THE EFFECT OF GUM ARABIC SUPPLEMENTS ON
SODIUM VALPROATE-INDUCED HEPATOTOXICITY IN
RATS

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Biochemistry

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DEDICATION

TO THE SOUL OF MY PARENTS
TO MY FAMILY AND MY FRIENDS

With love

Sitona
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ABSTRACT

Epilepsy is a common chronic neurological disorder and it imposes a big burden on health care systems. It is treated mainly with drugs but surgery may be used for severe cases. Sodium valproate is widely used as a major drug in the treatment of epilepsy, but the most serious adverse effect of chronic use is hepatotoxicity.

The objective of this study was to investigate the effects of Gum Arabic on sodium valproate-induced hepatotoxicity in the rat, by following liver function during treatment.

Twenty eight healthy rats weighing 80 – 160 g were divided into four groups and received treatment for 45 days. Group (A) was given sodium valproate orally at dose of 2.2 mg/kg body weight (B.wt) /rat/day. Group (B) was given sodium valproate 2.2 mg/kg body weight /rat/day and Gum Arabic 0.5 g/kg body weight (B.wt)/rat/day in drinking water simultaneously. Group (C) was given water and served as control for group (A). Group (D) received Gum Arabic at 0.5 mg/kg body weight (B.wt) /rat/day in drinking water. Alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase, (ALP), total plasma proteins, albumin and total bilirubin were estimated with Roche Diagnostic/ Hitachi (902) system.

The results showed that serum ALT and AST activities in group (A) receiving valproate increased significantly (P< 0.01) after 15 days, 30 days (P< 0.001) and 45 days (P< 0.0001).

Treatment with Gum Arabic in combination with valproate (group B) resulted in a significant increases in the level of ALT and AST after 30 and 45 days compared to the control D (P<0.01). Although levels of ALT and AST increased, the values were significantly decreased compared to the corresponding estimates of group (A).

A highly significant increase in the level of ALP was found in group (A) after 15, 30 and 45 days compared to the control. Although treatment with valproate in combination with Gum Arabic resulted in a significant rise in ALP level throughout the period of treatment; the rise was significantly lower than that recorded for group (A) at the respective times. Serum total protein concentration showed no significant difference between groups A, B, C, and D throughout treatment. However, a significant decrease in the level of albumin in group A was found after 30 and 45 days (P=0.01 and P= 0.001 respectively). Combination therapy restored the decrease that occurred
at 30 and 45 days in valproate treatment to normal. There was a highly significant increase in the level of total bilirubin concentration in group (A) after 30 days (P= 0.001) and 45 days (P=0.0001)). There was significant decrease in the level of total bilirubin concentration in group B compared to group A.

The present study showed that Gum Arabic has protective effect on sodium valproate- induced hepatotoxicity in rats. This is manifested by the effects on valproate treatment in reducing the increasing levels of transaminases and alkaline phosphatase, restoration of albumin as well as of bilirubin to almost normal levels.
الصرع من الإضطرابات العصبية المزمنة الأكثر شيوعاً، وهو يفرض عيناً كبيراً على أنظمة الرعاية الصحية، وهو يعالج بالعقاقير أساساً ولكن الجراحة قد تستعمل للحالات الحادة، وتستخدم فالباروات الصوديوم عقاراً رئيسيًا بشكل واسع في معالجة الصرع، لكن التأثير المضاد والأكثر خطورة للاستعمال المزمن هو تسمى الكبد.

أن الهدف من هذه الدراسة هو معرفة تأثير الصعم العربي على فالباروات الصوديوم المسببة لتسوس الكبد في الفترات، وذلك من خلال متابعة وظائف الكبد أثناء المعالجة.

في هذه التجربة تم إخضع ثمانية وعشرين فأرا ابيضاً سليماً للدراسة، توزن 80-160 جراماً وقد قسمت إلى أربع مجموعات وأعطيت معالجة لمدة 45 يوم مجموعة (أ) أعطيت فالباروات الصوديوم 2.2 ملجم/كم/يوم، أما المجموعة (ب) فقد أعطيت فالباروات الصوديوم 2.2 ملجم/كم/يوم وفي نفس الوقت أعطيت جرعة من الصعم العربي 5 جم/كم/يوم في الماء الصالح للشرب. وأما المجموعة (ج) أعطيت ماء واستخدمت كمجموعة تحكم. كما أعطيت المجموعة (د) جرعة صمغ عرقي 5 جم/كم/يوم في الماء للشرب.

لقد تم تحديد إنزيمات الانترنسيز، أنسامبيز، سترانسيريز، والفوستيزز البروتين الكلي، الألبومين، والبليبرين الكلي باستخدام نظام روش التشخيصي/ هيتدشي (902).

أوضحت النتائج زيادة معنوية في نشاط إنزيمات الانترنسيز، سترانسيريز والإسبارتيز أنسامبيز في المجموعة (أ) بعد 15 يوما، 30 يوما، وبعد 45 يوما.

أدت المعالجة بفاليباروات الصوديوم مع الصعم العربي (مجموعة ب) إلى زيادة معنوية في مستوى الأمينو والأسبارتيز بعد 30 يوما، وبعد 45 يوما مقارنة بمجموعة التحكم (د)، بالرغم من أن مستويات الأمينو والأسبارتيز زادت، إلا أن القيم نقصت معنوية مقارنة بتلك الفترات المطلقة في المجموعة (أ).

بُرِدَت هناك زيادة معنوية كبيرة في مستوى الفوستيزز القلوي في السيرم في المجموعة (أ) بعد 15، 30 و45 يوما مقارنة بمجموعة التحكم. وبالرغم من أن العلاج بفاليباروات الصوديوم مع الصعم العربي (مجموعة ب) أدى إلى ارتفاع معنوي في مستوى الفوستيزز القلوي خلال فترة المعالجة ما مرتبطة بمجموعة التحكم (د)، إلا أن هذا الارتفاع كان مخففاً معنوبًا على المستويات التي سجلت للمجموعة (أ) في نفس الأوقات. ليس هناك اختلافاً معنويًا عن تركيز البروتين الكلي في السيرم بين المجموعات، ب، ج، د خلال فترة المعالجة ولكن هناك نقص معنوي في مستوى الألبومين في المجموعة (أ) بعد 30 يوما، و45 يوما، ولقد أعاد العلاج بفاليباروات الصوديوم مع الصعم العربي لفترة 30 يوما و45 يوما في المجموعة المعالجة بفاليباروات الصوديوم للمستوي الطبيعي.
كان هناك زيادة معنوية عالية في مستوى تركيز البليروبين الكلي في المجموعة (أ) بعد 30 يوماً و45 يوماً. هناك نقصان معنوي في مستوى تركيز البليروبين الكلي في المجموعة (ب) مقارنة بالمجموعة (أ).

أوضحت الدراسة الحالية أن الصمغ العربي له تأثير وقائي ضد تسمم الكبد في الفترة. المعالجة بفالبروات الصوديوم يظهر ذلك بتاثيره على العلاج بالفالبروات في تخفيض المستويات المتزايدة للترانس أمينيز والفسفات القلوى، و إعادة الألبومين والبليروبين إلى المستويات الطبيعية.
INTRODUCTION

Acacia Gum is exudates of the stems and branches of acacia trees (family leguminaceae) and it is often referred to commercially as Gum Arabic. It is a fermentable polysaccharide resistant to gut enzymes and thus can be described as a dietary fiber (Phillips, 1998). It is a high molecular weight polysaccharide containing D-galactopyranose, D-glucuronic acid, L-rahamnopyanose and L-arabofuranose, beside a protein portion. The exact structure is not yet depicted but believed to be a protein core from which polysaccharide chains ramify.

It is extensively used in the food industry in a wide range of products including sweets, soft drinks and beverages, exploiting its demulcent, emulsifying and binding properties. It is also used in pharmaceutical preparations for the same properties. Recently it has been indicated as a supportive therapy in chronic renal failure (Ali et al., 2008). In 1999, Gum Arabic was introduced as a supportive therapy for chronic renal failure patients in Sudan by a group of doctors headed by Professor Salma M. Sulieman (Sulieman, 1999), a consultant medical Physician at Khartoum Dialysis and Kidney Transplantation Center.

Gum Arabic is generally recognized as safe with no recognized toxic effects (Sheu et al., 1986 and Campbell et al., 1997). It is neither teratogenic nor carcinogenic (National Toxicology Program, 1982) or allergic (Sander et al., 2006). Gum Arabic has been shown to have protective effect against the toxicity of a number of drugs. (Abd-Allah et al., 2002; Al-Majed et al., 2002; Gamaleldin et al., 2003 and Al-Majed et al., 2003)
Epilepsy:
Epilepsy is a common disorder, characterized by seizures, which take various forms and result from episodic neuronal discharges. It affects approximately 1% of the population. It is treated mainly with drugs but surgery may be used for severe cases. Current antiepileptic drugs such as phenytoin, carbamazepine, valproate and ethosuximide are effective in controlling seizures in about 70–80% of patients, but their use is often limited by side effects (Eadie and Vajda, 1999). One of the drugs extensively used is sodium valproate (VPA). Such extensive use is justified by the drug efficacy against a wide range of seizure disorders. It appears to be well tolerated in therapeutic doses; however, close monitoring of patients treated with the drug for hepatotoxic effects is a common practice by physicians and neurologists. It has been shown that valproate induces hepatotoxicity (Gerstner et al., 2008).

Independent risk factors for valproate hepatotoxicity are polytherapy with other antiepileptic drugs and over dosage (Kondo et al., 1992, Lippincott and Wikins, 2003). Since there are no laboratory tests, which detected early toxicity, it is prudent to avoid valproate in these risk patients.

It has been reported that Gum Arabic has hepatoprotective effects against certain drugs (Gamal-eldin et al., 2003). It is worth-while to probe this aspect in connection with valproate by investigating the effect of Gum Arabic supplementation in patients receiving valproate as a sole medication or in combination therapy. From a pure ethical stand one would rather do this protocol in an animal model that gives
results, which can be extrapolated to the human situation. The most eligible animal candidate in this situation is the rat.

**Objectives:**

General objective in this study is to investigate the effects of Gum Arabic oral treatment on sodium valproate hepatotoxicity in the rat, by following liver function during treatment with the drug.

Assessment of liver function will be followed by:

1. Measurements of the activity of liver enzymes such as Alanine Amino Transferase (ALT), Aspartate Amino Transferase (AST) and Alkaline Phosphatase.
2. Total proteins and Albumin.
CHAPTER ONE
LITERATURE REVIEW

1.1 Gum Arabic

1.1.1 Definition

Gum Arabic (GA) is a dietary fiber derived from the dried exudates obtained from the stems and branches of natural strains of *Acacia Senegal* (L.) or *Acacia seyal* (family leguminosae). Sudan is the world’s largest producer of Gum Arabic, principally in the traditional rainfed areas of western Sudan. The best grade of Gum Arabic comes from cultivated trees (garden gum or hashab), known as Kordofan Gum (Fig. 1).

Gum Arabic is generally recognized safe as a direct food additive, as it has shown negative genotoxicity effects (Sheu *et al.*, 1986). It has never proved to be a reproductive or developmental toxin (WHO, 2000) and is not carcinogenic (Campbell *et al.*, 1997) when given intraperitoneally or orally.

Acacia has long been used in traditional medicine. Arabic physicians treated a wide variety of ailments with Gum. To day, it is used widely in pharmaceutical and food industry as emulsifier, adhesive, and stabilizer (Leung 1980; Smolinske and Susan 1992).

1.1.2 Chemical constituents and properties

Acacia Gum is a brittle, odorless, pale colour, tasteless, and is soluble in water forming transportant viscous solution, but insoluble in ethanol and other organic solvents (FAO, 1995).
Fig. (1): Kordofan Gum Arabic (left raw Gum and right spray-dried powder)
Chemically, GA is complex polysaccharide consisting mainly of calcium salts of ploy Arabic acid, but also contains magnesium and potassium ions (Onishi et al., 2008; Nasir et al., 2008).

Gum Arabic described by Phillips and Williams (1993) as a large molecule with high molecular weight ranging from 300 to 800 Kilo Daltons. It consists of carbohydrate moieties made of L-rhaminose (12.2-14%), L-Arabnose (26-28%), D-galactose (40-44%) and D-glucuronic acid (15.5-16%). These carbohydrate moieties are covalently attached to a protein backbone (2%) which contains hydroxy proline (Anderson et al., 1985).

The main chemical and physical features of the *Acacia senegal* Gum and its molecular fractions isolated by chromatographies were determined using a wide variety of methods. Three main molecular fractions were isolated after hydrophobic interaction chromatography (HIC) and biochemical analyses confirmed the presence of an arabinogalactan-peptide (FI) an arabinogalactan-protein (FII), and a glycoprotein (FIII). Estimation of the polypeptide backbone length in the three fractions gave 43, 2253, and 4443 amino acid residues, respectively, hydroxyl proline (Hyp) and serine being the most prominent residues within FI and FII, Hyp and Asx (asparagine + aspartic acid) within FIII (Renard et al., 2006).

### 1.1.3 Biological aspects

#### 1.1.3.1 Absorption and metabolism

Gum Arabic is primarily indigestible to both human and animals as it is not degraded in the intestine, but fermented in the colon under the influence of microorganisms (Phillips, 1998). The literature indicates
that Gum Arabic can be fermented by intestinal bacteria to short chain fatty acid, particularly propionate (Kishimoto et al., 2006).

1.1.3.2 Toxicological studies
Gum Arabic has no teratogenic or carcinogenic properties (National Toxicology Program, 1982). In rats, GA has no histo-pathological or hematological toxicity when administered for 13 weeks at doses as high as 5 g/kg/day. It has been shown that GA has no effect on concentration of some free-scavengers (reduced glutathione, ascorbic acid, lipid peroxidation and the free radical scavenger enzyme superoxide dismutase) on the kidney and liver in healthy rats (Abd-Allah et al., 2002; Al- Majed et al., 2003).

1.1.4 Protective effects of Gum Arabic
Gum Arabic has been shown to have a protective effect against the toxicity of a number of drugs. GA has recently been claimed to be effective in preventing gentamicin-induced acute renal failure in rats. It has been suggested that the renoprotective effect of GA is possibly through inhibition of the production of free-radicals that cause peroxidation (Ali 2004). It has also been reported that GA has protective effect against cardiotoxicity induced by doxorubicin. This was evidenced by significant reduction in serum creatine kinase and cardiac lipid peroxides (Abd-Allah et al., 2002). In addition to the above GA is effective in protecting mice against acetaminophen-induced hepatotoxicity (Gamaleldin et al., 2003). Mice were given Arabic Gum orally at a concentration of 100 g per L, 5 days before a hepatotoxic dose of acetaminophen (500 mg per kg) that is administered intraperitoneally. Arabic Gum administration dramatically reduced acetaminophen-induced hepatotoxicity as
evidenced by reduced serum alanine (ALT) and aspartate aminotransferase (AST) activities. Nonetheless, Acetaminophen-induced hepatic lipid peroxidation was also significantly reduced by GA pretreatment (Gamaleldin et al., 2003).

1.1.5 Uses of Gum Arabic

1.1.5.1 Food industry

The major uses of Gum Arabic are in the food industry where it is used as food additive to improve desirable properties through its influence on viscosity, body and texture of food. In addition, it is non-toxic, odorless, colorless, tasteless, and completely water soluble and does not affect the flavor, odour, or color of the food to which it is added (Fig. 2).

* Confectionery: GA is extensively used in the confectionery industry because it has the ability to prevent crystallization of sugar also because of its thickening effect. It is used as a glaze in candy products (Leung, 1980).

* Dairy Products: It is used as emulsifier and stabilizer for frozen dietary products such as ice cream, because of its water absorbing properties (Leung, 1980).

* Bakery Products: Gum Arabic is widely used in the baking industry for its viscosity and adhesive property. It is used in glazes and toppings. It also adds smoothness when used as an emulsion stabilizer (Leung, 1980).

* Beverages: It also acts as a source of soluble fiber in low caloric and dietetic beverages (Phillips, 1998). Hence in such a manner it replaces calorific foods.
Fig (2): Uses of Gum Arabic in food industry
1.1.5.2 Medicinal uses

1.1.5.2.1 Renal

Gum Arabic was introduced as a supportive therapy for chronic renal failure patients in Sudan by a group of doctors headed by Professor Salma M. Suliman at Khartoum Dialysis and Kidney Transplantation Center (Sulieman, 1999). Results from other workers indicated significant increases in creatinine clearance and altered electrolyte excretion (Ali 2004; Ali et al., 2008; Nasir et al., 2008).

1.1.5.2.2 Cholesterol management

GA has been shown to lower the cholesterol levels in the blood (Ross et al., 1983; Mee and Gee, 1997). Specifically that fraction transported by low density lipoproteins (LDL) (Marlett, 2001). The mechanism by which these fiber sources lower blood cholesterol levels has been the focus of many investigations, and characteristics such as solubility in water, viscosity, fermentability, and the kinds and amounts of protein. The one characteristic common to all cholesterol-lowering fibers is viscosity (Marlett, 1998). This viscosity interferes with bile acid absorption from the ileum (Marlett et al., 1994). In response, LDL cholesterol is removed from the blood and converted into bile acids by the liver to replace the bile acids lost in the stool. Some evidence also indicates that ingestion of some viscous fibers slowing cholesterol synthesis (Moundras et al., 1994).

1.1.5.2.3 Blood sugar lowering effects

GA has been shown to lower the glucose levels in the blood (Freed and Joffe, 2000). Researchers found that Gum Arabic lowered postprandial serum glucose levels at least by three mechanisms. First, the dietary fiber increases the viscosity of small intestine juice and
hinders diffusion of glucose. Second, GA binds glucose and decreases the concentration of available glucose in the small intestine. Third, it retards $\alpha$-amylase action through capsuling starch and the enzyme and might directly inhibit the enzyme. All of these decreased the absorption rate of glucose and the concentration of postprandial serum glucose (Shyi et al., 2001).

The use of high-fiber diets in diabetic patients has long been indicated and well documented (Chandalia et al., 2000; Tabatabai and Li 2000 McIntosh and Miller 2001).

1.1.5.2.4 GA and electrolyte and water absorption
Gum Arabic is reported to enhance the movement of water and electrolytes such as water and sodium from the intestinal system to the blood (Turvil et al., 2000). Another benefit of the Gum Arabic is that its use does not change the viscosity of the final solution (Turvil et al., 2000).

1.1.5.3. Pharmaceutical Uses
Gum Arabic is listed in the U.S pharmacopeia as effective suspending aid and has been employed suspend insoluble drugs and to prevent the precipitation of the heavy metals from solution through formation of colloidal suspensions.

GA was one of best emulsifying agent and suspending agents for calamine suspensions, kaolin suspensions, liquid petroleum emulsions and cod liver oil emulsions.

Gum Arabic’s demulcent or soothing characteristics have led to its use in many pharmaceutical types of syrup, and used as an adhesive, binder for pharmaceutical tablets (Smolinske and Susan, 1992).
1.1.5.3.1. Improves gold Nanoparticles for cancer imaging

It has been shown that Gum Arabic could effectively stabilize gold nanoparticles in the body. The investigators noted that over 98 percent of the gold salt was converted to Gum Arabic-labeled gold nanoparticles. The resulting nanoparticles were stable in biological fluids for at least seven days (Kattumuri et al., 2007).

1.1.5.3.2. Cosmetics

Non toxic nature and free from dermatological and allergic reactions of Gum Arabic has made its use in cosmetics. In lotions and protective creams, Gum Arabic adds as smoothing feel to the skin and forms a protective coating. It also acts an adhesive in preparation of facial masks. It acts as a binder in face powder compacts, while in protective creams it is effectively used as stabilizer (Smolinske and Susan, 1992).

1.1.5.4. Other uses

- Paints
  Gum Arabic liquid should be mixed into paint before use, especially water-based paint. Gum Arabic makes the colors brighter and lighter.

- Inks
  GA has excellent protective colloidal properties. It is added to fabric and laundry marking inks to bring these to a desired viscosity for smooth printing on cloth. Water colour inks, quick dry inks, typographic inks, glass finish inks, all contain Gum Arabic.
• **Adhesives**

GA has been widely used in adhesives. Its glues are light in colour, odourless, very stable. The adhesive strength of the Gum can also be improved by addition of the certain metal salts, such as calcium nitrate and aluminum sulphate. GA also finds use in the wall paper, pastes, where it’s used in form of a mixture.

**1.2 Sodium Valproate**

Sodium valproate is a broad-spectrum antiepileptic drug (AED) that has been used for more than 30 years and is effective in the treatment of many different types of partial and generalized epileptic seizure (Johannessen and Johannessen, 2003). However, serious complications may occur in some patients receiving valproate chronically. These include fatal hemorrhagic pancreatitis (Moreiras *et al.*, 1999), bone marrow suppression (Kishi *et al.*, 1994), VPA-induced hepatotoxicity (VHT) (Gerstner *et al.*, 2008), and VPA-induced hyperammonaemic encephalopathy (VHE) (Young *et al.*, 2007; Rousseau *et al.*, 2009).

**1.2.1 Chemistry**

Valproic acid is one of a series of fatty carboxylic acids (2-propylpentanoic or di-n-propylacetic acid). Its structure is very similar to that of short chain fatty acids (Fig. 3).

The acid has antiseizure activity, which appears to be greatest for carbon chain lengths of five to eight atoms. Branching and unsaturation do not significantly alter the drug's activity but may increase its lipophilicity, by increasing its duration of action (Levy, 2002).
Fig. (3): Chemical structure of Sodium valproate.

1.2.2 Mechanism of action
VPA increases the levels of gamma-aminobutyric acid and prolongs the recovery of inactivated sodium channels. These properties may be responsible for its action as a CNS depressant.
* VPA interacts with voltage-sensitive sodium channels. Their blocking action shows the property of use-dependence, in other words, they block the excitation of cells that are firing repetitively and the higher frequency of the firing, the greater the block produced.
* VPA potentiates γ-aminobutyric acid (GABA) functions in some specific brain regions that are thought to be involved in the control of seizure generation and propagation by increasing both GABA synthesis and release (Bolans and Medina, 1997). It reduces the release of epileptogenic γ-hydroxybutyric acid and attenuates the neuronal excitation induced by N-methyl-D-aspartate (NMDA)-type glutamate receptors (Loscher, 2002).

1.2.3 Pharmacokinetic
1.2.3.1 Absorption
Non-enteric-coated preparations of VPA are rapidly and nearly completely absorbed from the gastrointestinal tract, with peak plasma concentrations occurring 1 – 4 hours after ingestion (Graudins and Aaron, 1996).

1.2.3.2 Distribution and protein binding
The volume of distribution (Vd) is 0.1-0.5 L/kg, with most of the quantity of VPA confined to the extracellular space. After an overdose, protein-binding sites are saturated, increasing the free fraction of VPA and Vd. At such therapeutic concentrations VPA is
80–90% bound to plasma proteins, but the percentage decreases at higher VPA levels (Levy, 2002).

**1.2.3.3 Metabolism**

VPA is extensively metabolized by the liver via glucuronic acid conjugation, mitochondrial β– and cytosolic (endoplasmic reticulum) ω-oxidation to produce multiple metabolites, some of which may be biologically active (Lheureux, *et al.*, 2005) (Fig. 4). Mitochondrial β-oxidation of VPA involves its transport within to the mitochondrial matrix, using the same pathway, as do long-chain fatty acid the 'carnitine shuttle' (Fig. 5).

Less than 3% of VPA is excreted unchanged in the urine (Brubacher *et al.*, 1999). Much of which is in the form of valproyl-carnitine (Hiraoka *et al.*, 1997).

Elimination of VPA follows first-order kinetics, with a half-life ranging from 5 to 20 hours (mean 11 hours). However, following overdose the half-life may be prolonged to as long as 30 hours (Mcnamara, 1996).

**1.2.3.4 Therapeutic levels and dosage**

Dosage of 400 – 600 mg/kg/d may be adequate in some patients but other may require over 600 mg/kg/d. Therapeutic serum concentrations range from 50 – 100 µg/ml (Levy, 2002).

**1.2.3.5 Drug interactions**

VPA increases serum levels of carbamazepine, Phenobarbital, and primidone mainly by inhibiting various cytochrome P450 (CYP450) isoenzymes involved in their metabolism. Cimetidine and ranitidine increase VPA levels by inhibiting hepatic mixed-function oxidase (thereby decreasing VPA metabolism). Drugs that slow the GI
Fig. (4): Liver metabolism of valproic acid.

Fig. (5): The carnitine shuttle

tract may delay absorption of VPA (opiates, antihistamines) during co-ingestion (Hachd 2002).

1.2.4 Toxicity
The most common dose related adverse effects of sodium valproate are nausea, vomiting and other gastrointestinal complaints such as abdominal pain and heartburn. A fine tremor is frequently seen at higher levels. Other reversible adverse effects seen in small number of patients include weight gain, increased appetite, and hair loss (Wallace, 2001). Serious complications may occur in some patients receiving VAP chronically, including hemorrhagic pancreatitis (Moreiras et al., 1999), bone marrow suppression (Kishi, et al., 1994) VAP induced hepatotoxicity (VHT) (Fernandez et al., 1995; Gerstner et al., 2008), and VAP induced hypermmonaemic encephalopathy (VHE) (Young et al., 2007; Rousseau et al., 2009).

1.2.4.1 Valproate-induced hepatotoxicity (VHT)
In up to 44% of patients chronic dosing with VPA may be associated with elevation in transaminases during the first months of therapy (Lippincott, 2003; Goto et al., 2008). The most common clinical presentation consists of lethargy, jaundice, nausea, vomiting, hemorrhaged, and worsening seizures and anorexia (Bryant and Dreifuss, 1996). Histological changes are similar to those observed in the Reye's syndrome, with early production of microvesicular steatosis accompanied by ultrastructural changes characterized by myeloid bodies, lipid vacuoles and mitochondrial abnormalities. Risk factors include age under 24 months (especially those with organic brain disease), developmental delay, coincident congenital metabolic disorders, previous liver dysfunction, or severe epilepsy treated with polytherapy or ketogenic diets (Kondo et al., 1992;
Gopaul, et al., 2003). The mechanisms of both sub-acute and idiosyncratic VHT remain incompletely understood, but it has been believed that hypocarnitinaemia, subsequent imbalance between \(\beta\)-oxidation and \(\omega\)-oxidation, and accumulation of 4-en-VPA are involved. Additionally, carnitine deficit may result in disruption of mitochondrial functions due to depletion in CoA (Coulter, 1991).

Reduced serum free carnitine as well as reduced levels of 3-keto-VPA, the main metabolite of \(\beta\)-oxidation of VPA, was first reported in 1982 by Bohles and coworkers (Bohles et al., 1982) in a 3-year-old girl who developed acute liver disease with typical features of Reye’s syndrome after treatment with VPA for 6 months. Reduced free carnitine and increased serum and urine acylcarnitine levels were also demonstrated in patients with VPA-induced Reye-like syndrome (Matsuda et al., 1986). In a patient with fatal VHT, Krahenbuhl et al., 1995 demonstrated a reduction in free and total carnitine in plasma and liver. Other mechanisms such as VPA-induced lipid peroxidation and glutathione depletion could also contribute to hepatotoxicity (Raza et al., 1997 and Zeitlhofer et al., 2000). Indeed, 4-en-VPA is transformed through \(\beta\)-oxidation to reactive intermediates such as 2-propyl-2, 4-pentadienoic acid (2, 4-dien-VPA) that are capable of depleting mitochondrial GSH, as suggested by rat studies (Tong et al., 2005). Unsaturated VPA metabolites (4-en-VPA and 2, 4-dien-VPA) are potent inducers of microvesicular steatosis in rats. Studies in rats also suggested that both VPA and its unsaturated metabolites inhibit \(\beta\)-oxidation through different mechanisms, such as sequestration of CoA-SH and direct inhibition of specific enzymes in the \(\beta\)-oxidation sequence by CoA esters, particularly 4-en-VPA figure (6).
Fig. (6): Effects of decreased β-oxidation and increased ω-oxidation of fatty acids and VPA on the urea cycle.

2.1 Materials

2.1.1 Animals
Twenty-eight healthy Wister albino rats of both sexes weighing 80-160 g were used. They were kept in cages and housed in standard environmental conditions of temperature, humidity and light. The rats were left for fifteen days adaptation and supplied with standard diet and water.

2.1.2 Gum Arabic
Gum Arabic was obtained from Gum Arabic Company in Khartoum as spray dried powder.

2.1.3 Sodium Valproate
Sodium valproate Syrup was obtained from Medico Laboratorys-Homs- Syria. Oral solution is an antiepileptic drug for oral administration, contains the equivalent of 200 mg/5 ml.

2.1.4 Equipments
i. Collection containers.
ii. Centrifuge.
iii. General laboratory equipments.
iv. Roche Diagnostic/Hitachi (902) Analyzer
It is an analyzer, which is used to report results on various body fluid samples for a wide range of analyses (Fig. 7). It is fully automated, computerized and performs photometric assays. It consists of photometric measuring potentiometric system, analytical processing units, screen and printer. The analyzer characteristics
Fig. (7): Roche Diagnostic/Hitachi (902) Analyzer
include: 200 tests per hour and refrigerated storage for 40 reagents containers.

2.1.5 Reagents

2.1.5.1 Reagents for enzyme analysis

2.1.5.1.1 Reagents for Alanine aminotransferase (ALT) analysis
Tris (hydroxymethyl)-amino methane (TRIS) buffer: 125 mmol/l, pH 7.3; L-alanine: 625 mmol/l; NADH: 0.23mmol/l (yeast); Lactate dehydrogenase (LDH) > 1.5 u/ml; Ketoglutarate: 94 mmol/l.

2.1.5.1.2 Reagents for Aspartate aminotransferase (AST)
TRIS buffer: 100 mmol/l, PH 7.8; L-aspartate: 300 mmol/l; NADH (yeast): 0.23 mmol/l; MDH (porcine heart \(\geq 0.53 \mu \text{ml} \)); LDH \(\geq 0.75 \text{ u/ml} \); Ketoglutarate: 75 mmol/l.

2.1.5.1.3 Reagents for Alkaline phosphatase (ALP) analysis
2-Amino-2- methyl-1-propanol: 1.12 mol/l, PH 10.44 (30°C); magnesium acetate: 2.49 mmol/l; zinc sulfate: 0.50 mmol/l; N- (2-hydroxyethyl)-ethylnediamine triacetic acid: 2.49 mmol/l; p-Nitrophenyl phosphate: 99.5 mmol/l, PH 8.50 (25°C).

2.1.5.2 Reagents for Total protein estimation
R1 (Sodium hydroxide: 400 mmol/l; potassium sodium tartrate: 89 mmol/l). R2 (Sodium hydroxide: 400 mmol/l; potassium tartrate: 89 mmol/l; potassium iodide: 61 mmol/l; copper sulfate: 24.3 mmol/l.

2.1.5.3 Reagents for Albumin estimation
R1 (Citrate buffer: 95 mmol, PH 4.1), R2 (Citrate buffer: 4.1; bromocresol green: 0.66 mmol/l.

2.1.5.4 Reagents for Total bilirubin estimation
R1 (Sodium acetate buffer: 85 mmol/l; sulfamic acid: 110 mmol/l; surfactant; solubilizer, R2 (HCL: 100 mmol/l; diazonium ion: 3 mmol/l).
2.2 Methods
Activity of enzymes, total protein, albumin, total bilirubin were measured by using fully automated apparatus Roche Diagnostics/Hitachi Analyzer (902).

2.2.1 Experimental design
At the end of the adaptation period, the rats were divided into four groups named, A, B, C, and D. Weighing 80–160g.
- Group (A) of eight rats, were treated with sodium valproate at a dose of 2.2 mg/kg body weight (B.wt)/rat/day for 45 days orally.
- Group (B) of eight rats, were given sodium valproate at a dose of 2.2 mg/kg (B.wt)/rat//day and at the same time received Gum Arabic at a dose 0.5 g/kg (B.wt)/rat/day in drinking water for 45 days
- Group (C) of six rats, were given water for 45 days (served as control).
- Group (D) of six rats, received Gum Arabic at a dose 0.5 mg/kg B.wt /rat/day (B.wt) in drinking water 45 days (as a base line).

2.2.2 Samples
Blood samples were collected from orbital plexus vein in early morning every fifteen days for 45 days. Sera were analyzed for activities of transaminase ALT and AST, alkaline phosphatase, total protein, albumin and total bilirubin.

2.2.3 Estimation of enzymes activities
1. Estimation of Alanine aminotransferase (ALT):
The Roche Diagnostic/Hitachi (902) system is used for the measurement of ALT in serum or plasma. The time required to
complete an assay is approximately 10 minutes. Concentration range was 4-600 IU/L with the lower detection limit approximately 4 IU/L.

**Principle:**
ALT catalyzes the transformation of L-Alanine and Oxoglutarate at optimal PH (7.3). The pyruvate release in the reaction is transformed by Lactate dehydrogenase (LDH) in the presence of NADH coenzyme to L-lactate, while the NADH/NAD+ oxido-reductive process show a decrease in absorbance at 340 nm. The change in absorbance correlates with serum ALT activity (Riemtman and Frankel, 1957).

\[
\text{L -Alanine} + \alpha\text{-Ketoglutarate} \rightleftharpoons \text{Pyruvate} + \text{L-Glutamate}
\]

\[
\text{Pyruvate} + \text{NADH} + \text{H}^+ \rightleftharpoons \text{Lactate} + \text{NAD}^+
\]

2. Estimation of Aspartate aminotransferase (AST):
The Roche Diagnostic/Hitachi (902) system is used for the measurement of AST in serum or plasma. The time required to complete an assay is approximately 5 minutes. Concentration range was 4-800 IU/L with the lower detection limit approximately 4 IU/L.

**Principle:**
Two substrates participate in the reaction catalyzed by AST. L-aspartate and Oxoglutarate with the help of NADH coenzyme. Malate dehydrogenase (MDH) contained in the reagent catalyses the transformation of Oxalactate released in the first reaction. The oxido-reductive process of NADH/NAD+ is indicated by a decrease in the absorbance at 340 nm. The change in absorbance correlates with AST activity (Riemtman and Frankel, 1957).

\[
\alpha\text{-Ketoglutarate} + \text{L}\text{-Aspartate} \rightleftharpoons \text{L-Glutamate} + \text{oxaloacetate}
\]

\[
\text{Oxaloacetate} + \text{NADH} + \text{H}^+ \rightleftharpoons \text{L-malate} + \text{NAD}^+
\]
3. **Estimation of alkaline phosphatase (ALP):**

The Roche Diagnostic/Hitachi (902) system was used for the measurement of ALT in serum or plasma. The time required to complete an assay is approximately 10 minutes. Concentration range was 1-1200 IU/L with the lower detection limit approximately 0.67 IU/L.

**Principle:**

In the presence of magnesium and zinc ions, p-nitrophenyl phosphate is cleavage by phosphatases into phosphate and p-nitrophenol. The p-nitrophenol released is proportional to the ALT activity and is measured photometrically (Chemie 1972).

\[
P- \text{nitrophenyl phosphate} + \text{H}_2\text{O} \xrightarrow{\text{ALP}} \text{phosphate} + \text{p-nitrophenol}
\]

4. **Estimation of total protein (T PRO):**

The Roche Diagnostic/Hitachi (902) system was used for the measurement of total protein in serum or plasma. The time required to complete an assay is approximately 5 minutes. Concentration range was 0.2-15 g/dl with the lower detection limit approximately 0.2 g/dl.

**Principle:**

Divalent copper reacts in alkaline solution with protein peptide bonds to form the characteristic purple-colored biuret complex. Sodium potassium tartrate prevents the precipitation of copper hydroxide and potassium iodide prevents auto reduction of copper. The color intensity is directly proportional to protein concentration, which can be determined photometrically (Reinhold, 1953).

\[
\text{Protein} + \text{Cu}^{2+} + \xrightarrow{\text{alkaline solution}} \text{Cu- protein complex}
\]
5. Estimation of albumin (ALB):
The Roche Diagnostic/Hitachi (902) system was developed for measurement of Albumin in human serum or plasma. The time required to complete an assay is approximately 10 minutes. Concentration range was 1-7 g/dl with the lower detection limit approximately 0.2 g/dl.

**Principle:**
At PH= 4.2, albumin bind with bromocresol green to produce a blue-green complex. The change in absorbance at 628 nm correlates with the concentration of albumin (Spencer and Price, 1977).

\[
\text{Albumin} + \text{BCG} \xrightarrow{\text{PH 4.1}} \text{albumin BCG complex}
\]

The analyzer automatically calculates the analyte concentration of each sample. Conversion factors: g/dl × 10 = g/l.

6. Estimation of Total Bilirubin (T.BIL):

**Principle:**
Total bilirubin in the presence of a suitable solubilizing agent, is coupled with a diazonium ion in strongly acidic medium (pH= 1-2). The intensity of the colored bilirubin produced is Proportional to total bilirubin concentration and can be measured photometrically (Jandrassik and Grof, 1938).

2.2.4. Statistical analysis
Data were entered and analyzed using SPSS (Statistical Package for Social Sciences) version 13. Numerical data were expressed as means, standard error. Significance of difference between means was tested by Student’s Samples t test, or one-way ANOVA, depending on the number of compared groups; with a p value of ≤ 0.05 considered statistically significant.
CHAPTER THREE
RESULTS

3.1 Liver Function Tests:
3.1.1 Enzymes Activities

Table (1, 2 and 3) show the serum level of ALT, AST and ALP of experimental rats after 15, 30 and 45 days treatment. Group (A) of 8 rats received valproate (2.2 mg/kg/Bwt daily for 45 days. Group (B) received Gum Arabic (0.5g/kg) simultaneously with valproate (2.2 mg/kg/day) for 45 days. Group (C) of 6 rats received water for 45 days. Group (D) of 6 rats received Gum Arabic (0.5g/kg/day in drinking water for 45 days.

3.1.1.1. Measurement of serum ALT

Table (1) shows the serum level of ALT in rats receiving valproate alone and in combination with Gum Arabic and their respective controls. Serum ALT activity was measured at day 0 before starting the treatment. There was no significant difference in the four groups (A, B, C and D). A significant increase in the level of ALT was found in group (A) after 15 day (69.6 ± 2.7 U/L) P<0.01, then ALT showed a steady increase after 30 day (261 ± 7.4 U/L) with higher level of significant P<0.001 and the level reached a peak at day 45 of treatment reaching (302 ± 12.8 U/L) P<0.0001 compared to control. Serum ALT level in Group (A) of rats that received sodium valproate alone daily for 30 days was almost four times greater than that of the control. Upon extension of the treatment to 45 days for the same group, serum ALT level was almost five times greater than that of the control.
Table (1): Changes in serum ALT activity in rats treated with sodium valproate alone and in combination with Gum Arabic

<table>
<thead>
<tr>
<th>Groups</th>
<th>ALT U/L (Mean ± SE)</th>
<th>Day 0</th>
<th>Day 15</th>
<th>Day 30</th>
<th>Day 45</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 0</td>
<td>Day 15</td>
<td>Day 30</td>
<td>Day 45</td>
</tr>
<tr>
<td>A</td>
<td>58.8±0.14</td>
<td>69.6±2.7*</td>
<td>261.0±7.4**</td>
<td>302±12.8***</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n=8</td>
<td>n=8</td>
<td>n=8</td>
<td>n=4</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>59.3±0.33</td>
<td>61.20±1.6</td>
<td>146.8±1.3**</td>
<td>128.5±15.7**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n=8</td>
<td>n=8</td>
<td>n=8</td>
<td>n=6</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>57.8±0.2</td>
<td>58.7±0.2</td>
<td>58.5±0.2</td>
<td>58.4±0.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n=6</td>
<td>n=6</td>
<td>n=6</td>
<td>n=6</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>58.4±0.4</td>
<td>59.6±0.3</td>
<td>59.9±0.2</td>
<td>59±0.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n=6</td>
<td>n=6</td>
<td>n=6</td>
<td>n=6</td>
<td></td>
</tr>
</tbody>
</table>

Key:
* P ≤ 0.01  ** P ≤ 0.001  *** P ≤ 0.0001

Group (A) = Received sodium valproate.
Group (B) = Received sodium valproate +Gum Arabic.
Group (C) = Received water (control).
Group (D) = Received Gum Arabic
n = Number of rats
Treatment with Gum Arabic daily for 15 days in combination with valproate resulted in no significant difference in the level of ALT compared to the control group (D). However after 30 and 45 days of treatment, the level of ALT in group (B) was (146.83 ± 1.3 U/L) and (128.5±15.7 U/L) respectively. This increase was very significant (P<0.001) compared to the control D. However, after 45 days of the combination treatment ALT dropped (128.5±15.7 U/L) from the level attained after 30 days. It is worthwhile to note that both the magnitude and pattern of change in the level of ALT after 30 and 45 days of treatment with valproate on its own is significantly different from that recorded for the treatment with valproate in combination with Gum Arabic. The levels of ALT in group (A) both at 30 and 45 days were significantly higher than the corresponding levels in group (B); almost doubled and tripled in value respectively. Moreover, while the level of the enzyme increased steadily during the treatment with valproate reaching 302 ± 12.8 U/L at day 45; the level in the combination therapy rose to 146.8 ± 1.3 U/L after 30 days and started to fall there after, reaching 128.5 ± 15.7 U/L after 45 days.

Figure (8) illustrates the findings in Table (1) showing ALT levels in the four groups over a period of 45 days.
Fig. (8): The level of ALT as affected by treatment with sodium valproate alone and in combination with Gum Arabic for 45 days.
3.1.1.2 Measurement of serum AST

Table (2) shows the serum level of AST in rats receiving valproate alone and in combination with Gum Arabic plus the corresponding controls. Serum AST activity was measured at day 0 before starting the treatment, there was no significant difference in the four groups. A significant increase in the level of AST (172.0 ± 0.8 U/L) was found in group (A) after 15 days (P< 0.01). More significant increases were recorded after 30 days (420.5 ± 6.8 U/L) and 45 days (453.8 ± 19.2 U/L) of administration of sodium valproate compared to control (P< 0.001 and P< 0.0001 respectively). Serum AST levels in group (A) rats that received sodium valproate daily for 45 days was almost five times greater than that of the control. This parallels the changes in ALT levels described in the previous section.

Treatment with Gum Arabic in combination with valproate resulted in a significant increase (131.3 ± 1.5 U/L) (P< 0.01) after 15 days compared to the control D. However, the level of AST in group (B) was (319.9 ± 1.4 U/L) and (235 ± 20.4 U/L) after 30 and 45 days of the combination treatment respectively. This increase was very significant compared to the control D P< 0.0001 and P< 0.001 respectively. However at 45 days of treatment AST dropped (235 ± 20.4 U/L) from the level attained after 30 days.

It is worthwhile to note that both the magnitude and pattern of change in the level of AST after 30 and 45 days of treatment with valproate on its own were different from that recorded for the treatment with valproate in combination with Gum Arabic.
Table (2): Changes in serum AST activity in rats treated with sodium valproate alone and in combination with Gum Arabic

<table>
<thead>
<tr>
<th>Groups</th>
<th>AST U/L (Mean ± SE)</th>
<th>Day 0</th>
<th>Day 15</th>
<th>Day 30</th>
<th>Day 45</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td></td>
<td>81.6±0.3</td>
<td>172.0± 0.8*</td>
<td>420.5±6.8**</td>
<td>453.8±19.2 ***</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n=8</td>
<td>n=8</td>
<td>n=8</td>
<td>n=4</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td>83.4±0.4</td>
<td>131.3±1.5*</td>
<td>319.9 ±1.4 ***</td>
<td>235 ± 20.4 **</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n=8</td>
<td>n=8</td>
<td>n=8</td>
<td>n=6</td>
</tr>
<tr>
<td>C</td>
<td></td>
<td>83.3±0.4</td>
<td>82.8 ± 0.5</td>
<td>81.4±0.3</td>
<td>82.6 ± 0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n=6</td>
<td>n=6</td>
<td>n=6</td>
<td>n=6</td>
</tr>
<tr>
<td>D</td>
<td></td>
<td>81.8±0.3</td>
<td>88.4 ± 2.1</td>
<td>86.0 ± 0.8</td>
<td>84.7 ± 0.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n=6</td>
<td>n=6</td>
<td>n=6</td>
<td>n=6</td>
</tr>
</tbody>
</table>

Key:
* P ≤ 0.01    ** P ≤ 0.001    *** P ≤ 0.0001

Group (A) = Received sodium valproate.
Group (B) = Received sodium valproate +Gum Arabic.
Group (C) = Received water (control).
Group (D) = Received Gum Arabic.
n = Number of rats.
The level of AST increased steadily to reach 453.8 ± 19 U/L after 45 days of treatment with valproate alone. With the combination treatment the highest level was reached at day 30 (319.9 ± 1.4 U/L) and dropped there after 45 to (235 ± 20.4 U/L). The levels of AST in group (A) were significantly (P< 0.01 and P< 0.001 respectively) higher at day 30 and day 45 compared to the corresponding levels of group (B) recorded for the same duration. For example AST level was found to be 420 ± 6.8 U/L after 30 days of treatment with valproate alone where as for the same duration of treatment using combination therapy the level recorded 319.9 ± 1.4 U/L. The reduction in the level of AST after 45 days in the combination therapy (235 ± 20.4 U/L) was even more pronounced when compared to the level when valproate was administered alone.

Figure (9) illustrates the findings in Table (2) showing AST levels in the four groups over a period of 45 days.
Fig. (9): The level of AST as affected by treatment with sodium valproate alone and in combination with Gum Arabic for 45 days
3.1.1.3 Measurement of serum ALP

Table (3) shows the serum level of ALP in groups of rats receiving sodium valproate alone and in combination with Gum Arabic and their respective controls. Serum ALP activity was measured at day 0 before starting the treatment. There was no significant difference between the four groups. The level of ALP in group (A) measured after 15 and 30 days of treatment with sodium valproate was (326.7 ± 1.2 U/L) and (335.8 ± 3.4 U/L) respectively. This is a highly significant increase (P< 0.001) compared to the control. An even higher level of significance in the increase of serum ALP level was observed after 45 days of treatment reaching 405 ± 15 U/L (P< 0.0001).

Group (A) rats that received sodium valproate daily for 15 and 30 days, serum ALP level was almost three times that of the control. Upon extension of the treatment to 45 days for the same group, serum ALP level was almost four times of the control.
Table (3): Changes in serum ALP activity in rats treated with sodium valproate alone and in combination with Gum Arabic

<table>
<thead>
<tr>
<th>Groups</th>
<th>ALP U/L (Mean ± SE)</th>
<th>Day 0</th>
<th>Day 15</th>
<th>Day 30</th>
<th>Day 45</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 45</td>
<td>Day 30</td>
<td>Day 0</td>
<td>Day 15</td>
</tr>
<tr>
<td>A</td>
<td>119.0± 0.3</td>
<td>326.7±1.2</td>
<td>335.8 ±3.4</td>
<td>405±15.5*</td>
<td>335.8 ±3.4</td>
</tr>
<tr>
<td></td>
<td>n=8</td>
<td>n=8</td>
<td>n=8</td>
<td>n=4</td>
<td>n=8</td>
</tr>
<tr>
<td>B</td>
<td>119.7 ±0.7</td>
<td>202.2±1.4</td>
<td>218.1±2.4</td>
<td>306.2±9.3</td>
<td>202.2±1.4</td>
</tr>
<tr>
<td></td>
<td>n=8</td>
<td>n=8</td>
<td>n=8</td>
<td>n=6</td>
<td>n=8</td>
</tr>
<tr>
<td>C</td>
<td>119.3±0.4</td>
<td>118.7±0.4</td>
<td>119.0±0.3</td>
<td>119.3±0.2</td>
<td>118.7±0.4</td>
</tr>
<tr>
<td></td>
<td>n=6</td>
<td>n=6</td>
<td>n=6</td>
<td>n=6</td>
<td>n=6</td>
</tr>
<tr>
<td>D</td>
<td>118.6±0.3</td>
<td>123.3±1.2</td>
<td>122.4±1.0</td>
<td>119.8±0.4</td>
<td>123.3±1.2</td>
</tr>
<tr>
<td></td>
<td>n=6</td>
<td>n=6</td>
<td>n=6</td>
<td>n=6</td>
<td>n=6</td>
</tr>
</tbody>
</table>

Key:
* P ≤ 0.01      ** P ≤ 0.001      *** P ≤ 0.0001

Group (A) = Received sodium valproate.
Group (B) = Received sodium valproate + Gum Arabic.
Group (C) = Received water (control).
Group (D) = Received gum Arabic.
n = Number of rats.
Treatment with valproate in combination with Gum Arabic resulted in a significant rise in ALP level after 15 days (202.2 ± 1.4 U/L) and 30 days (218.1 ± 2.4 U/L) that was almost double the level of serum ALP in the control D (P< 0.01). However, a highly significant increase in the level of serum ALP level was recorded (306.2 ± 9.3 U/L) after 45 days of treatment that was three times the level in the control P< 0.001. Comparing this increase in ALP in group (B) with that of group (A) at corresponding duration of treatment, showed that there was a 66.6% drop in ALP level when combination therapy was applied for 15 and 30 days reaching a 75% reduction after 45 days of treatment.

Figure (10) illustrates the findings in Table (3) showing ALP levels in the four groups over a period of 45 days.

3.1.1.4. Comparing Enzymes Activities:

The pattern of elevation in the level of these three liver enzymes, however, differed in magnitude as relevant to treatment duration (Fig. 11).
Fig. (10): The level of ALP as affected by the treatment with sodium valproate alone and in combination with Gum Arabic for 45 days
Fig. (11): Comparison of the patterns of change in the activity of ALT, AST and ALP during valproate / valproate + Gum Arabic treatment during a period of 45 days
3.1.2 Protein

3.1.2.1 Total protein

Table (4) shows the serum level of total protein in rats treated with valproate alone and in combination with Gum Arabic. Serum total protein concentration was measured at 0, 15, 30 and 45 days of treatment with valproate. Treatment with valproate for 45 days resulted in no significant difference in the level of total protein compared to the control.

Treatment with valporate in combination with Gum Arabic for 15, 30 and 45 days resulted in no significant difference in the level of total protein compared to the control group (D).

Figure (12) illustrates the findings in Table (4) showing total protein concentration in the four groups over a period of 45 day.
Table (4): changes in serum total protein value in rats treated with sodium valproate and in combination with Gum Arabic

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total protein g/dl (Mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
</tr>
<tr>
<td>A</td>
<td>7.3±0.02  n=8</td>
</tr>
<tr>
<td>B</td>
<td>7.3±0.01  n=8</td>
</tr>
<tr>
<td>C</td>
<td>7.3±0.01  n=6</td>
</tr>
<tr>
<td>D</td>
<td>7.3±0.01  n=6</td>
</tr>
</tbody>
</table>

Key:
ns = not significant
Group (A) = Received sodium valproate.
Group (B) = Received sodium valproate +gum Arabic.
Group (C) = Received water (control).
Group (D) = Received gum Arabic.
n = Number of rats
Fig. (12): Total protein level as affected by the treatment with sodium valproate alone and in combination with Gum Arabic for 45 days
3.1.2.2 Albumin concentration

Table (5) shows the serum level of albumin in rats treated with valproate alone and in combination with Gum Arabic. After fifteen days of treatment with valproate alone no significant difference in the level of albumin was observed when compared to control (C). A highly significant decrease in the level albumin was found in group (A) after 30 (2.8 ± 0.2 g/dl) and 45 (2.4 ± 0.04 g/dl) days of administration of sodium valproate compared to control (P< 0.01 and P< 0.001 respectively).

Treatment with valproate in combination Gum Arabic daily for 15 days (3.4 ± 0.04 g/dl), 30 days (3.3 ± 0.03g/dl) and 45 days (3.4 ± 0.1g/dl) resulted in no significant difference in the level of albumin compared to the control group (D). The level of albumin after 45 days of treatment with valproate in combination Gum Arabic is significantly (P< 0.01) increased compared to that recorded for the treatment with valproate alone at 45 days.

Figure (13) illustrates the finding in Table (5) serum albumin level in groups over a period of 45 days.
Table. (5): Changes in serum albumin value in rats treated with sodium valproate alone and in combination with Gum Arabic

<table>
<thead>
<tr>
<th>Groups</th>
<th>Day 0</th>
<th>Day 15</th>
<th>Day 30</th>
<th>Day 45</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>3.4±0.02 n=8</td>
<td>3.3±0.1 n=8</td>
<td>2.8±0.2* n=8</td>
<td>2.4±0.04** n=4</td>
</tr>
<tr>
<td>B</td>
<td>3.4±0.02 n=8</td>
<td>3.4±0.04 n=8</td>
<td>3.3±0.03 n=8</td>
<td>3.4±0.1 n=6</td>
</tr>
<tr>
<td>C</td>
<td>3.5±0.01 n=6</td>
<td>3.4±0.1 n=6</td>
<td>3.5±0.1 n=6</td>
<td>3.5±0.1 n=6</td>
</tr>
<tr>
<td>D</td>
<td>3.4±0.01 n=6</td>
<td>3.5±0.02 n=6</td>
<td>3.4±0.1 n=6</td>
<td>3.5±0.1 n=6</td>
</tr>
</tbody>
</table>

Key:
* P ≤ 0.01    **  P ≤ 0.001

Group (A) = Received sodium valproate.
Group (B) = Received sodium valproate + gum Arabic.
Group (C) = Received water (control).
Group (D) = Received gum Arabic.
n = Number of rats.
Fig. (13): Albumin level as affected by sodium valproate alone and in combination with Gum Arabic for 45 days
3.1.3 Total bilirubin concentration

Table (6) shows the changes of serum bilirubin concentration in rats treated with sodium valproate alone and in combination with Gum Arabic. After 15 days of treatment with valproate alone there was significant ($P < 0.01$) increase in the level of total bilirubin when compared to control. Total bilirubin showed a steady increase after 30 days ($0.3 \pm 0.02 \text{ mg/dl}$) that was significant at $P < 0.001$ and it reached a higher level at day 45 of treatment ($0.4 \pm 0.001 \text{ mg/dl}$) which was significant ($P < 0.0001$) compared to the control. The rise in serum total bilirubin in group (A) rats receiving sodium valproate daily for 45 days was almost four fold that of the control.

Supplementation of Gum Arabic with valproate after 15, 30 and 45 days resulted in no significant difference in the level of total bilirubin compared to the control group (D). Total bilirubin level in group (A) was found to be ($0.18 \pm 0.01 \text{ mg/dl}$, $0.3 \pm 0.02 \text{ mg/dl}$ and $0.4 \pm 0.001 \text{ mg/dl}$) after 15, 30 and 45 days of treatment with valproate alone where as the corresponding levels for group (B) treated with a combination therapy recorded ($0.13 \pm 0.02 \text{ mg/dl}$, $0.14 \pm 0.02 \text{ mg/dl}$ and $0.16 \pm 0.003$ respectively). Comparing these sets of data there a significantly ($P < 0.001$) remarkable reduction in total bilirubin concentrations when Gum Arabic is supplemented. There was a 33.3% drop in level total bilirubin when combination therapy is applied for 15 and 30 days reaching a 50% reduction after 45 days of treatment.

Figure (14) illustrates the findings in Table (6) showing total bilirubin concentration in the four groups over a period of 45 days.
Table (6): Changes in serum Total bilirubin concentration in rats treated with sodium valproate alone and in combination with Gum Arabic

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total bilirubin mg/dl (Mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
</tr>
<tr>
<td>A</td>
<td>0.13±0.03</td>
</tr>
<tr>
<td></td>
<td>n=8</td>
</tr>
<tr>
<td>B</td>
<td>0.13±0.02</td>
</tr>
<tr>
<td></td>
<td>n=8</td>
</tr>
<tr>
<td>C</td>
<td>0.13±0.01</td>
</tr>
<tr>
<td></td>
<td>n=6</td>
</tr>
<tr>
<td>D</td>
<td>0.14±0.01</td>
</tr>
<tr>
<td></td>
<td>n=6</td>
</tr>
</tbody>
</table>

Key:
* P ≤ 0.01  ** P ≤ 0.001  *** P ≤ 0.0001

Group (A) = Received sodium valproate.
Group (B) = Received sodium valproate + gum Arabic.
Group (D) = Received gum Arabic.
Group (C) = Received water (control).
n = Number of rats
Fig. (14): Total bilirubin level as affected by sodium valproate and Gum Arabic treatment for 45 days
CHAPTER FOUR
DISCUSSION

Epilepsy is one of the most common and serious neurological disorders. Genetic, congenital, and developmental conditions are mostly associated with it among younger patients. Although epilepsy occurs in adults, 25% of all cases develop before the age of five (Hitiris et al., 2007). Incidence rate increase in resource-poor countries; socioeconomically deprived people are at higher risk (Sander, 2003). Considering treatment, potential side effects can be worse than the disease itself (Hitiris et al., 2007).

Sodium valproate is widely used as a major drug in the treatment of epilepsy, but the most serious adverse effect is hepatotoxicity (Goto et al., 2008; Genstner et al., 2008). As clinical usage of VPA increased, reports of its hepatotoxicity began to appear. This toxicity ranges from mildly increased aminotransferase enzymes in 15 to 30 percent of patients to liver failures (Browne, 1980), and death in some patients (Zimmerman and Ihsak, 1982; Konig et al., 1994). Valproate toxicity is thought to be idiosyncratic and metabolic with an immunologic basis (Zafrani and Berthelot, 1982; Zimmerman and Ihsak, 1982).

The mechanisms of both sub-acute and idiosyncratic valproate hepatotoxicity (VHT) remain incompletely understood, Hepatotoxicity is suspected to result from the formation of toxic valproate metabolites, (Siemens et al., 1993) or altered antioxidant enzyme activities (Graf et al., 1998). It has been believed that hypocarnitinaemia, subsequent imbalance between β-oxidation and ω-oxidation, and accumulation of 4-en-VPA are involved. Additionally,
carnitine deficit may result in disruption of mitochondrial functions due to depletion in CoA (Coulter, 1991). Valproate hepatotoxicity is more common in persons with mitochondrial enzyme deficiencies and may be ameliorated by IV administration of carnitine, which valproate therapy can deplete.

Other mechanisms such as VPA-induced lipid peroxidation and glutathione depletion could also contribute to hepatotoxicity (Raza et al., 1997 and Zeitlhofer et al., 2000). Indeed, 4-en-VPA is transformed through β-oxidation to reactive intermediates such as 2-propyl-2, 4-pentadienoic acid (2, 4-dien-VPA) that are capable of depleting mitochondrial GSH, as suggested by rat studies (Tong et al., 2005).

The current study was carried out to investigate the effect of Gum Arabic on sodium valproate induced-hepatotoxicity in rats.

4.1 Effects of Gum Arabic on valproate induced hepatotoxicity

It has been suggested that overdoses of valproate are potentially toxic (Geoffrey et al., 2003). Induced alterations in the biochemical parameters, usually tested to assess liver function, upon administration of sodium valproate were investigated in this study. These included enzymes such as alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP), total protein, albumin and bilirubin.

In this study hepatotoxicity induced by valproate treatment has been clearly observed and documented particularly with chronicity of the treatment characterized by elevation of ALT, AST, ALP, bilirubin and reduction in albumin. This in fact agrees with findings of experiments performed in a similar setting and experimental design (Muhamma et
al., 2006). This is usually taken care of by physicians treating epileptic patients by close monitoring their liver function to make necessary dose adjustments (Konig et al., 1998).

4.1.1 The transaminases (ALT and AST)

Serum transaminases level is most widely used as a measure of hepatic injury. In this study serum transaminases were significantly increased in sodium valproate-treated rats. The study demonstrated significant hepatocellular damage. This finding is in good agreement with previous findings (Genstner et al., 2008). The present study showed that administration of Gum Arabic in combination with valproate in drinking water daily for 45 days to Wistar rats produced significant reduction of valproate-induced hepatotoxicity as evidenced by the decrease in serum alanine and aspartate aminotransferase activities. This indicates that Gum Arabic has a successful hepatoprotective effect in averting the elevation of both transaminases studied induced by valproate. The protective effect being particularly pronounced for ALT (a lowering effect ranging from 50% to over 60%). The protective effect on AST ranged from 25 to 50%. The pattern of activity reduction is similar in both enzymes. For both enzymes it seems that Gum Arabic exerts its protective effect gradually as reflected by a decline after 30 days of continuous combination therapy which opposes the steady rise in activity of these enzymes measured during single valproate treatment. Whether this pattern of change will continue towards normalization of activity as the treatment goes on remains to be investigated. This needs to be verified by checking the levels of these enzymes after 60, 75, 90 days and so on. This finding is in good agreement with previous findings
(Gamaleldin et al., 2003) who investigated the effect of Gum Arabic on acetaminophen-induced hepatotoxicity in rats. These authors reported that ALT, AST were significantly (P<0.001) decreased in mice pretreated with Gum Arabic compared to acetaminophen alone. If this proves true it provides venues for new epilepsy therapeutic formulations. The prospect isn’t far fetched if one takes into consideration the use of Gum Arabic both in the food and drug industries (Phillips 1998).

4.1.2 Alkaline phosphatase

This enzyme is usually found at high concentrations in liver, bone, and placenta and in bile ducts. Increased blood ALP can indicate damaged or diseased liver tissue but is also seen during normal bone growth.

Serum alkaline phosphatase level in this study like ALT and AST increased during the treatment period. The pattern of elevation in the level of these three liver enzymes, however, differed in magnitude as relevant to treatment duration (Fig. 11).

While there was a gradual rise in the level of ALT and AST from a low at day 15 to a high at day 45, ALP showed a three fold increase at day 15 and remained almost unchanged after 30 days of treatment with valproate, then increased to reach a higher level that is almost four fold of its level at the start of treatment. The lag phase may reflect some biochemical changes pertaining to phase I and phase II metabolism of valproate and how this may affect cytochrome P450 induction. This is in agreement with previous observations by (Tutor-Crespo, et al., 2002) who studied some indirect biochemical markers
in the evaluation of enzymatic induction caused by antiepileptic drugs. The pertinent question to the raise about the behavior of the three liver enzymes is whether such elevation in activity is due to induction of these enzymes or due to liver cell injuries that releases them into the circulation. Production of reactive oxygen species has been implicated in hepatocytes damage (Thomas et al., 2006). 4-en-VPA is transformed through β-oxidation to reactive intermediates such as 2-propyl-2, 4-pentadienoic acid (2, 4-dien VPA) and believed to be capable of depleting mitochondrial GSH in rat studies (Tong et al., 2005). Of course GSH is an important cellular antioxidant particularly in prevention of biomembranes damage via lipid peroxidation. The administration of Gum Arabic in combination with valproate to the rats under investigation produced significant reduction in serum ALP activities. The overall pattern of reduction in ALP activity when combination therapy is used parallels that recorded for ALP activity during valproate treatment on its own; a steady rise in the enzyme activity throughout the investigation period. However, it differs from the pattern of reduction exhibited by the transeaminases; where a significant drop was obtained. These findings may give an important signal to the protective effect of the soluble fiber (GA) against liver damage, particularly if one considers the importance of ALT and AST as biomarkers of liver cell injuries rather than ALP.

If one considers the generation of free radicals hypothesis as a cause of valproate hepatotoxicity and if one rules out liver enzyme induction during valproate administration in the present study, it may be suggested that Gum Arabic has antioxidant properties. Other workers in fact have suggested this (The superoxide scavenging effect of GA
may explain, at least in part, the protective effect of AG against cardiotoxicity induced by DOX (Abd-Allah et al., 2002). The protection offered by Gum Arabic does not appear to be caused by a decrease in the formation of toxic acetaminophen metabolites, which consumes glutathione, because Gum Arabic did not alter acetaminophen-induced hepatic glutathione depletion. Acetaminophen increased nitric oxide synthesis as measured by serum nitrate plus nitrite at 4 and 6 h after administration and Gum Arabic pretreatment significantly reduced their formation (Gamaleldin et al., 2003).

4.1.3 Total protein and albumin
There was no significant difference in total protein in all the groups at the different durations. However, administration of valproate for 45 day caused a significant decrease in albumin. This result is in agreement with other workers findings (Attilakos et al., 2007). The reduction in serum albumin was restored to normal level after 30 and 45 days of combination treatment. The reduction in albumin is surprising in the light of no change in total plasma protein. However, it appears inconsistent with oxidative stress resulting in liver cell dysfunction that might have affected the production of other proteins as well. Formation of toxic metabolites may be involved in the protection offered by Gum Arabic does not appear to be caused by a decrease in the formation of toxic metabolites, which consumes glutathione, because Gum Arabic did not alter acetaminophen-induced hepatic glutathione depletion.

4.1.4 Bilirubin
Significantly elevated serum bilirubin was evident in valproate treatment as reflected by a four-fold increase in the metabolite serum
concentration by the end of the investigation period of 45 days. This is indicative of severe hepatotoxicity. This finding is in agreement with previous observations (Lippincott Williams and Wikins, 2003), which suggested that bilirubiaemia may result from hepatocellular dysfunction. The observation that treatment with Gum Arabic resulted in significant decreases of serum bilirubin is suggestive of its protective effect against valproate-induced hepatotoxicity. This in agreement with previous findings. Ray et al., 2006 found that administration of the extract of *Acacia catechu* at doses of (250 mg/kg) for seven days showed significant hepatoprotective activity (P<0.001) in albino rats.

Valproate treatment resulted in reduction of albumin and an increase in bilirubin concentration. Supplementation with Gum Arabic almost restored the two biochemical indices to almost their normal values.

### 4.2 The effect of valproate prolonged administration

Duration of valproate administration has significant effects on liver function. This is obvious from the results of the serum enzymes ALT, AST and bilirubin levels recording 81, 82 and 75% increase respectively after 45 days of valproate treatment as compared to the same group tread for 30 days (78, 80, and 66% respectively), and 15 days (16, 53 and 44% respectively). Administration of GA (0.5 g/kg) with sodium valproate (group B) produced a significantly protection against hepatotoxicity induced by valproate. This was evidenced by significant reductions in serum ALT, AST and bilirubin when compared to valproate group.
Conclusions
The potential value of Gum Arabic supplements in preventing adverse effects of sodium valproate-induced hepatotoxicity has been clearly revealed in this study. Its amelioration of valproate-induced hepatotoxicity is evident liver function tests as it reduced the level of serum aminotransferases, alkaline phosphatase, bilirubin and restored serum albumin levels.

It has been proven that Gum Arabic supplementation produces no adverse or harmful effects on human health evidenced by its extensive use in both the food and pharmaceutical industries. It emerges from this study that it could be a beneficial supplement that can effectively counter the valproate-induced hepatotoxicity.

Recommendations
Because of the scarcity of data on the protective effects of Gum Arabic and exact mechanisms by which it exerts its action the author finds that the following recommendations are worthwhile to consider.

- Extension of the duration of treatment to further assess the protective effect of Gum Arabic in liver transaminases and alkaline phosphatase and to examine its potential in restoring their serum level to the normal reference value.

- Further studies are needed to address the reduction in serum albumin and to determine the mechanisms involved in VPA-mediated decrease in serum albumin concentrations.

- Initiatives by governmental bodies and private sector to make use of this important national natural product is highly demanded in the direction of designing pharmaceutical formulation of Gum Arabic for therapeutic purposes.
REFERENCES


