydrolysis of camel urine and chloroformic extract:

Two to three drops 0.6 N HCl were added to a few ml's of camel urine, chloroformic extract each, and heated at 120°C for 3 hours. The hydrolysate obtained, the references solution and authentic samples of six amino acids (glycine, DL-serine, L-Tyrosine, L-Alanine histadine monochloride, 4-Aminobuteric acid and L-Tryptorphan) were chromatographer on silica gel plates using propanol-water, Butanol-acetic acid and water as developing systems. The developed plates were dried, sprayed with ninhydrin reagent and the Rf values of the detected spots were calculated.

2.9. Analytical screening of camel urine

Analytical screening for the constituents present in the camel urine extracts were carried out using the methods of (Ibrahim 1991). These studies were carried out on the liquid sample of urine and on the successive extracts of camel and cattle urine.

2.9.1. Tests of alkaloids and/or nitrogenous bases:-

a) The residues obtained from 20ml of Alcoholic extract, (ethanol and water; 35:15) was dissolved in 2ml dilute hydrochloric acid, and tested with Mayer’s and Wagner reagents (1.3g of mercuric chloride were dissolved in 60ml of
water, and 5g of potassium sulphite (KS) in 10ml of water. The two solutions are mixed and diluted to 100ml with distilled water).

b) Urine samples of camel (from adult and young female camel) and cattle were extracted with chloroform (v/v). Chloroformic layer was then evaporated, using steam to evaporate chloroform, the residue was dissolved in few mls of methanol. Then each sample was spotted on TLC plate and developed with methanol and ammonium water (100:1.5) to a suitable distance. The plates were then air dried, visualized under U.V. (Bright-Blue spots were seen), sprayed with Dragndoff reagent. The Rf of the detected compounds were calculated.

2.10. Experimental design and treatment protocol

Experiments:
1. Physical and biochemical analysis of camel, cattle, goat and human urine.
2. Time course of hepatotoxic effect of (CCL₄) on rat liver.
3. Hepatoprotective effect against carbon tetrachloride (CCL₄) pre and post treatment with camel urine, and urine extracts.
4. Hepatoprotective effect against paracetamol compared with Silymarin natural hepatoprotective drug.
5. Clinical trials on naturally infected calves (fascioliasis).
6. *In vitro* effects of camel urine on cell culture and isolated strips.
7. Chromatography and electrophoresis.
8. Phytochemical analysis of camel urine.

**Experiment (1)**

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dosage</th>
<th>Route</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2% carboxyl methyl cellulose (CMC)</td>
<td>10ml/kg B.W.</td>
<td>Orally</td>
</tr>
<tr>
<td>2</td>
<td>2% (CMC) + CCL₄</td>
<td>10ml 2% (CMC/kg 150µl +kg B.W.)</td>
<td>Orally</td>
</tr>
<tr>
<td></td>
<td>Description</td>
<td>Amount/Concentration</td>
<td>Route</td>
</tr>
<tr>
<td>---</td>
<td>------------------------------------------------------------------------------</td>
<td>----------------------------------------------------------</td>
<td>-----------</td>
</tr>
<tr>
<td>3</td>
<td>Urine + CCL₄</td>
<td>2ml of urine 150µl+kg B.W.</td>
<td>Orally</td>
</tr>
<tr>
<td>4</td>
<td>Urine + CCL₄</td>
<td>4ml of urine 150µl+kg B.W.</td>
<td>Orally</td>
</tr>
<tr>
<td>5</td>
<td>Urine + CCL₄</td>
<td>8ml of urine 150µl+kg B.W.</td>
<td>Orally</td>
</tr>
<tr>
<td>6</td>
<td>Corn oil</td>
<td>10ml 1kg B.W.</td>
<td>Orally</td>
</tr>
<tr>
<td>7</td>
<td>Urine + CCL₄ before 1 hour</td>
<td>8ml of urine + 1ml 1kg B.W.</td>
<td>Orally</td>
</tr>
<tr>
<td>8</td>
<td>Urine + CCL₄ before 1 hour</td>
<td>16ml of urine + 1ml 1kg B.W.</td>
<td>Orally</td>
</tr>
<tr>
<td>9</td>
<td>Urine + CCL₄ before 1 hour</td>
<td>32ml of urine + 1ml 1kg B.W.</td>
<td>Orally</td>
</tr>
<tr>
<td>10</td>
<td>CCL₄ 5% (v/v) in corn oil</td>
<td>1ml/kg B.W.</td>
<td>I/P</td>
</tr>
<tr>
<td>11</td>
<td>Chloroformic extract (C.E.) + CCL₄ before 1 hour</td>
<td>100mg C.E. + ml CCL₄/kg B.W.</td>
<td>I/P</td>
</tr>
<tr>
<td>12</td>
<td>CCL₄ before 1 hour</td>
<td>200mg C.E. +ml CCL₄/kg B.W.</td>
<td>I/P</td>
</tr>
<tr>
<td>13</td>
<td>CCL₄ before 1 hour</td>
<td>404mg C.E. +ml CCL₄/kg B.W.</td>
<td>I/P</td>
</tr>
<tr>
<td>14</td>
<td>Corn oil</td>
<td>ml/kg B.W.</td>
<td>I/P</td>
</tr>
<tr>
<td>15</td>
<td>CCL₄ + corn oil</td>
<td>ml/kg B.W.</td>
<td>I/P</td>
</tr>
<tr>
<td>16</td>
<td>Corn oil</td>
<td>ml/kg B.W.</td>
<td>I/P</td>
</tr>
<tr>
<td>17</td>
<td>CCL₄ + in corn oil</td>
<td>ml/kg</td>
<td>I/P</td>
</tr>
<tr>
<td>18</td>
<td>CCL₄ + urine after 1 hour</td>
<td>ml +8ml/kg B.W.</td>
<td>I/P orally</td>
</tr>
<tr>
<td>19</td>
<td>CCL₄ + urine after 1 hour</td>
<td>ml +16ml urine/kg B.W.</td>
<td>I/P orally</td>
</tr>
<tr>
<td>20</td>
<td>CCL₄ + urine after 1 hour</td>
<td>ml (CCL₄) +32ml/kg B.W.</td>
<td>I/P orally</td>
</tr>
<tr>
<td>21</td>
<td>CCL₄ + C.E after 1 hour</td>
<td>ml (CCL₄) +100mg/kg B.W.</td>
<td>I/P orally</td>
</tr>
<tr>
<td>22</td>
<td>CCL₄ + C.E after 1 hour</td>
<td>ml (CCL₄) +200mg C.E./kg B.W.</td>
<td>I/P orally</td>
</tr>
</tbody>
</table>
23 | CCL₄ + C.E after 1 hour | ml (CCL₄) +400mg C.E./kg B.W. | I/P orally |
24 | CCL₄ + corn oil | ml /kg B.W. | I/P |
25 | CCL₄ + corn oil | ml /kg B.W. | I/P |

**Experiment II paracetamol**

**Examination of camel urine**

1- Microscopically examination

2- Cultural examination

a) Maconkey agar

b) Blood agar

   All the methods, were described in details in chapter 2.2.

**Experiment III**

**Experimental design:**

The positive calves were randomly assigned into 4 groups of 4 calves, the 4 additional calves were held as replacements. And the remainder were healthy normal calves. The calves were treated according to the scheme shown below.

<table>
<thead>
<tr>
<th>A</th>
<th>Infected untreated</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>Infected treated with 2ml/kg camel urine B.W.</td>
</tr>
<tr>
<td>C</td>
<td>Infected treated with 4 ml /kg camel urine B.W.</td>
</tr>
<tr>
<td>D</td>
<td>Infected treated with</td>
</tr>
</tbody>
</table>
Animals of Group I (infected untreated control) were given the placebo as adrench at a dose rate of 2ml /kg B.W. group II and III received fresh early morning urine of Young she-camel at a dose rate of 2 and 4ml/kg B.W. respectively. Group IV was given Rafoxanide 3, 5, dinitro-3’-chloro-4P-chlorophenoxy(salycianide) as adrench at a dose of 4ml/10kg B.W. group V stands as normal healthy calves.

2.11. Statistical analysis:

Data were expressed as (Means ± SEM). Statistical comparisons between groups were done using one way analysis of variance (ANOVA) followed by multiple comparison test to compare differences between groups. Significance was accepted at P<0.05.

CHAPTER THREE

RESULTS

3.1. Urine analysis

3.1.1. Physical properties of urine

Physical properties of camel cattle, goat, and human urine shows that most samples have a varying degree of colour intensities. It ranges from colourless to slight yellow and watery for human and younger animals. Amber yellow to ferrous in case of adult camel. On long standing the colour may change to deep brown or red. The pH of camel urine was alkaline with an average of 9.5±0.5, when compared to other species urine sample pH as shown in table (1).

Table (1) The physical properties of different urines:

<table>
<thead>
<tr>
<th>Type</th>
<th>Sex</th>
<th>Temperature</th>
<th>PH</th>
<th>Colour</th>
</tr>
</thead>
<tbody>
<tr>
<td>rafoxoniale 4ml/10kg B.W.</td>
<td>E</td>
<td>Healthy not treated</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
3.1.2. Qualitative analysis of sulphates, bicarbonates, phosphates and chlorides.

Crude camel urine sample or freeze-dried urine sample, when examined qualitatively proved the predominance of sulphates followed by phosphates, bicarbonates and chlorides.

3.1.3. Biochemical constituents of camel urine compared to that of other species.

3.1.3.1. Determination of organic compounds of camel urine.

The results of organic constituent (Protein and non-protein nitrogen) such as total protein (TP), Albumin, urea, uric acid, creatinine and creatine were shown in table (2), with the mean and SEM of the six compared animals.

Table (2) Organic constituents of different species urine
3.1.3.2. Determination of electrolytes and trace element constituents of camel urine:

Table (3) showed the concentration of the electrolytes sodium, potassium in mmol/L, magnesium (Mg) and calcium (Ca), in mg/L. the microelements zinc (Zn) and iron concentrations in mmol/L the results of the above minerals concentration in the six animal species were comparable to Ohaj 1993, 1998. Table (4) represents the concentration in percentage of mineral contents of some preferable plants browsed by camel in the Sudan.

Table (3) Electrolytes and Mineral contents of Camel Urine Compared to Other species urine

<table>
<thead>
<tr>
<th>Species</th>
<th>G/L Albumin</th>
<th>G/L Total protein</th>
<th>Mg/L Urea</th>
<th>Mg/L Uric acid</th>
<th>Mg/L Creatinine</th>
<th>Mg/L Creatine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>7.8 ± 1.6</td>
<td>8.87 ± 3.3</td>
<td>14.2 ± 3.3</td>
<td>182.25 ± 23.4</td>
<td>127.17 ± 18.65</td>
<td>17.75 ± 3.2</td>
</tr>
<tr>
<td>Goat</td>
<td>8.7 ± 0.84</td>
<td>9.4 ± 0.6</td>
<td>15.66 ± 0.69</td>
<td>236.3 ± 33.98</td>
<td>144 ± 26.63</td>
<td>20.9 ± 1.5</td>
</tr>
<tr>
<td>Camel</td>
<td>7.27 ± 1.24</td>
<td>8.7 ± 1.25</td>
<td>12.3 ± 2.55</td>
<td>214.5 ± 62.01</td>
<td>136.88 ± 30.35</td>
<td>23.11 ± 8.67</td>
</tr>
<tr>
<td>Camel Young</td>
<td>4.54 ± 1.17</td>
<td>7.34 ± 0.28</td>
<td>10.5 ± 2.76</td>
<td>171 ± 10.53</td>
<td>126 ± 11.93</td>
<td>15 ± 0.31</td>
</tr>
<tr>
<td>Man</td>
<td>6.95 ± 0.77</td>
<td>8.6 ± 0.56</td>
<td>10.35 ± 0.21</td>
<td>149.5 ± 0.7</td>
<td>132.5 ± 3.53</td>
<td>14.3 ± 0.84</td>
</tr>
<tr>
<td>Woman</td>
<td>6.4 ± 0.28</td>
<td>8.15 ± 0.49</td>
<td>9.7 ± 0.28</td>
<td>145.5 ± 0.7</td>
<td>137 ± 0.0</td>
<td>14.35 ± 0.63</td>
</tr>
<tr>
<td>Animal</td>
<td>Value 1</td>
<td>Value 2</td>
<td>Value 3</td>
<td>Value 4</td>
<td>Value 5</td>
<td></td>
</tr>
<tr>
<td>-------------</td>
<td>---------</td>
<td>---------</td>
<td>---------</td>
<td>---------</td>
<td>---------</td>
<td></td>
</tr>
<tr>
<td>Cattle</td>
<td>9.6±3.56</td>
<td>154.25±22.69</td>
<td>206.75±15.04</td>
<td>6.77±1.31</td>
<td>3.0±0.6</td>
<td></td>
</tr>
<tr>
<td>Camel</td>
<td>9.13±0.38</td>
<td>167.66±23.93</td>
<td>198.17±13.19</td>
<td>7.25±0.65</td>
<td>2.0±0.8</td>
<td></td>
</tr>
<tr>
<td>Camel young</td>
<td>8.27±0.65</td>
<td>175.13±19.67</td>
<td>200.87±10.25</td>
<td>7.75±0.47</td>
<td>2.0±0.6</td>
<td></td>
</tr>
<tr>
<td>Goats</td>
<td>7.74±0.43</td>
<td>142.2±8.01</td>
<td>172.8±7.59</td>
<td>5.44±0.35</td>
<td>1.04±0.4</td>
<td></td>
</tr>
<tr>
<td>Man</td>
<td>7.45±0.35</td>
<td>148.0±2.83</td>
<td>174±7.07</td>
<td>5.15±0.07</td>
<td>2.0±0.08</td>
<td></td>
</tr>
<tr>
<td>Woman</td>
<td>7.35±0.21</td>
<td>145.0±9.89</td>
<td>171±1.41</td>
<td>5.25±0.35</td>
<td>2.0±0.05</td>
<td></td>
</tr>
</tbody>
</table>


Table 4 Mineral concentration of some important plants browsed by camel in the Sudan

<table>
<thead>
<tr>
<th>Botanical name</th>
<th>Ca%</th>
<th>Mg %</th>
<th>K%</th>
<th>Mn%</th>
<th>Zn%</th>
<th>Cu%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acacia seyal var. fistula</td>
<td>10.16</td>
<td>0.21</td>
<td>0.67</td>
<td>0.0101</td>
<td>0.118</td>
<td>0.0632</td>
</tr>
<tr>
<td>Acacia seyal var. seyal</td>
<td>14.5</td>
<td>0.62</td>
<td>0.49</td>
<td>0.0162</td>
<td>0.166</td>
<td>1.050</td>
</tr>
<tr>
<td>Acacia Senegal</td>
<td>6.3</td>
<td>0.76</td>
<td>0.61</td>
<td>0.006</td>
<td>0.081</td>
<td>0.0187</td>
</tr>
<tr>
<td>Acacia mellifera</td>
<td>5.85</td>
<td>0.73</td>
<td>0.80</td>
<td>0.0045</td>
<td>0.101</td>
<td>0.0156</td>
</tr>
<tr>
<td>Acacia seiberaine</td>
<td>9.4</td>
<td>1.8</td>
<td>1.061</td>
<td>0.0048</td>
<td>1.22</td>
<td>0.0416</td>
</tr>
<tr>
<td>Acacia nubiea</td>
<td>6.8</td>
<td>2.1</td>
<td>0.89</td>
<td>0.0105</td>
<td>0.1706</td>
<td>0.0104</td>
</tr>
<tr>
<td>Acacia tortilis</td>
<td>5.07</td>
<td>0.47</td>
<td>0.29</td>
<td>0.0047</td>
<td>0.1062</td>
<td>0.0499</td>
</tr>
<tr>
<td>Ziziphus-spina christi</td>
<td>8.16</td>
<td>1.53</td>
<td>1.32</td>
<td>0.0214</td>
<td>0.373</td>
<td>0.0624</td>
</tr>
<tr>
<td>Balanites aegyptica</td>
<td>7.62</td>
<td>1.7</td>
<td>1.32</td>
<td>0.0193</td>
<td>0.137</td>
<td>0.0395</td>
</tr>
<tr>
<td>Tomerindus indicus</td>
<td>8.14</td>
<td>1.2</td>
<td>1.25</td>
<td>0.0037</td>
<td>0.120</td>
<td>0.0364</td>
</tr>
</tbody>
</table>

3.2. Pharmacology of camel urine

Toxicity results:

Oral administration of CCL₄. In 2% sodium carboxy methyl cellulose (CMC) (1ml/kg) produces 100% mortalities in group 1 which received CCL₄ 1ml /100g B.W. & group 2 which received 1ml of camel urine plus 1ml CCL₄. 60% recovery was achieved from administration of 2ml of urine and 100% recovery was achieved by administration of 4ml of urine as shown in table (5) below:

**Table (5) CCL₄ toxicity and percentage recovery achieved by camel urine.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Log-dose</th>
<th>% recovery</th>
<th>Probit</th>
<th>Calculated probit</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCL₄+ 0ml</td>
<td>-</td>
<td>0.00</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CCL₄+ 1ml</td>
<td>0.0000</td>
<td>0.00</td>
<td>-</td>
<td>0.5</td>
</tr>
<tr>
<td>CCL₄+ 2ml</td>
<td>0.3010</td>
<td>60.00</td>
<td>5.25</td>
<td>4.2</td>
</tr>
<tr>
<td>CCL₄+ 4ml</td>
<td>0.6021</td>
<td>100.00</td>
<td>7.33</td>
<td>7.9</td>
</tr>
</tbody>
</table>

3.2.1. Assessment of Hepatoprotective effect of Twenty four hour collected camel urine (24hcu) against CCL₄ toxicity:

Oral administration of adult and Young (24hcu) in a dose rate of 2, 4, and 8ml /100g B.W., one hour before CCL₄ administration, showed that the only dose which ameliorate hepatotoxicity of CCL₄ (by reducing the elevated activities of ALT and AST) was 4ml/100 B.W. of both (Adult and Young camel urine). The concentrations of detected enzymes (ALT& AST) were summarized in Table (6 a,b and c). and represented in Fig.(1a).

3.2.2. Assessment of Hepatoprotective effect of Early Morning camel urine (EMU) against CCL₄ toxicity:
Oral administration of camel urine (EMU) at a dose rate 1, 2 and 4ml/100g B.W. one hour before CCL$_4$ administration reduces the highly elevated activities of AST and ALT where as the dose 2ml/100G B.W. was showing more reduction in the elevated liver enzymes. The mean concentration of rat liver enzymes were summarized in table(6 a,b and c) and represented in Fig.(1b).

**Table (6.a.) Effect of young 24 hcu, EMU and chloroformic extract on rat liver (GPT) one hour before CCL$_4$ induced hepato toxicity.**

<table>
<thead>
<tr>
<th>Treats /drugs</th>
<th>24hcu</th>
<th>EMU</th>
<th>CE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cont</td>
<td>17.2±3.03</td>
<td>18.22±5.02</td>
<td>28.4±6.19</td>
</tr>
<tr>
<td>CCL$_4$</td>
<td>33.0±2.72</td>
<td>37.0±4.53</td>
<td>69.0±12.27</td>
</tr>
<tr>
<td>Dose (1) 2ml/100 of urine</td>
<td>28.2±3.42</td>
<td>22.2±2.39</td>
<td>43.8±2.59</td>
</tr>
<tr>
<td>4ml</td>
<td>19.7±**</td>
<td>20.74± **</td>
<td>39.4±3.51*</td>
</tr>
<tr>
<td></td>
<td>4.92</td>
<td>11.5</td>
<td></td>
</tr>
<tr>
<td>8ml</td>
<td>24.4±*</td>
<td>23.0±3.54</td>
<td>35.2±5.25**</td>
</tr>
<tr>
<td></td>
<td>7.96</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

-Values are means (5 rats) ± SEM
4ml, 2ml and 0.2µl /100 gm B.wt are the more effective doses of 24hcu, EMU and CE respectively.

** Sig. (P < 0.01)
* Sig (P < 0.5)
24hcu: twenty four hour collected urine
EMU: early morning urine
CE: chloroformic extract
Table (6.b) The effect of 24 hcu, EMU and chloroformic extract on GOT activity before one hour CCL₄ induced hepatotoxicity.

<table>
<thead>
<tr>
<th>Treats/drugs</th>
<th>24hcu</th>
<th>EMU</th>
<th>CE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 0.1 corn oil</td>
<td>19.0±2.47</td>
<td>39.0±3.72</td>
<td>116.8±14.27</td>
</tr>
<tr>
<td>CCL₄ 15% (v/v) in corn oil 0.1ml</td>
<td>32.80±4.09</td>
<td>51.1±3.85</td>
<td>157.6±2.07</td>
</tr>
<tr>
<td>Dose (1) 2ml/100g</td>
<td>33.6±3.91</td>
<td>42.6±9.94</td>
<td>(0.1ml) 150.0±7.18</td>
</tr>
<tr>
<td>Dose (2) 4ml/100g</td>
<td>22.8±4.15*</td>
<td>33.75±3.06*</td>
<td>(0.2ml)* 120.0±7.71</td>
</tr>
<tr>
<td>Dose (3) 8ml/100g</td>
<td>33.5±11.9</td>
<td>43.25±5.56</td>
<td>(0.4ml)* 135.0±5.57</td>
</tr>
</tbody>
</table>

Values are means±SEM (5 rats) for each treatment.
* Sig (P < 0.5)

24hcu: twenty four hour collected urine
EMU: early morning urine
CE: chloroformic extract

Table (6.c) The effect of CE on GOT and GPT activity after one hour of CCL₄ I.P. induced hepatic-toxicity

<table>
<thead>
<tr>
<th>Treatment/ enzyme</th>
<th>Control ALT</th>
<th>CCL₄ ALT</th>
<th>0.1 ml/100g ALT</th>
<th>0.2 ml/100g ALT</th>
<th>0.4 ml/100g ALT</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT</td>
<td>28.33±2.08</td>
<td>73.33±14.98</td>
<td>148.23±5.0</td>
<td>145.2±29.89</td>
<td>94.77±23.16</td>
</tr>
<tr>
<td>AST</td>
<td>109.6±12.66</td>
<td>159.0±1.0</td>
<td>310.23±2.0</td>
<td>183.47±38.88</td>
<td>296.40±2.54</td>
</tr>
</tbody>
</table>

Values are means±SEM.

ALT = Alanine aminotransferase
AST = Aspartate aminotransferase.

The effect of early morning camel urine on ALT (GPT) and AST (GOT) activies one hour before Carbon tetrachloride IP injection.
Fig (2)
3.2.2 Assessment of Hepatoprotective effect of early morning camel urine (EMU):
Oral administration at a dose rate of 1, 2 and 4 ml/100 g B.W. one hour before CCL4 administration reduces the highly elevated activities of AST and ALT whereas the dose 2 ml/100 g B.W. was showing more reduction in the elevated enzymes. The mean concentration of the reduced enzymes were summarized in Table (6) and represented in Fig. (6b).

**Fig (3)**

G: Group
G-1 = Control (corn oil .1micro litre/ 100g BW)
G-2 = Control (Carbon tetrachloride .1micro litre/ 100g BW)
G-3 = Treated with (2ml /100g BW) (24 hcu) camel urine.
G-4 = Treated with (4ml /100g BW) (24 hcu) camel urine.
G-5 = Treated with (8ml /100g BW) (24 hcu) camel urine.
3.2.3 Assessment of Hepatoprotective effect of chloroformic extract (CE) of camel urine.

Intraperitoneal administration (I.P.) of chloroformic extract (CE) in a dose rate of 0.1, 0.2 and 0.4ml/100g B.W. one hour before CCL$_4$ I.P. injection results in a marked reduction in AST elevated activity at the dose 0.2ml/100 B.W., while a linear decline response of ALT activities according to the increase in the dose rate was shown in Table (6 a,b and c) and represented in Fig.(1c).

**Ant hepatotoxic effect of chloroformic extract of camel urine (CE) on liver ALT and AST activities one hour before CCL intoxoxation**

Control (Complex tetrachloride .1micro litre/ 100g BW )
Control (corn oil .1micro litre/ 100g BW )

Treated with(.1,.2,.4ml /100g BW ) Chloroformic extract of camel urine .
(Numbers between prackets represent dosage for each treatment)
3.2.4. Time course of hepatotoxic effect of CCL₄ on rat liver:

Enzyme levels in 5 rats serum were returned to normal levels after 96 hours, as shown in table (7) Fig.(2).

Table (7) Time course of hepatotoxic effect of CCL₄ on rat liver:

<table>
<thead>
<tr>
<th>Time/ enzyme</th>
<th>0 hour</th>
<th>24 hours</th>
<th>48 hour</th>
<th>72 hours</th>
<th>96 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPT (ALT)</td>
<td>28.33± 2.08</td>
<td>88.0±18.36</td>
<td>65.33±5.68</td>
<td>48.27±2.41</td>
<td>28.67±6.5</td>
</tr>
<tr>
<td>GOT (AST)</td>
<td>109.6±12.66</td>
<td>208.13±8.95</td>
<td>181.27±8.73</td>
<td>161.93±10.55</td>
<td>146.67±6.11</td>
</tr>
</tbody>
</table>

- Values are means ± SEM

Fig (2): Time course of hepatotoxic effect of CCL₄ on rat liver

3.3. Effect of Camel urine (EMU) on serum AST, ALT and LDH against paracetamol hepatotoxicity:
Table (8) showed the effect of camel urine on AST, ALT and LDH, the percentage of reduction in the activities of these enzyme were 71%, 79% and 45% respectively.

**Table (8) Hepatoprotective effect of camel urine against paracetamol toxicity compared with silymarin**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>AST</th>
<th>ALT</th>
<th>GGT</th>
<th>LDH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cont. 1ml/kg</td>
<td>21.66±3.6</td>
<td>17.66±0.39</td>
<td>----</td>
<td>322.5±47.3</td>
</tr>
<tr>
<td>Uml + P g/kg for 3 day</td>
<td>20±0.33</td>
<td>4.71±0.66</td>
<td>19.83±2.27</td>
<td>280±46.9</td>
</tr>
<tr>
<td>Sml +P g/kg</td>
<td>22.7±0.78</td>
<td>6.66±0.91</td>
<td>32.92±0.62</td>
<td>313.25±56</td>
</tr>
<tr>
<td>Paracetmol g/kg</td>
<td>62.3±3.38</td>
<td>43.67±5.94</td>
<td>70.61±16.13</td>
<td>382±24.2</td>
</tr>
</tbody>
</table>

Results are men ± SEM of treated groups each group of (5) rats.
U-urine 2ml/100g, S silymarin 1ml/100g, P- paracetamol g/kg

**3.4. Pharmacological screening**

**3.4.1. Effect of camel urine and camel urine protein precipitant (CUPP) on isolated rat fundus:**

The rat fundus strip is highly sensitive to 5-hydroxytryptamine (5-HT) (Rang and Dale 1986). Fig. (3) demonstrates the effect of 5-HT on isolated fundus strip in a dose of (400ng/ml) stimulated the fundus strip while camel urine 0.8ml and (CUPP) (using 40% ammonium sulphate) stimulated the rat fundus at a dose of 0.1 ml and 0.4ml/bath respectively. The stimulant effect of the fundus was completely blocked by cyproheptadine (2µg/ml).
3.4.2. Comparison of the effect of camel, cattle and human urine protein precipitant (UPP) on rat duodenum:

Camel urine and (CUPP) and that of other animals urine showed a relaxant effect on rat duodenum (0.1ml/bath) as presented in Fig. 4 (a, b and c).

**Fig (4)**
Rat duodenum isolated stripts were inhibited by 40% ammonium sulfate protein precipitate of camel, cattle & human urine samples as showed in figures (A-B-C) respectively.
influence of (CUPP) on contracting rabbit jejunum. This effect was completely blocked by atropine (2.5µg/ml). Camel urine induced transient relaxation followed by increased contraction.
3.4.4. Effect of camel urine and (CUPP) on isolated rabbit rectum

Fig. (5 b.) showed the influence of CUPP on isolated rabbit rectum.
3.4.5. Effect of camel urine chloroformic extract (CE) on the isolated stripts (rat funds, rat dudenum, rabbit jejunm and rectum).

CE of camel urine showed no effect on each of the pre mentioned isolated strip

3.5 Camel urine in vitro tests on different cell cultures:

3.5.1. Effect of concentrated camel urine (crude urine) and diluted urine (1:1;1:3) v/v on liver cell culture.

Inoculation of 100%, 50% and 25% camel urine at a dose of 1ml/cell flask, when incubated at 37°C for 24 hours showed high, moderate and slight cell toxicity respectively.

Cells inoculated with concentrated (100%) urine showed progressive cellular changes including swollen cells, cell rounding, cell degeneration and distortion and Karyorrhectic nuclei Diluted (50% and 25%) inoculated in liver cell cluture showed less toxic effect as shown in Fig. (6 a, b and c).
A- showed the effect of inoculated 25% of camel urine on liver cell culture
B- showed the effect of inoculated 50% of camel urine on liver cell culture
C- showed the effect of inoculated 100% of camel urine on liver cell culture
3.5.2. Effect of (CUPP) on bovine kidney cell culture:

Bovine kidney cell culture inoculated with 1ml CUPP showed moderate changes when observed over a period of five successive days. The monolayer showed swollen to rounded cell decreasing gradually to normal cell sheet by the fifth day Fig. (7 a,b and c.).

Fig. (7)
A- showed normal Bovine kidney cell culture (control)
B&C- showed Bovine kidney cell culture inoculated with40%ammonium sulfate precipitate of camel urine(ASPPCU) , moderate changes were observed over aperiod of five successive dayes.
3.6. Clinical effect of camel urine treatment:
3.6.1. Pretreatment clinical observation for selecting naturally infected animals.
clinical signs are more or less the same in all naturally infected animals. Although the intensity of chronicity is varied between the infected calves. The pre-dominant observations were loss condition, emaciation, and rough coats in some cases. As shown in Fig.(8).

![Fig.(8)](image)

*Fig.(8)*

*Showed Body Score Condition of infected untreated calves.*
3.6.1.1. Parasitological examination:-

sedimentation and floatation methods of faecal samples resulted in 15 positive samples with *Faschiola gigantica* and 2 cases with mixed infection faschiola and *Schistosoma bovis*, 8 calves were infected with *Strongylus* and 3 of them have faschiola. One animal showed Tricuris and two were infected with Theileria after blood film examination (Table 9).
Table (9) examination of fecal sample for parasitological infection

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Fasciola eggs</th>
<th>Schistosoma</th>
<th>Others in blood faecal</th>
</tr>
</thead>
<tbody>
<tr>
<td>35</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>36</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>37</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>38</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>39</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>40</td>
<td>+</td>
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<td></td>
</tr>
<tr>
<td>41</td>
<td>+</td>
<td></td>
<td>+++</td>
</tr>
<tr>
<td>42</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>43</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>44</td>
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<td>46</td>
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</tr>
<tr>
<td>47</td>
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<td>-</td>
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</tr>
<tr>
<td>50</td>
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<td>51</td>
<td>+</td>
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<tr>
<td>52</td>
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<td>-</td>
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<tr>
<td>53</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>54</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>55</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>56</td>
<td>-</td>
<td>-</td>
<td>Strongylus</td>
</tr>
<tr>
<td>57</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>58</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>59</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

3.6.1.2. Biochemical assessment of liver function test:

Biochemical assay of serum enzymes activity, such as aspartate amino transferase (AST), alanine amino transferase (ALT), Alkaline
phosphates (ALP), lactate dehydrogenase (LDH) and Gamma glutamate transferease (GGT) revealed an increase in the enzyme activities and slight increase in the concentrations of serum electrolytes and minerals.

3.6.1.3. **Haematological examination:**

Anaemia with a reduced haemoglobin (Hb) concentration and red blood cell (RBC) count was evident, and a parallel fall in PCV was detected. Prothrombin time result in slight change in clothing time.

Haematological and biochemical results were represented in table (10 and 11).
Table (10) Haematological and biochemical analysis of naturally infected calves
(from white Nile province- Sudan)

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Mean values for the diagnosed animal</th>
<th>Reference values</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCV %</td>
<td>18</td>
<td>28-38</td>
</tr>
<tr>
<td>leukocytes TWBC (µl)</td>
<td>3450±150.1</td>
<td>4000-10000</td>
</tr>
<tr>
<td>Total billirubin (g/L)</td>
<td>0.2 ± 0.01</td>
<td>0.8-8.6</td>
</tr>
<tr>
<td>AST (µ/l)</td>
<td>89 ± 11.4</td>
<td>40-80</td>
</tr>
<tr>
<td>GGT (u/L)</td>
<td>3.6 ± 0.2</td>
<td>6-17</td>
</tr>
<tr>
<td>Urea (mmol/L)</td>
<td>1.8 ± 0.05</td>
<td>2.5-7.5</td>
</tr>
<tr>
<td>Sodium (mmol/L)</td>
<td>155 ± 5.1</td>
<td>140-150</td>
</tr>
<tr>
<td>Potassium (mmol/L)</td>
<td>6.7 ± 1</td>
<td>4-5</td>
</tr>
<tr>
<td>Calcium (mmol/L)</td>
<td>8.7 ± 2.2</td>
<td>2-2.6</td>
</tr>
<tr>
<td>Phosphates (mmol/L)</td>
<td>4.9 ± 1.02</td>
<td>1.3-1.25</td>
</tr>
<tr>
<td>Magnesium (mmol/L)</td>
<td>2.2 ± 0.92</td>
<td>0.7-1.1</td>
</tr>
<tr>
<td>Units ALP</td>
<td>97 ± 3.9</td>
<td>0.0-500</td>
</tr>
<tr>
<td>Units LDH</td>
<td>464.42 ± 36.5</td>
<td>692-1445</td>
</tr>
</tbody>
</table>
Table (11). Prothrombin time.

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Clot in second (1)</th>
<th>No. CP (2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>38</td>
<td>21</td>
<td>10</td>
</tr>
<tr>
<td>39</td>
<td>19</td>
<td>10</td>
</tr>
<tr>
<td>40</td>
<td>20</td>
<td>11</td>
</tr>
<tr>
<td>42</td>
<td>12</td>
<td>9</td>
</tr>
<tr>
<td>46</td>
<td>19</td>
<td>7</td>
</tr>
<tr>
<td>47</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td>48</td>
<td>13</td>
<td>11</td>
</tr>
<tr>
<td>55</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>56</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>57</td>
<td>10</td>
<td>7</td>
</tr>
</tbody>
</table>

3.6.1.4. Ultrasonographic examination

Ultrasonic scanning Fig. (9-13) revealed homogenous liver texture, dilatation of hepatic veins (congestion), and distended gall bladder in most cases. Calcified and necrotic foci with acoustic shadowing in two calves, a liver cyst were found in 4 calves. In most cases there is hepatomegally (round edges of the liver) and tissue coarse texture in two cases. As shown in figures below:
Fig. (9 a) calf No. 347 shows a dilated Gall bladder with thick wall, coarse texture, non haemogenous.

Fig. (9 b) calf No. 337 shows a dilated Gall bladder and congested liver.

Fig. (9 c) Animal No.357; sonograph shows presence of fibrotic, congested portal vein, enlarged gall bladder 3.4 cm with thickened wall and congestion.
Fig. (10.1) Animal No.345; sonograph shows coarse texture, congestion and granulated congested veins. A cyst with 75 cm diameter and distended gall bladder.

Fig. (10.2) Animal No.351; Sonograph showed hyperechoic liver, congested, hepatomegally and fibrotic foci.

Fig. (10.3) Animal No.50 shows normal liver size and texture, enlarged gall bladder.

Fig. (10.4) Animal No.352; sonograph shows normal liver gall bladder mildly large in size 3.7 cm, nephritis and liver cyst 8.4 cm in length.
Fig. (11 a) Animal No. 346 shows dilated Gall bladder 4.4 cm enlarged and congested hepatic veins, congested liver.

Fig. (11 b) Calf No. 353 shows coarse, no Haemogenous liver texture and distended gall bladder, 3.66cm

Fig. (11 c) Calf No. 351 viewed hyper echoic. Congested and enlarged liver with fibrotic foci and calcification.

Fig. (11 d) Calf No. 338 shows Normal liver.
Fig. (12 e) Calf No. 355 shows congested veins, fibrotic foci, liver cyst (8.9)cm diameter.

Fig. (12 f) Calf No. 347 shows a dilated Gall bladder with thickened wall, coarse texture, non haemogenous.

Fig. (12 g) Calf No. 359 showed normal liver texture and size enlarged Gall bladder i.e. cholecystitis, thick wall 3.8cm

Fig. (12 h) Calf No. 339 shows hyper echoic liver, Gall bladder with thickened wall, congested cystic ducts.
Fig. (13 i) Calf No. 337 shows dilated gall bladder and congested liver.

Fig. (13 j) Calf No. 340 shows dilated portal vein slightly enlarged liver. Normal texture.

Fig. (13 k) Calf No. 344 shows moderately enlarged liver, coarset texture and dilated gall bladder.

Fig. (13 l) Calf No. 357 shows congested portal veins, enlarged gall bladder 3.4 cm and thickened wall. Fibrerotic foci and congested liver.
3.6.2. Observations and Post treatment examination

3.6.2.1. Toxicity of camel urine (EMU)

Among the calves in group II, III and IV, no signs of toxicity were seen during the course of camel urine (EMU) treatment. Except during week one and two a mild diarrhoeal effect was observed in group II and III.

3.6.2.2. General appearance of treated animals (calves):

Calves in group II and III exhibited gain in body weight, ranging up to 13% among the treated calves table (12) and Fig(14-18)

Table (12). Average body weight

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>In. w.</th>
<th>Fin w.</th>
<th>A</th>
<th>%</th>
<th>Animal No.</th>
<th>Int. w.</th>
<th>Fin w.</th>
<th>A</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>35</td>
<td>120</td>
<td>136</td>
<td>128</td>
<td>13</td>
<td>39</td>
<td>110</td>
<td>120</td>
<td>115</td>
<td>9</td>
</tr>
<tr>
<td>36</td>
<td>130</td>
<td>136</td>
<td>130</td>
<td>0</td>
<td>40</td>
<td>140</td>
<td>155</td>
<td>145.5</td>
<td>10</td>
</tr>
<tr>
<td>37</td>
<td>135</td>
<td>150</td>
<td>142.5</td>
<td>11.1</td>
<td>46</td>
<td>155</td>
<td>160</td>
<td>157.5</td>
<td>3</td>
</tr>
<tr>
<td>50</td>
<td>150</td>
<td>150</td>
<td>150</td>
<td>0</td>
<td>47</td>
<td>160</td>
<td>180</td>
<td>170</td>
<td>12.5</td>
</tr>
</tbody>
</table>

Int. w. initial weight
Fin. w. final weight
A. average weight
- Fig. (14)

(1) Body Score Condition before treatment with EMC.
(2) Body Score Condition of the same animal after treatment with 4ml/kg of fresh early morning camel urine.
- Fig.(15)

(1) Body Score Condition before treatment with EMC.
(2) Body Score Condition of the same animal treated with 4ml/kg of fresh early morning camel urine (EMU) Two weeks after treatment.
(3) Body Score Condition of the same animal treated with 4ml/kg of fresh early morning camel urine (EMU) four weeks after treatment.
(4) Body Score Condition of the same animal treated with 4ml/kg of fresh early morning camel urine (EMU) eight weeks after treatment.
- Fig. (16)

(1) Body Score Condition before treatment with EMC.
(2) Body Score Condition of the same animal treated with 4ml/kg of fresh early morning camel urine (EMU) two weeks after treatment.
(3) Body Score Condition of the same animal treated with 4ml/kg of fresh early morning camel urine (EMU) four weeks after treatment.
(4) Body Score Condition of the same animal treated with 4ml/kg of fresh early morning camel urine (EMU) eight weeks after treatment.
- Fig. (17)

(1) Body Score Condition before treatment with EMC.
(2) Body Score Condition of the same animal treated with 4ml/kg of fresh early morning camel urine.
- Fig. (18)

(1) Body Score Condition before treatment with EMC.
(2) Body Score Condition of the same animal treated with 4ml/kg of fresh early morning camel urine.
3.6.2.3. Faecal examination:

Treated calves in group II, III and IV when examined for fluke egg, no fluke egg was found. They were completely disappeared at the 4\textsuperscript{th} week of treatment table (13).

Table (13): Post treatment faecal examination

<table>
<thead>
<tr>
<th>Group</th>
<th>Calves No.</th>
<th>Age (year)</th>
<th>Oral dose</th>
<th>Duration of treatment</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>51,52,53,54</td>
<td>1-2</td>
<td>2ml/kg B.W. saline</td>
<td>8 weeks</td>
<td>++ -</td>
</tr>
<tr>
<td>II</td>
<td>35,36,37, 50</td>
<td>1-2</td>
<td>2ml/kg B.W. EMU</td>
<td>8 weeks</td>
<td>- +</td>
</tr>
<tr>
<td>III</td>
<td>39,40,46, 47</td>
<td>1-2</td>
<td>4ml/kg B.W.EMU</td>
<td>8 weeks</td>
<td>- ++</td>
</tr>
<tr>
<td>IV</td>
<td>44, 55,57,59</td>
<td>1-2</td>
<td>4ml/10kg B.W.Rafoxaid</td>
<td>4 weeks</td>
<td>- -</td>
</tr>
</tbody>
</table>

3.6.2.4. Biochemical investigations:

Serum ALT, AST, LDH, ALP and GGT enzyme activities were examined weekly, as shown in Fig (19-23) enzyme activities started to decline at the second week and reach its maximal reduction at the 4\textsuperscript{th} and 5\textsuperscript{th} weeks, then increased gradually till the 6\textsuperscript{th} week beyond which no more changes was observed.

The result of serum total protein albumin, creatinine, urea uric acid and bilirubin were presented in fig (24-29). Serum electrolytes and mineral shows the same pattern of alteration during the 4\textsuperscript{th} and 5\textsuperscript{th} week of camel urine and standard Rafoxanide drug.
Time course of the effect of urine (2.4 ml/kg) and Rafoxanide 4 ml/10 kg on serum GPT.

* sig (p < 0.05)
Fig(20)

Time course of the effect of urine (2,4ml/kg) and Rafoxanide 4ml/10kg on serum GOT.

* sig (p < 0.05)
The level of alkaline phosphatase in different treatment groups

Fig(21.a)
Time course of the effect of urine (2.4 ml/kg) and Rafoxanide 4 ml/10 kg on serum ALP.
* sig (p < 0.05)
**Fig(21.b)**

Time course of the effect of urine (2,4ml/kg) and Rafoxanide 4ml/10kg on serum PO$_4$.

* sig (p < 0.05)
The level of creatine in the different treatment groups

![Graph showing the level of creatine over weeks for different treatment groups.]

**Fig(22)**

Time course of the effect of urine (2.4ml/kg) and Rafoxanide 4ml/10kg on serum Creatine.

*sig (p < 0.05)
Fig(23)

Time course of the effect of urine (2.4ml/kg) and Rafoxanide 4ml/10kg on serum total protein.
* sig (p < 0.05)
The level of Calcium in the different treatment groups

![Graph showing the level of Calcium over weeks for different treatment groups.]

**Fig(24)**

Time course of the effect of urine (2,4ml/kg) and Rafoxanide 4ml/10kg on serum Calcium.

* sig (p < 0.05)
Fig(25)
Time course of the effect of urine (2,4ml/kg) and Rafoxanide 4ml/10kg on serum Uric acid
*sig (p < 0.05)
Fig(26)
Time course of the effect of urine (2,4ml/kg) and Rafoxanide 4ml/10kg on serum K.
* sig (p < 0.05)
Fig(27)
Time course of the effect of camel urine (2,4ml/Kg) and Rafoxanide 4ml/10Kg on serum Uric acid
The mean difference is significant at p<0.05
Fig(28)

Time course of the effect of camel urine (2,4ml/Kg) and Rafoxanide 4ml/10Kg on serum sodium

The mean difference is significant at p<0.05
3.6.2.5. Haematological findings:

Haematological parameter resulted in increased Hb and PCV. Also some animals showed an increase in RBCs and WBCs count as shown in Fig.(30-34).
3.7. Post mortem clinical examination

3.7.1. Macroscopic lesions

3.7.1.1. Macroscopic lesions in calves naturally infected with *F. gigantica* and untreated:

The liver was extremely affected, congested, firm and hard in cut. Both heart and spleen were enlarged. The lung was heavy and oedematous. Congestion was seen in lung, intestine and kidney group I (Animal No. 51, 54, 55, 59).

3.7.1.2. Macroscopic lesions in calves naturally infected with *F. gigantica* and treated with (4ml/kg B.W.) camel urine (EMU):

No significant gross pathological changes were observed in liver, intestine and spleen. The lung was slightly oedematous and the thorathic cavity contained little fluid. Kidney was normal in size and pale, the heart slightly flabby.

3.7.1.3. Macroscopic lesions in calves naturally infected with *F. gigantica* and treated with Rafoxanide 4ml/10 kg B.W.

The most prominent lesion was noticed in the liver, which revealed congestion. The lung appeared normal, spleen pale, the heart flabby, kidney also showed congestion, hydroperitonium was noticed and the thoracic cavity contained little fluid. Kidney was normal in size and pale, the heart slightly flabby.

The macroscopic lesions of the liver, kidney and heart for the infected untreated and treated (4ml/KgB.W. EMU) calves are shown-in Fig. (35,a,b,andc).
Fig. (35a)

- A&B showed macroscopic photographs of infected untreated calves hepatomegaly, enlarged gall blader and pale liver.
- C - showed photograph of treated calf liver with 2ml/kg. camel urine.
- D - showed photograph of treated calf liver with 4ml/kg. camel urine.
Fig. (53 d)
Photographs A&B showed enlargedment, congestion and pale anemic kidneys of infected untreated calves.
C - showed calves (kidney) treated with camel urine 2ml/kg body weight.
D - showed calves (kidney) treated with camel urine 4ml/kg body weight.
- a & b & c showed macroscopic photograph of infected untreated calves (flabby hearts).
- d showed macroscopic photograph of treated calf (heart) with camel urine 4ml/kg B.W.
3.7.2. Histopathological findings:

3.7.2.1. Histopathological lesion induced in calves infected with *F. gigantica* and untreated:

The liver was severely infected and exhibited hepatocellular necrosis with vacules in the cytoplasm. Congestion was noticed in the centnolabular vein. Haemorrhage and fibrous tissue also observed. Kidney showed interstitial nephritis with sever haemorrhage in the medulla. Intestine revealed inflammatory cells, mainly plasma cells, eosinophils and mononuclear cells. The blood capillary was congested. The lung manifested interlobular oedema and thickening of the alveolar septa, some RBCs were detected. Heart muscle exhibited degeneration and congestion of blood vessels. Fig.(36.a,b,c,d and e) shows the histopathological changes of different organs from the infected untreated calves.
Fig. (36 a) Liver showed necrosis and cytoplasmic vaculation, congestion, Hemorrhage and fibrosis. Stain H & E x 100

Fig. (36 b) kidney exhibited interstitial nephritis and severe haemorrhage in the medulla stain H & Ex 100.
Fig (36 c) intestine showed epithelial cells necrosis and congestion of blood vessel. Stain H & E x 100.

Fig (36 d) lung showed oedema, congestion and thickening of the alveolar septa
stain H and Ex 400
Fig. (36 e) Heart showed congestion, muscular degeneration stain H&E x400.

Fig. (36 f) showed congestion of centrolobular vein, Haemorrhage, hepatic cells necrosis. fibrous tissues found around bile duct. Stain H&E x 100.
3.7.2.2. Histopathological lesions induced in calves naturally infected with *F. gigantica* and treated with (4ml/Kg B.W.) camel urine (EMU).

Congestion of centrolobular vein, extravasation, and hepatic cell necrosis was detected. Some fibrous tissues observed around the bile ducts. Kidney displayed necrosis of the epithelial cells of the renal tubules and haemorrhage was noticed. The intestine revealed congestion, necrosis of the epithelial cells and infiltration with some inflammatory cells. Lung showed congestion and oedema. The heart showed degeneration and congestion of blood vessels. Fig. (37.a,b,c,d and e) showed the histopathological changes of different organs from the infected and treated calves (4ml/KgBW EMU).
FIG. (37 a) Kidney exhibited necrosis of epithelial cells of the renal tubules and Haemorrhage. Stain H and E x 100.

FIG. (37 b) Intestine revealed, congestion, epithelial cell necrosis and infiltration with inflammatory cells Stain H & E x 400.
3.7.2.3. Histopathological lesions induced in calves naturally infected with *F. gigantica* and treated with Rafoxanide (4 ml/10kB.W.).

Fig. (37 c) Lung showed congestion and oedema. Stain H and E x 100.

Fig. (37 d) Liver showed fibrosis, congestion, liver cells necrosis, cytoplasmic vaculation and inflammatory cells also noticed. Stain H and E x 100.

with *F. gigantica* and treated with Rafoxanide (4 ml/10kB.W.).
Liver showed congestion, fibrosis and inflammatory cells. Hepatocellular necrosis was observed and some vacuoles were noticed on the cytoplasm. Intestine revealed congestion of blood vessel and some inflammatory cells. Kidney exhibited severe haemorrhage in the medulla and necrosis of the epithelial cells and renal tubule.

The lung showed oedema and congestion. Fig(38.a,b,c,d and e) show the histopathological changes of different organs from the infected and treated calves.(4ml/10Kg.B.W.Rafoxanide).
Fig. (38 a) Intestine showed congestion, inflammatory cells & necrosis of the epithelial cells. Stain H and Ex 100.

Fig. (38 b) kidney reflected severe Haemorrhage in the medulla and necrosis of renal tubles epithelium. Stain H and Ex 100.
Fig. (38 c) treated Lung exhibited oedema and congestion stain H & E x 400.

Fig. (38 d) Heart muscle showed degeneration & congestion stain H & E x 100.

3.6. Chromatography of camel urine
3.8.1. Thin layer chromatogram of Camel urine, its chloroformic extract, cattle urine (CE) and human urine (CE).

Camel urine, its chloroformic extract and that of cattle and human urine when chromatographed on TLC it revealed a positive reaction with Ninhydrin reagent, with one oval yellow spot for each sample, whereas human urine showed no reaction. The RF values were 0.81, 0.79 and 0.6 for camel. As presented in Fig.(39.a and b).

Also chromatogram of (CE) hydrolysate by using 0.6 N HCL revealed one yellow spot after Ninhydrin spraying with R = 0.65 Fig (39.c.).

3.8.2. Thin layer chromatogram of camel and cattle urine (CE) in methanol:

Thin layer chromatogram of camel urine (CE), cattle urine (CE) were dissolved in drops of methanol, they revealed a light blue-small rounded-spots under short UV light [254mm]. When sprayed with Ninhydrin a yellow 5 spots with RF values of 0.6, 0.7, 0.75, 0.8, and 0.85, for she-camel 1 & 3 years old and cattle (young and adult) Fig. (40).

3.8.3. Thin layer chromatogram of camel urine butanolic fraction:

Butanolic fraction (1,2,3) (4,5) (6,7,8) (9) (10,11,12) revealed one violet spot for each, with RF equal 0.24. Fraction 13, 14, 15 resulted in three components of violet, pink and yellow colouration after, Ninhydrin spraying their Rf values were 0.24, 0.82, 0.88 respectively. Crude urine
showed the same zone of separation with addition of one more yellow spot with 0.92 Rf value.

With Dragindoff reagent one yellowish to orange spot was appeared against each stain spot with Rf of 0.6, 0.7, 0.75, 0.8 and 0.85 Fig. (41.a.). Another plate was exposed to potassium iodide vapor a positive orange spots were seen (spot IA and 3B) Fig.(41.b.).

3.8.4. Thin layer chromatogram of camel urine ethanolic fraction:

Preparative thin layer chromatography (PTLC) of ethanolic fractions and that of chloroformic extract spotted on silica gel (0.5mm) and soaked in Butanol: acetic acid: water solvent system BAW (4:1:5;4:1:1) for 45 minutes showed bright blue colour under short UV light (254nm). When sprayed with ninhydrin and activated at 110 C, Butanolic fraction (1-5) showed one violet spot of 0.24 Rf value. Fraction 13,14,15 gives 3 bands of violet, pink and yellow coluration Rf values 0.24, 0.82 and 0.88. crude urine spot of young she-camel revealed eight bands of 0.08, 0.17, 0.25, 0.67, 0.79, 0.88 and 0.92 Rf values. Most of which were yellow and pink in colour. Ethanolic fraction resulted in 3 bands of 0.21, 0.82 and 0.88 Rf values with the same colouration, where chloroformic extract revealed a large round pink spot and slight yellow band above it with Rf values of 0.8 and 0.88 respectively Fig.(42).

3.8.5. Ultraviolet visible spectrophotometry (UV. V/S)

UV spectrophotometer [200-400] nm revealed components ranged between [288-237] nm. Methanolic and methanol in HCl (99;1) fraction resulted in the presence of amino acids as compared to 13 standard amino acids ranged between 200-400 nm as shown in Fig. (44.a and b.) table (14). Most of which are similar to the reference amino acids.

Table (14) Represent UV absorbance of methanolic, methanol in hydrochloric acid and hydrolysate of camel urine
<table>
<thead>
<tr>
<th>Standard Amino acid</th>
<th>Methanol in HCL (99:1)</th>
<th>(M) Methanol</th>
<th>Hydrolysate</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>0</td>
<td>200-220</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>1.3</td>
<td>230-240</td>
</tr>
<tr>
<td>8,9,12</td>
<td>66</td>
<td>10</td>
<td>240-250</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>3-7,9</td>
<td>250-260</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,2,5,7,13</td>
<td>67</td>
<td>2</td>
<td>260-270</td>
<td></td>
</tr>
<tr>
<td>6,10,11</td>
<td>70-75,75-77</td>
<td>1,45-55</td>
<td>2</td>
<td>270-280</td>
</tr>
<tr>
<td></td>
<td>79</td>
<td>11,12</td>
<td>6</td>
<td>280-290</td>
</tr>
<tr>
<td>4</td>
<td>61,63-65,74,78,80</td>
<td>14-44</td>
<td>4</td>
<td>290-300</td>
</tr>
<tr>
<td></td>
<td>68,69</td>
<td></td>
<td>300</td>
<td></td>
</tr>
</tbody>
</table>

3.8.6. Infra red spectrophotometry (IR):

Infra red of chloroformic extract and that of 4 lyophilized camel urine as KBr disc or NaCl cell recorded on a perkin Elmer 2Lambda Spectra. 580 Infra red spectrophotometer Neelfur gave the spectera data in table (15) Fig.(45 a,b,c,d and e).

Table (15) Infra red spectrophotometery data

<table>
<thead>
<tr>
<th>Frequency (cm⁻¹)</th>
<th>Type of vibrate</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>3600-2400</td>
<td>O-H</td>
<td>H₂O</td>
</tr>
<tr>
<td>1680-1600</td>
<td>C=O</td>
<td>CaOH group</td>
</tr>
</tbody>
</table>
**Remarks of IR analysis:**

All the spectra showed the following characteristic absorption bands:

1- 3600-2400 trade band characteristic of O-H st. vib. H banded mainly of acids if H2 O is present.

2- 1680 cm$^{-1}$ and neighbours, are of C= O St. vib.

* more than one C = O St. vib.

III – 1124 cm$^{-1}$ charac. Of C- O St. vib.

** I, II, III strongly pointed to – COOH carboxylic acid.

IV 1600 m 1500 1484 stands mainly for C- C of aromatic system.

773, 691 bonding and pattern of subs.

V- To large degree sample zone 4 identical 1, 2 similar to 4 but not identical.
Fig (39) Shows achromotogram of chlorformic extract (CE) and crude camel urine (CU) treated with 6 (NHCL).
Fig. (40 a ) shows achromotogram of chlorformic extract (CE) and crude camel urine (CU).

Fig. (40 b ) shows achromotogram of different chlorformic extract of camel, cattle, human, and young she camel urine. 1, 2, 3, and 4, 5 respectively. On silica gel plates using BAW as solvent system.
Dragndoff detection of Alkaloid Camel and cattle

Chloroformic extract

Fig. (41)

Spots A (1-5) Represent Chloroformic extract of camel urine,
Spots B (1-5) Represent Chloroformic extract of cattle urine,
Dragndoff reagent revealed positive reaction (orange colour).
Spots A (1-5) represent Chloroformic extract of camel urine, Spots B (1-5) represent Chloroformic extract of camel urine. Dragnoff reagent revealed a positive reaction (orange colour).

Fig. (42 a)
Preparative thinlayer chromatography of Butanolic, ethanolic, chloroformic extracts of camel urine vis youn she - camel crude urine by using (BAW) system in ratio of 4:1:1

(1-5) Butanolic fractions
(6) chloroformic extract
(7) ethanolic extract (crude urine)
(8) ethanolic extract (lyfolysed urine)
(9) young she - camel crude urine

Fig. (42 b)
Preparative thinlayer chromatography of Butanolic, ethanolic, chloroformic extracts of camel urine vis young she - camel crude urine
Fig. (43) represents an electrophoreies of 40% ammonium sulphate precipitant of urine & milk of different spp. compared to human serum.
8- camel milk protein precipitate (run).
Fig (43 b) GAS analysis of a camel urine aminoacid
Fig. (44 a) UV Spectrophotometry of methanolic in HCL (99 : 1) extract of Camel urine
Fig. (44 b) UV Spectrophotometry of methanolic extract of Camel urine.
Fig. (45 b) Infra Red spectra of lyophilized camel urine
Fig. (45c) Infrared spectra of lyophilized camel urine
Fig. (45c) Intra Red spectra of lyophilized camel urine
DISCUSSION

Medicinal usage of plant and/or animal products, represents one of the most essential sources of drugs. In Sudan and many other countries camel owners use camel urine to treat a wide range of health problems. Camel urine has antibacterial, antifungal, antiviral, antineoplastic and therapeutic applications on ascitic patients as reported by Ohaj (1998), Amer and Alhendi (1996) and Wisal (2002).

No pharmacological investigation has been published on livestock urine, so the aims of the present study are to make more considerable physical, biochemical, pharmacological and clinical investigations on camel urine.

4.1. Physiochemical Investigations

4.1.1. Physiological properties

Most animal's urine is clear watery solutions with exception of Equine urine which is viscous due to the presence of mucoproteins, while birds urine is creamy and thick due to formation of uric acid, which is excreted with faeces, (Ibrahim 1989). Camel urine is amber yellow to deep brown in colour, with offensive odour. This is in agreement with observation reported by Ohaj (1989) and Wisal (2002).

In this study camel urine gave an alkaline pH with a mean value of 9.5±0.5 this likely due to the presence of greater reservoirs of bicarbonate in the intestine of camel (Maloiy and Clemens -1980), which is slightly higher than the pH (7.42) reported by Wermery and Wensvoort (1992) in Blood and Urine of camel Amer and Alhendi (1996) reported acid pH of (6.5) in 14 male urine samples while 6 samples showed an alkaline pH. In this study, the pH of young female camel. (6 month up to 1 year old) urine sample was (6.7), this is in agreement with pH 6.5 that was reported by Manefield and Tinson (1996). This variation in pH may be due to:
(1) feed intake (High energy diets), such diets can cause metabolic acidosis and mineral disturbances.

(2) environmental condition.

(3) different areas of sample collection.

4.1.2. Chemical and biochemical analysis

Human urine is the most analyzed urine, whereas camel and goat urine were studied as a tools of dehydration effects on desert animals, Musa (1978) and Yagil (1984 and 1994).

Potassium was the most predominant electrolyte followed by sodium and calcium. Camel in their normal habitat are exposed to dehydration salty bushes or salty water Dahl and Hjout (1980) and Yagil (1985). The well adapted and functioning camel kidney which has a relatively thick medulla (Medulla cortex 4.1cm) with long loop of Henle for water and sodium reabsorption and hence the ability to concentrate urine Wilson (1989).

Urea is the major end product of nitrogen metabolism, as summarized in table (2) this appears reasonable because of the counter current metabolism for urine concentration, which permits to excrete highly soluble urea with very little water. The average urea level 2-3g/dl and 10.5g/dl for male and female respectively which was in agreement with Ohaj (1998) reports but disagrees with Amer and Alhendi (1996) who reported the mean value to be 1.95g/dl. Bolyne (1977) and Etzon and Yagil (1986) reported a mean value of 1.8g.dl in control and 1.4g/dl in dehydrated ones.

In this study, the results showed, that goat urine has higher values of urea followed by cattle, camel and human. Wilson (1989) reported higher levels of camel urine sample urea of dehydrated and fully watered camels 5.7g/dl and 3.7g/dl respectively. Our result is in contrast with the result of Read (1925) who found no urea in camel urine.
Uric acid was found to be higher in goat and camel, than in other animal’s urine. These results were similar to that reported by Ohaj (1998), and higher than that reported by Amer (1996), Uric acid mean values were 236.3, 214.5, 182.25, 145.5 mg/L in goat, camel, cattle and human urine respectively.

Murphy et al. (1969) and Cracer et al. (1988) found low level concentration of uric acid in cow, sheep, goat, pigs and horse respectively, this may be attributed to the action of liver uricase enzyme which converts uric acid into allanatoine (Margon, 1959). It is assumed that camel livers do not release uricase enzyme (Murphy et al. 1969 and Cracer et al. 1988). The mean value of creatinine and creatine were higher in males, because the male has more muscles. This result was in accordance with Ohaj (1988) and Wisal (2002) findings.

4.2. Pharmacological screening of camel urine:

The pharmacological screening was carried out to evaluate if the twenty four hour (24hcu) collected urine early morning urine (EMU), Ammonium Sulphate Protein Precipitate of camel urine (ASPPOCU), and chloroformic extract (CE) of camel urine used have other activity that might be considered of interest. and to establish explain the general effects experimentally on blood and liver enzymes of normal and intoxicated rats.

4.2.1. Hepatoprotective effect of camel urine and its extract:

In this study camel urine, (ASPPOCU), and chloroformic extract (CE) were screened for their hepatoprotective activity against CCL₄ as standard hepatotoxic agent Handa, (1986) and Mansour, (2000) and paracetamol as a necrotic agent at high doses (1g/kg) induces hepatocellular damage Tee, et al (1983), compared to silymarin (1g/kg) standard anti-hepatotoxic agents. Such a screening process represents an essential stage for selecting the most potent hepatoprotective form of
camel urine for further detailed pharmacological and chromatographical investigation.

4.2.1.1. CCL₄ toxicity:

CCL₄ is a colourless volatile liquid with characteristic sweet odor, it is miscible with most aliphatics and stable in the presence of air and light. Decomposition may produce phosgene, CO and hydrochloric acid Phol et al. (1983) and Watanable et al. (1982). The final step in the biotransformation of CCL₄ is catalysed by cytochrome p450 enzyme leading to formation of reactive trichloromethyl radical.

In the oxidative biotransformation, the most important pathway of the trichloromethyl radical is the formation of the more reactive trichloromethyl peroxide, which covalently links to macromolecules, and lipid peroxidation occurs via metabolic intermediates of CCL₄, (Dezwat et al. 1977; Nagi et al. 1999; Mansour, 2000). Liver and kidney are the target organs for CCL₄ toxicity.

4.2.1.2. Time course of CCL₄ hepatotoxic effect on rat liver

A daily decrease of CCL₄ elevated levels of ALT and AST were showed in Fig. (4) after ninety six hours ALT and AST were reduced to normal levels. Similar findings were reported by Teschke, et al (1933) and Murphy, et al (1969)

4.2.1.3. Paracetamol toxicity

Decomposition of paraminophenol compounds may produce its analgesic and antipyritic agent, in this group of compounds the analgesic effect is due to N-acetyl paraminophenol (paracetamol) that found in the liver, also it has slight toxic effect. The sulphate ester and glucuronide of paracetamol account for about 75-80% of ingested drug Jewis,(1964).

4.2.1.4. Hepatoprotective effect of camel urine against CCL₄ and paracetamol toxicity:
Different doses of (24HCU), (EMU) and chloroformic extract of camel urine were tested against liver damage induced by CCl₄ in two ways, one hour prior and one hour after CCl₄ administration. The doses that protect liver against CCl₄ toxicity (in rats) were 4ml/100g BW of all (24hcu) tested urine 2ml and 0.2ml of (EMU) and chloroformic extract respectively.

Camel urine and its chloroformic extract significantly (P<0.05) reduced the highly elevated levels of ALT and AST induced by CCl₄.

The result of the present study indicated that I.P. administration of CCL₄ at a dose rate of 0.1ul/100g B.W. induced damage of liver cells of experimental rats, which was manifested biochemically by significant elevation of AST, ALT enzyme activities (P<0.05).

These forms of camel urine, significantly reversed the elevated level of serum ALT and AST activity induced by CCL₄ at 4ml and 2ml/100g B.W. fig. (1, 2 and 3). Other doses did not protect liver cells, whereas 2ml dose of (24hcu) may be below the therapeutic value while the 8ml of the (24hcu) was found to be too high or may be due the long storage of urine lead to some changes in 24hcu urine constituents, since the rats died in less than 55 minutes. On the bases of these result we could clearly suggested that camel urine has an active component (s) which may have an important role as an endogenous anti-oxidant and/or could act as cytoprotective agent against tissue damage mediated by toxic substances. The percentage of hepatic protection with camel urine is 82%, and with CE is 95% against CCL₄ induced toxicity.

There is no published data concerning camel urine hepatoprotective activity, whereas similar hepatoprotective effects were reported by Ali et al (2001). Wen (1999) and Shuang (2003) reported similar findings by using human urine extracts. Findings were reported by many authors dealing with some plants having hepatoprotective activity, such as

4.2.2. Response of isolated tissue strips to camel urine and its extracts:

Serotonin causes increased gastrointestinal motility and contraction of isolated strips of intestine, this being partly due to a direct effect on smooth muscle cells and partially due to indirect effect on enteric neurones Rang and Dale, (1987).

Cyproheptadine the standard amide at concentration of (4.4x10^{-5} M) significantly inhibits the contractile response induced by 5-HT (5x10^{-5} M) Rang and Dale (1987).

4.2.2.1. Isolated rat duodenum

Produces spontaneous contractile action. Addition of 0.1ml/bath of camel urine protein precipitate (CUPP), abolished the contractile responses of this tissue this suggest that (CUPP) may possess adrenaline like substances.

4.2.2.2. Isolated rabbit jejunum

It was directly stimulated by (CUPP) and diluted urine (DU, 1:3), at dose rates of 0.1 ml/bath and 0.8ml/bath respectively, camel urine exerts a daul effect on rabbit jejunum. The stimulant effect appeared to be mediated via muscarinic receptor stimulation as the effect was blocked by atropine 0.2ml/bath (5x10^{-5}M), which implies an acetylcholine like action. This was similar to result reported by Fischer and his Co.
worker.(1983). Crude urine 0.1ml/bath (CU) cause marked relaxant effect on isolated rabbit jejunum followed by a transient contraction after wash suggesting that concentrated urine has an irritant effect.

Addition of small, medium and high dose of (CE). 0.1, 0.2, 0.4µl/bath respectively producing slight effect on the isolated rectum of rabbit and chick, this suggest that camel urine may contain polar and non polar substances that affect the isolated tissue than do the (CE).

4.2.2.3. Isolated rat Fundus

Camel urine precipitant 0.8ml/bath significantly stimulated the isolated rat fundus (P<0.05). Moreover the diluted camel urine (1:3) significantly stimulated the isolated rat fundus strip at doses of 0.2, 0.4, and 0.8ml/bath. No effect was produced when CE was administrated, similar findings were reported by Guthum, (1958). We might suggest that camel urine may contain 5-HT like substances and / or mediated amines.

4.2.3. Cytotoxic effect in vitro test of camel urine (ASPPCU) on bovine kidney and liver cell culture:

Concentrated camel urine when inoculated in tissue culture (BLCS) caused 100% cytopathic effect after 24 hours, while 1:10 caused damage to the cell culture at 50% where diluted urine (DU, 1:3) shows growth action of the liver cell culture which was in agreement with Umberto et al.(2000). These observations suggest that camel urine and (CUPP) may contain some components which enhance or initiate cell growth or alter cell membrane permeability.

4.3. Camel urine therapy:

4.3.1. Biological Role of Macroand Micro elements

Macro elements are commonly described; essential and nonessential. The first one so defined because their lack leads to functional disorders, and complementation with a physiological dose can prevent or cure these disorder (Mertz, 1981). These elements are
generally included in enzyme molecules, for example, copper in cytochrome oxidase, Alkaline phosphates, DNA and RNA polymerases, and dehydrogenases, Manganese in pyruvate carboxylase and selenium in glutathione peroxidase. Some are included also in hormones (iodine in thyroid hormone), vitamins (cobalt in VB₁₂) or metaloproteins (iron in haemoglobin and myoglobin).

4.3.2. Effect of (EMU) camel urine on the selected parameters

Results of the parameter selected in this study, may be used as a surveillance tools for detecting the incidence of curelly and clinical status of the animal health. Camel urine (EMU) at a dose rate of 2 and 4 ml/kg B.W. changed serum enzymes, AST, ALT, ALP, GGT, and LDH; electrolytes (Na and K); minerals, Ca, P, and Mg. Among the mentioned parameters, AST and ALT showed a significant (P<0.01) reduction at 4th week along with Rafoxanide treatment. Change in serum Ca, P, Na, and K concentrations also decreased at week 3 and 4; whereas gradual increase in Na concentration was observed. Although according to these results, camel urine in general observations, started to exert its effect at the first week this may be attributed to its high concentrations of K, Na, Ca and Mg; and their salts e.g. NaCl, MgSO₄, NaH₂O, which have a pronounced physiological and pharmacological effect on the potentiality of cell membrane permeability and ion exchange system. Moreover, the increased influence of camel urine may be referred to alteration in its absorption and local metabolic degradation, taking into account the changes in Bovine ruminal microflora environment. Rumen pH generally varies from 6.3 to 7.0, because the composition of fibrous feeds usually leads to considerable recycling of buffer in saliva resulting from the greater time of mastication and regurgitation. Also mineral acids reduces the ruminal pH Dunand and Kawashima, (1980). A significant lowering of enzyme levels (Table 1) (P<0.05) compared to infected untreated
control animals. Changes in serum constituents were significantly correlated at week 3 and 4 rather than in week eight. AST, and ALT showed a highly significant correlation (P<0.01), r = 0.778 and 0.639 in week 3 and 4. Also there is a highly interaction between GGT and urea (P<0.01) at week eight r = 0.731, while total protein and albumin were significantly correlated (P<0.01), with time r = 0.656 at the first and 8th week followed by no significant interaction at week 3 and 4. Total protein and creatinine were significantly correlated (P<0.01) and (P<0.05) in the 3rd and 4th week respectively. Ca and P showed no significant correlation in first and 8th week, whereas a highly significant correlation (P<0.01) was obtained in the 3rd and 4th week, r= 0.690 and 0.635.

Urea is formed solely in the liver from the catabolism of amino acids, and it is the main excretion product of protein metabolism. Urea diffuses freely at the same concentration throughout the body water.

Blood urea concentration is influenced by the extent to which amino acids are oxidized and on the absorption of NH₃ from the rumen, Wallace (1979). Uric acid is a principal end-product of nucleic acid and purine metabolism in man, via final common pathway of conversion of xanthine by means of xanthine oxidase to uric acid.

The rise in RBCs and WBCs concentration in group II and III may indicate that camel urine has some factor(s) (e.g. Interlukine) which initiate or enhance haemopoietic stem cells to promote synthesis of new RBC, and WBC cells; also it may indicate that microflora were generating antibodies against the given (EMU) of camel and by the 4th week, the animal internal organs acid base balance adapted and equilibrated the change in buffer system. Microflora too become totally adapted to the changed environmental condition.

Results of histopathology revealed slight recovery in the hepatocyte texture. Camel urine as observed from the results of the
treated calves showed a parallel correlations with that of Rafoxanide. This suggests that camel urine may contain active substances present in Rafoxanide i.e. substituted phenols, salicylanide or nitrnoxil.

4.3.3. Camel urine chromatography

Extraction of active substances from camel urine, using the method described by Ibrahim (1989), showed that Chloroformic extract gave poor yield. Precipitation with ether appears to be an inefficient way to recover the active material from camel urine. Much better recovery has been obtained by ethanolic, methanolic and chloroform in methanol fraction, which provides the presence of alkaloids and which was similar to the observation found by Umberto et al. (1987) using HPLC analysis of camel and zebo cattle.

Elusion of camel urine from sephadex DAEA [25-150] was found more satisfactory than the butanolic elution, recovery was good and the active material (s) were concentrated in a few fractions. As shown in this study columns of IRC 60 mesh and sephadex have the advantage that active ingredient can be adsorbed from small volumes of urine. If elution is carried out with acid little activity is lost.
Conclusion

The results of experimentally examined camel urine, camel urine chloroformic extract and camel urine protein precipitant when compared to standard drugs on hepatoprotective effect, responses to various isolated strips, chromatographic and curative effect show certain similarities to many investigators using human and rat urine Gaddum (1953) and Horton (1958).

In this investigation diluted camel urine appeared to be more active than protein precipitant, crude urine and chloroformic extract on in vitro experiments. Early morning urine of young female camel is the most effective on in vivo one’s the CUPP and camel urine seems to have a similar role of action to that of 5-HT, parallel assay provided more evidence of response relationship of all these mediated peptides and amino acids.

From all these biological and biochemical comparisons it is concluded that the three forms of camel urine do have the same active principle(s) that are responsible for the activity of camel urine studied, more over camel urine of four different ages female camel emphasizing their similarity without implying identity by using IR technique.

Indeed, these experiments will suffice to illustrate the hypothesis that camel urine may cure and prevent liver and internal organ disorder. The knowledge gained from this study could improve the application of camel urine for animal health and nutrition, as well as to assist in the alleviation of diseases in significant measures. These goals have been realized in this research of camel urine physiology, biochemistry, pharmacology and chromatography which could be summerised in:
1) Camel urine has an alkaline pH.

2) Camel urine has a pronounced level of potassium and urea.

3) Camel urine consists of bicarbonates, sulphates, phosphates, and chlorides.

4) Antioxidant agents.

5) Purgative and little laxative effects.

6) Alkaloids – nitrogenous basis.

7) Promoting or supporting agent(s).
Recommendations for future work:

More considerable investigation must be done to know:

1) The structure of the macromolecules of camel urine

2) How the potential energy available from oxidation of camel urine can derive the main-fold energy requiring processes of the living cells. (its action on liver metabolizing enzyme).

3) The primary strands that the genetic apparatus encodes instruction for the precise ordering of the amino acids in the primary strands of protein.

4) Quantitative biological activity-directed fractionation of (CE) of camel urine.

5) Determination of the most potent fraction of GC-MS.

6) Synthesis and optimisation of the active constituent(s)

7) Formulation of the active ingredients in the pharmaceutical dosage.
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