A Study on the Potential Hypoglycemic Effect of Feeding Nigella sativa
(Black cumin) Seeds in Rats

By

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DEDICATION

To the candles which had brightened my days,
The soul of my mother ….

My father ….
My sisters and brothers…..

My friends…..
My nephews…..

With all love…..

Mawda
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ABSTRACT

The present study was designed to evaluate the hypoglycemic effect of *Nigella sativa* (Black cumin) seeds and to explore how the induction of hypoglycemia by feeding seeds can modulate glucose metabolism in animals. This effect was compared with a reference of a known hypoglycemic drug Glibenclamide.

Thirty female Wister albino rats were used as experimental animals and divided into five groups (A, B, C, D and E) of six rats each. Each rat was fed 8 g for 30 days as follows: In group A, the rats were fed basal diet and kept as control, group B was fed Glibenclamide (hypoglycemic drug) at the rate of 10 mg/kg body weight calculated as part from the rat basal diet, group C was fed *Nigella sativa* (50 mg/day as part from the basal diet), group D was fed *Nigella sativa* (50 mg/day + glucose 2g/kg body weight as part from the basal diet) and group E was fed glucose (2g/kg body weight calculated as part from the rat basal diet). Blood glucose levels were measured weekly. Insulin concentration was recorded at days 14 and 28. At the end of the experimental period, blood hemoglobin concentration was measured and oral glucose tolerance test was conducted for all rats. Glucose-6-phosphate dehydrogenase and Alanine aminotransferase activities were estimated, and a histopathological examination of the liver and the pancreas was done for all groups. Animal's body weight was reported at the beginning and at the end of the experiment.
All the treated groups showed significant reduction in blood glucose concentration after one week of treatment, and it was more pronounced in group B. At the second week and until the end of the experiment, significant reduction compared to the control group were maintained only in groups B and C which received Glibenclamide and *N. sativa* respectively. By the end of the experiment, groups D and E showed similar levels of glucose to the control group. In group D, feeding high glucose dose was not associated with the application of *N. sativa* to the diet. When insulin concentration was measured after two weeks, no significant increase was observed in all groups except groups D and E. By the end of the experimental period, all treated groups showed significantly higher insulin levels and it was significantly higher in group E.

After four weeks of treatment, no significant changes were noticed in Glucose-6-phosphate dehydrogenase activity in all groups, only higher values were found in groups D and E, being two folds higher than group B. The activity of Alanine aminotrasferase was significantly increased in the group receiving *N. sativa* but was significantly decreased in the groups treated with Glibenclamide and glucose respectively. The performance of the groups receiving Glibenclamide or only the *N. sativa* was similar when Glucose Tolerance Test was carried out, but addition of extra glucose to the rat basal diet plus the *N. sativa* abolished this effect. Hemoglobin concentration and animal’s body weight were not influenced by all treatments applied. No histopathological changes were
noticed in the liver and pancreas of *Nigella sativa* treated groups compared with the other groups and the control.

The results showed that *Nigella sativa* at 50 mg/day can exert potential hypoglycemic effects in rats. The hypoglycemic effect of the seeds may be mediated, at least in part, by decreasing glucose concentration and increasing insulin level. Glucose uptake and absorption were significantly influenced by feeding *N. sativa* to the rats. The increase of Alanine aminotransferase activity in *N. sativa* treated groups, with no histopathological changes, may indicate slight effects on the integrity of liver cells.
المستخلص

صممت الدراسة الحالية لتقييم تأثير الكمون (الجلوكوز) في خفض سكر الدم، وتناول كيفية إحداث خفض سكر الدم بتغيير أيض الجلوكوز في الحيوانات التي أعطيت البذور، ومقارنة هذا التأثير بداء الجلنكلاميد المعروف بخفضه لسكر الدم.

استخدمت 30 أنثى جرذ وستة ألبينو كحيوانات تجارب وقسمت إلى خمس مجموعات (أ، ب، ج، د، ه). أعطيت كل جرذ 8 جرام لمدة 30 يوماً، حيث أطعمت الجرذان في المجموعة أ الأكل الأساسي واستخدمت كمجموعة شاهد، وأعطيت المجموعة ب الجلنكلاميد (دواء خفض لسكر الدم) بجرعة 10 ملجم/كم من وزن الجسم (حسب كجزء من الأكل الأساسي)، وأعطيت المجموعة ج الكمون بمعدل 50 ملجم/اليوم كجزء من الأكل الأساسي، وأعطيت المجموعة د الكمون بمعدل 50 ملجم/اليوم إضافة إلى الجلوكوز (2جرام/كم من وزن الجسم كجزء من الأكل الأساسي)، وأعطيت المجموعة ه الجلوكوز (2جرام/كم من وزن الجسم) وتم حسابها كجزء من الأكل الأساسي. سجل مستوى جلوكوز الدم أسبوعياً وتركيز هرمون الإنسولين في اليوم 14 و 28. في نهاية مدة التجربة سجل تركيز هيموغلوبين الدم، وإجراء اختبار تحمل الجلوكوز لكل الجرذان. تم تقييم نشاط إنزيم الجلوكوز-6-فوسفات ديهيدروجيناز والأمينوتراسفيراز، كما فحصت الأنسجة المرضية للكبد والبنكرياس لكل المجموعات وسجلت أوزان الحيوانات في بداية ونهاية التجربة.

كانت الألبينو أظهرت كل المجموعات المعالجة نقصاً معيناً في تركيز جلوكوز الدم بعد إسبوع من التجربة، وكان أكثر وضوءاً في المجموعة ب، وكان هناك انخفاضاً في المجموعتين ب وج (المجموعة المعالجة بالجلوكلازميد والمجموعة المعالجة بالكمون على التوالي) في الأسبوع الثاني وحتى نهاية التجربة مقارنة مع مجموعتين الشاهد. بنهاية التجربة أظهرت مجموعتين الاختبار د و ه مستوى جلوكوز مشابهة لمجموعة الشاهد. إعطاء جرعة عالية من الجلوكوز للجموتيين د و ه نبينه مدة الشاهد، إعطاء جرعة عالية من الكمون معينه في كل المجموعات غير المجموعتين د و ه. بنهاية مدة المعالجة أظهرت كل المجموعات زيادة في مستوى هرمون الإنسولين وزيادة أكبر في المجموعة ه.
لم تلاحظ أي تغيرات معنوية في نشاط إنزيم الجلوكوز-6-فسفوت ديهيدروجيناز في كل المجموعات بعد أربعة أسابيع من المعالجة، وقد لوحظت الزيادات فقط في المجموعة D و E التي بلغت ضعف المجموعة B. حدثت زيادة معنوية في نشاط إنزيم الألفين أمينوتراينسيريز في المجموعة التي أعطيت الكمون بينما لوحظ نقص معنوي في المجموعات المعالجة بالجلينكلاميد والجلوكوز على التوالي. عند إجراء اختبار تحمل الجلوكوز كان الأداء متساوي في المجموعات التي أعطيت الجلبنكلاميد والكمون فقط، لكن إضافة الجلوكوز لأكل الجرذان الأساسي بالإضافة للكمون الغي هذا التأثير. لم يتأثر تركيز الهيموغلوبين وأوران الحيوانات بالمعاملات. لم تلاحظ أي تغيرات أنسجة مرضية في الكبد والبنكرياس للمجموعات المعالجة بالكمون مقارنة بالمجموعات الأخرى ومجموعة الشاهد.

أظهرت النتائج أن الكمون بمعدل 50ملجرام/اليوم قد يظهر تأثيراً بخفض سكر الدم في الجرذان. وقد يكون تأثير البذور الخفيف لسكر الدم، على الأقل جزئياً، بخفض تركيز الجلوكوز زيادة مستوى هرمون الإنسولين. وهناك تأثير معنوي لإعطاء الكمون على استهلاك وإمتصاص الجلوكوز في الجرذان. زيادة نشاط إنزيم الألفين أمينوتراينسيريز في المجموعات المعالجة بالكمون، مع عدم وجود تغيرات أنسجة مرضية، قد يدل على التأثير الطيفي على سلامة خلايا الكبد.
INTRODUCTION

Diabetes Mellitus is a serious, complex metabolic disorder of multiple etiologies. It has a significant impact on the health, quality of life and life expectancy of patients, as well as on the health care system (Kwon et al., 2008). Although different types of hypoglycaemic agents such as thiazolidinediones, insulin, biguanides and sulphonylurea are available, but there is growing interest in herbal remedies due to the side effects associated with these therapeutic agents, beside their limitations in managing the disease effectively. Diabetes Mellitus characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion (B-cell dysfunction), insulin action (insulin resistant) or both (Kardesler et al., 2008).

Type 2 insulin-resistant diabetes mellitus accounts for 90-95% of all diabetes. Both genetic and environmental factors play an important role in the onset of type 2 insulin-resistant diabetes (Lima et al., 2008). The microvascular and macrovascular complications of diabetes including cardiovascular disease, blindness, renal failure and peripheral nerve damage (Taskinen, 2002). Recent estimates indicate there were 171 million people in the world with diabetes in the year 2000 and this is projected to increase to 366 million by 2030 (Wild et al., 2004). Around 3.2 million deaths every year are related to a complication of diabetes, which equals six deaths each minute (WHO, 2007).

Renewed attention to alternative medicine and natural therapies has stimulated new researches to look for more efficacious agents with lesser side effects (Kim et al., 2006). According to the world ethno botanical information reports, almost 800 plants may possess antidiabetic potential. *N. sativa* is one such herbal product which has been in use as a spice from ancient times. Its medicinal value to treat various ailments is also well-known (Khanam and Dewan, 2008). *N. sativa* and several species of plants have been described as having antidiabetic property.
(Uddin et al., 2002; Uddin et al., 2005). Naturally occurring antidiabetic plant are relatively non toxic, inexpensive and available in an ingestive form. It used where low and middle-income populations are important (Gazioano et al., 2007).

The objectives of the present study are to investigate about the hypoglycemic effect of *N. sativa* seeds on glucose absorption uptake and metabolism in Wister albino female rats. The effects of feeding the seeds will be compared to the effect of the Glibenclamide (hypoglycemic drug). The parameters to be measured include:

- Plasma glucose concentration.
- Serum insulin concentration.
- Glucose-6-phosphate dehydrogenase activity.
- Blood Hemoglobin (Hb).
- To perform oral glucose tolerance test (OGTT).
- To measure Alanine aminotransferase (ALT) activity.
- To measure changes in animal’s body weight.
- To examine histopathological changes related to treatments.
CHAPTER ONE
LITERATURE REVIEW

1.1 *Nigella sativa*

*Nigella sativa* (*N. sativa*) commonly known as black seed or black cumin is a small plant originating in the Middle East and is found widely in Egypt, Asiatic Turkey and the Balkan states. It has been traditionally used in the Indian subcontinent (Nadkarni, 1976), Arabian countries (Sayed, 1980) and Europe (Lautenbacher, 1997) for culinary and medicinal purposes as a natural remedy for number of illnesses and conditions.

Abu Huraira (may God be pleased with him) narrated that God’s Messenger (pbuh) said, “Use this black seed regularly because it contains a cure for every disease except death.” *N. sativa* seeds are claimed to have bronchodilatory, hypotensive, antibacterial, antifungal, analgesic, anti-inflammatory and immunopotentiating properties (Schmall, 2007). The seed extracts from this plant are used in traditional medicine (Hakims or tabibs) in the treatment of several medical disorders including dyslipidemia, obesity and hypertension (Le et al., 2004).

*Nigella sativa* seed is herbaceous annual plant belonging to the Ranunculaceae family; it is cultivated in several countries in the Mediterranean region and Asia. In South Asia, it is called *kalanji*, its Arabic name is habbat el Baraka or habbah saouda and in English it is known as black cumin. The seeds of *N. sativa* have been employed for thousands years as a spice and food preservative (Aboutabl et al., 1986; Hanafy and Hatem, 1991). Additionally, it used in the traditional medicine applications (Abdulelah and Zainal-Abidin, 2007).

The seeds of *Nigella sativa* (*N. sativa*) has been showed to contain >30% of fixed oil and 0.4 – 0.45% wt/wt of volatile oil, protein, alkaloid, flavonoid and saponins
(Winkler et al., 2005). The volatile oil has been showed to contain 18.4 – 24% thymoquinone (2-isopropyl-5-methyl-1, 4-benzoquinone) and 46% monoterpenes such as p-cymene and &-piene (El-Tahair et al., 1993). The biological activity of the seeds has been showed to be due to thymoquinone, which is also present in the fixed oil (Ghosheh et al., 1999).

*N. sativa* has been known for it is hypotensive (El-Tahir et al., 1993), exhibited hepatoprotective effect against liver damage (Al-Gharably et al., 1997; Nagi et al., 1999; El-Dakhakhny et al., 2002), antimicrobial (El-Alfy et al., 1975), antibacterial (El-Kamali et al., 1998), antioxidant (Burits and Mucar, 2000), anti-inflammatory (Houghton et al., 1995) and antitumoral (Worthen et al., 1998). Besides, the essential oil was showed to have anthelmentic activity (Agarwal et al., 1979) and the seeds were effective against cestodes and nematodes (Akhtar and Rifaat, 1991).

*N. sativa* seeds have also been reported to have hypoglycemic activities (Al-Hader et al., 1993; El-Dakhakhny et al., 2002), which are principally related to the lipidic compounds of the seeds.

### 1.1.1 Scientific classification

- **Kingdom:** Plantae
- **Division:** Magnoliophyta
- **Class:** Magnoliopsida
- **Order:** Ranunculales
- **Family:** Ranunculaceae
- **Genus:** Nigella
- **Species:** *N. sativa*

Binomial name: *Nigella sativa* L.

### 1.1.2 Botanical description
*N. sativa* is an annual herb 35-50 cm tall, branching at the top, stems green, round, hairy, 2-5 mm diameter, internodes 2-5 cm long, flowers regular bisexual terminal on branches, white or greenish white about 3 cm diameter, long stalked, pedicels 1.5-5.5 cm long, becoming longer as the fruit matures (El-Dakhakhny *et al.*, 2000). *N. sativa* reproduces with itself and forms a fruit capsule which consists of many white trigonal seeds. Once the fruit capsule has matured, it opens up and the seeds contained within are exposed to the air, becoming black in color (Schleicher and Saleh, 1998).

### 1.1.3 Chemical composition

The multiple uses of *N. sativa* seeds in the folk medicine encouraged many researches to identification and isolation to the major constituent of it and to understand the pharmacological action. The seeds of *N. sativa* are the source of the active ingredients of this plant (thymoquinone). The oil and seed constituents have showed potential medicinal properties in traditional medicine (Salem, 2005). *N. Sativa* seeds contain 36%-38% fixed oils, proteins, alkaloids, saponins and 0.4%-2.5% essential oil (Lautenbacher, 1997).

The fixed oil is composed mainly of unsaturated fatty acids, including the unusual C20:2 arachidic and eicosadienoic acids (Houghton *et al.*, 1995). The essential oil was analyzed by Burits and Bucar (2000). Many components were characterized, but the major ones were thymoquinone (27.8%- 57.0%), ñ-cymene (7.1%-15.5%), carvacrol (5.8%-11.6%), t-anethole (0.25%-2.3%), 4-terpineol (2.0%-6.6%) and longifoline (1.0%-8.0%). Thymoquinone readily dimerizes to form dithymoquinone (El-Dakhakhny, 1965). Four alkaloids have been reported as constituents of *N. sativa* seeds. Two, nigellicine and nigellidine have an indazole nucleus, whereas nigellimine and its N-oxide are isoquinolines (Atta-ur-Rahman *et al.*, 1985; 1992; 1995).
In spite of there were no lead, cadmium and arsenic were found in the seeds. Zinc, calcium, magnesium, manganese and copper were found at lower level while potassium, phosphorus, sodium and iron are predominant elements. Linoleic and oleic acids were the major unsaturated fatty acids while palmitic acid was the main saturated one. Glutamic acid, arginine and aspartic acid were the main amino acids present while cystine and methionine were the minor amino acids (Al-Jassir, 1992).

1.1.4 Distribution

*N. sativa* is native to southwest Asia, also it grows in Mediterranean countries and it is also cultivated in the south of Algerian (Winkler *et al.*, 2005). It is also found in southern Europe and northern Africa.

1.1.5 Pharmacological properties

This plant has been a focus of much research, particularly during the past two decades. It has several traditional uses and consequently has been extensively studied for its chemical constituents and biological activities on various body systems in vivo or in vitro. A lot of animal studies have already been done to identify the various activities of *N. sativa* oil on different components of the metabolic syndrome, for example blood glucose (Bamosa, 1997).

The *N. sativa* seeds, seeds extract and thymoquinone have long been used in the Middle and Far East as a traditional medicine for a wide range of illnesses including bronchial asthma, headache, dysentery, infections, obesity, back pain, hypertension and gastrointestinal problems (Schleicher and Saleh, 1998; Al-Rowais, 2002). Until now it have antihypertensive (Rashid *et al.*, 1987), antibacterial (Hanafy and Hatem, 1991; Morsi, 2000), antiviral (Salem and Hossain, 2000), antidiabetic (Uddin *et al.*, 2002; Uddin *et al.*, 2005) and lipid lowering (Shaha *et al.*, 2004). Renoprotective (Ragheb *et al.*, 2009) potentialities have been obtained through research.
Various therapeutic effects, such as antiepileptic effects in children with refractory seizures (Akhondian et al., 2007), anti-inflammatory and analgesic actions (Khanna et al., 1993; Mutabagani and El-Mahdi, 1997; Abdel-Fattah et al., 2000) and antihistaminic (El-Dakhakhny et al., 2000; Kanter, 2006) have been described for *N. sativa*. Additionally, it has been shown that *N. sativa* has protective effect against ischemia reperfusion injury to liver and various organs (El-Abhar et al., 2003). Its use in skin condition as eczema has also been recognized worldwide (Goreja, 2003).

Besides these effects, *N. sativa* also demonstrates anti-parasitic effects. For instance, its administration decreases the number of eggs as well as worms in schistosomiasis, which tends to affect hepatic and intestinal tissues (El Shenawy et al., 2008). Intraperitoneal and oral administration of ethanol, chloroform and aqueous seed extracts of *N. sativa*, showed antimalarial activity against *Plasmodium berghei* in mice (Abdulelah and Zainal-Abidin, 2007). In fact, *N. sativa* attenuates the damage to β-cells of the pancreas following exposure to metal and toxic elements (Kanter et al., 2005). Similarly, *N. sativa* administration attenuates the ulcerative effects of ethanol on gastric mucosa by decreasing the glutathione-S transferase levels in gastric mucosa (Kanter et al., 2005). *N. sativa* oil was found to be effective as an add-on therapy in patients of insulin resistance syndrome (Najmi et al., 2008).

It has been reported that thymoquinone prevents oxidative injury in various in vitro and in vivo studies in rats (Daba and Abdel-Rahman, 1998; Mansour et al., 2001). It has been suggested that thymoquinone may act as an antioxidant agent and prevents membrane lipid peroxidation in tissues (Mansour et al., 2002). Moreover, it has been demonstrated that *N. sativa* can significantly prevent hepatotoxicity (Al-Gharably et al., 1997; Nagi et al., 1999) and might have protective effects against nephrotoxicity induced by either disease or chemicals (Abdel-Fattah et al., 2000).
The protection was suggested to be related to the ability of thymoquinone to inhibit lipid peroxidation. Also black seed oil was shown to be an effective adjuvant for the treatment of patients with allergic diseases (Kalus et al., 2003). In another clinical study, significant benefits of *N. sativa* extract in the treatment of acute tonsillopharyngitis was shown (Dirjomuljono et al., 2008). Furthermore, black seed preparations may have a cancer chemo preventive potential and may reduce the toxicity of standard antineoplastic drugs (Salomi et al., 1991). In fact, topical application of a black seed extract inhibited the two stage initiation-promotion of skin carcinogenesis in mice by croton oil (Salomi et al., 1991). *N. sativa* decreases DNA damage and thereby prevents initiation of carcinogenesis in colonic tissue secondary to exposure to toxic agents such as a zoxymethane (Al-Johar et al., 2008). In fact, sustained delivery of thymoquinone is almost as effective in causing apoptosis of colon cancer cells as sustained delivery of 5-fluorouracil (Norwood et al., 2007).

1.1.6 Toxicological properties
The seeds extract and its constituents appear to have a low level of toxicity. The administration of *N. sativa* seed extract (50 mg/kg) intraperitoneally to rats for 5 days did not significantly affect the activities of several enzymes and metabolites indicative of hepatic and renal function (El-Daly, 1998). Oral administration of the seed oil at dose up to 10 ml/kg in rats and mice did not cause any mortality or overt toxicity during the observation period of 2 day (Khanna et al., 1993). This was confirmed with a work of Zaoui et al. (2002) on the same dose for up to 12 weeks. It was shown that oral administration of *N. sativa* oil did not cause any mortality or significant alteration of the key hepatic enzymes in rats.

1.2 Effects of *Nigella sativa* on glucose metabolism

1.2.1 Hypoglycemic effect
Hypoglycemia is a syndrome characterized by a reduction in plasma glucose concentration to a level that may induce symptoms of low blood sugar. Hypoglycemia typically arises from abnormalities in the mechanisms involved in glucose homeostasis.

The effect of *N. sativa* on some of the complications of experimental diabetic rabbits has been investigated by a number of workers (e.g. Al-Hader *et al.*, 1993; El-Zawahrawy and Al-Zahraa, 1998; Meral *et al.*, 2001). Al-Hader *et al.* (1993) reported that intraperitoneal administration of the volatile oil of *N. sativa* seeds (50 mg/kg) significantly reduced the fasting blood glucose concentration in the normal and hypoglycemic rabbits. Insulin concentration was unaffected by the treatment.

In rats, an aqueous extract of the seeds of *N. sativa* was administered orally under light ether anesthesia for 7 and 14 days. Blood glucose, insulin level and key hepatic enzyme concentrations in serum a histopathological change in the liver in both treatment groups was done by El- Daly, 1994. A significant elevation of certain key hepatic enzymes and varying degrees of histopathological changes in the liver were documented following oral administration of boiling water extracts of seed of *N. sativa*. An increase in gamma-glutamyl transpeptidase in the absence of hepatocyte degeneration were observed following oral administration of *N. sativa* extract and this suggests that these enzymes may have been released due to hepatocellular damage caused at the molecular level.

Prolonged exposure (9 days) of beta-cell aggregates to 20 mmol/L glucose was used by Ling and Pipeleers 1996 to examine the effects of chronically elevated glucose levels on the survival and function of purified rat beta-cells. It did not lead to cell losses, but reduced the amount of insulin secreted in response to glucose. This decrease was not caused by cellular desensitization but resulted from the lower cellular insulin content after a prolonged imbalance between stimulated rates of insulin synthesis and release. It is concluded that chronic exposure of rat beta-
cells to elevated glucose levels induces a prolonged state of beta-cell activation and glucose hypersensitivity rather than glucotoxicity or glucose desensitization. Many studies have also examined the antidiabetic effects of *N. sativa* in normal and in diabetic animal models. Crud aqueous extract of *N. sativa* restore glucose homeostasis (Labhal *et al*., 1997). *N. sativa* was also shown to enhance liver cell insulin sensitivity (Le *et al*., 2004). The effect of different *N. sativa* seed extract (acidic, neutral and basic) on insulin secretion was studied by Rchid *et al*. (2004). The insulin secretory effects of these extracts were evaluated individually at concentrations 0.01, 0.1, 1 and 5 mg/ml, in vitro in isolated rat pancreatic islets in the presence of 8.3 mmol/l glucose. It was postulated that the antidiabetic properties of *N. sativa* seeds may be, at least partly, mediated by stimulated insulin release, and that the basic sub fraction largely contributes to this stimulatory effect (Rchid *et al*., 2004).

Study on protective effects of the volatile oil of *N. sativa* seeds on insulin immunoreactivity and ultra structural changes of pancreatic b-cells in STZ-induced diabetic rats was done by Mehmet *et al*. (2010). It is suggest that *N. sativa* treatment exerts a therapeutic protective effect in diabetes by decreasing morphological changes and preserving pancreatic b-cell integrity. Consequently, *N. sativa* may be clinically useful for protecting b-cells against oxidative stress.

Abdelmeguid *et al*. (2010) investigate the effect of *N. sativa* extract, oil, and thymoquinone on serum insulin and glucose concentration in streptozotocin diabetic rats. Which have been significantly decreased the elevated serum glucose level and significantly increased the serum insulin levels. In addition, the hypoglycemic effect observed could be due to amelioration of beta cells thus leading to the increased insulin levels.

**1.2.2 Effects of *N. sativa* seeds on Oral Glucose Tolerance Test**
The oral glucose tolerance test (OGTT) measures the body's ability to use a type of sugar, called glucose, which is the body's main source of energy. OGTT, a test of immense value and sentiment, in favor of using fasting plasma glucose concentration alone was seen as a practical attempt to simplify and facilitate the diagnosis of diabetes.

*N. sativa* have gotten a great therapeutic benefit in diabetic individuals and those with glucose intolerance, as it accentuates glucose-induced secretion of insulin, besides having a negative impact on glucose absorption from the intestinal mucosa (Rchid *et al.*, 2004; Meddah *et al.*, 2009). However, to date, little attention has been paid to the effects of *N. sativa* on intestinal glucose absorption. One of this was done by Meddah *et al.*, 2009 on the effects of the crude aqueous extract of *N. sativa* seeds on intestinal glucose absorption in vitro using a short-circuit current technique and in vivo using an oral glucose tolerance test. It showed directly inhibits the electrogenic intestinal absorption of glucose in vitro. On the other hands there was improvement of glucose tolerance and body weight in rats after chronic oral administration in vivo.

### 1.2.3 Effects of *N. sativa* seeds on glucose-6-phosphate dehydrogenase activity

G6PDH is the key rate-limiting enzyme of the pentose phosphate pathway in glucose metabolism. One of the important roles of this pathway is the generation of NADPH in the cytoplasmic fraction of the cell. The reducing power of NADPH is utilized for various synthetic processes, especially for synthesis of fatty acids. Thus G6PDH participates in the regulation of both lipogenesis and glucose metabolism (Kelley and Kletzien, 1984).

Chandrasekaran and Leelavinothan (2009) reported that an oral administration of Thymoquinone (major constituent of *N. sativa* seeds) for 6 weeks, dose dependently improved the glycemic status in streptozocin-nicotinamide induced diabetic rats. The levels of insulin, Hb increased with significant decrease in
glucose and HbA1c levels. The altered activities of carbohydrate metabolic enzymes were restored to near normal. In diabetic state the activities of hexokinase and glucose-6-phosphate dehydrogenase are decreased as a result of total absence or insufficiency of insulin. No significant changes were noticed in normal rats treated with thymoquinone. These result show that thymoquinone at 80 mg/kg is associated with beneficial changes in hepatic enzyme activities and there by exert potential antihyperglycemic effects.

1.3 Effects of *N. sativa* seeds on Hemoglobin level

The effect of *N. sativa* seeds fixed oil in rats has been investigated by Zaoui *et al.* (2002) by monitoring blood homeostasis and body weight as well as toxicity. Daily treatment by an oral dose of 1ml/kg body weight of *N. sativa* seeds fixed oil for 12 weeks. Significantly increased level of Hematocrit and Hemoglobin was found by 6.4% and 17.4% respectively.

Treatment of rats with the seed extract for up to 12 weeks has been reported to induce changes in the hemogram that include an increase in both the packed cell volume (PCV) and hemoglobin (Hb), and a decrease in plasma concentrations of cholesterol, triglycerides and glucose (Ali and Gerald, 2003).

1.4 The effect of *N. Sativa* seeds on serum Alanine transaminase (ALT)

The Alanine transaminase (ALT) enzyme is found principally in the liver with only small amounts being present in other organs. When there is liver cell damage, the serum or plasma levels of the enzyme are raised (Cheesbrough, 1989). Al-Jishi and Abuo Hozaifa, (2003), investigated the effects of *N. sativa* powdered seeds on blood coagulation and some liver function tests in male rats. The result showed an increase in ALT activity. In other study an aqueous extract of the *N. sativa* seeds was administered orally for 7 and 14 days. The result showed a significant increase in serum ALT concentration (El-Daly, 1994). Also serum ALT concentration was
significantly increased when aqueous extracts of the seeds of *N. sativa* were administered orally to male rats (Tennekoon, *et al*., 1991).

1.5 **The effects of *N. sativa* seeds on body weight**

The animal body weight is an important indicator of general health. *N. sativa* diets did not adversely affect growth (Al-Homidan *et al*., 2002). Another three studies with regard to the effect of *N. sativa* (10 and 20 g/kg) on growth were carried out with broilers over periods of 35 days. The body weight did not show any differences between control and *N. sativa* treated groups at the end of the experimental period (Brake, 2004).

1.6 **Glibenclamide**

Glibenclamide, also known as glyburide, is an anti-diabetic drug in a class of medications known as sulfonylureas. It is sold in doses of 1.25 mg, 2.5 mg and 5 mg, under the trade names Diabeta, Glynase and Micronase in the United States and Daonil, Semi-Daonil and Euglucon in the United Kingdom. It is also sold in combination with metformin under the trade name Glucovance.

1.6.1 **Uses of Glibenclamide**

It is used in the treatment of type II diabetes. Since 2007, it is one of only two oral anti-diabetics in the World Health Organization Model List of Essential Medicines. Sulfonylureas have been used for type 2 diabetes for over 50 year and are still the leading class of oral anti hyperglycemic agents. Their popularity is based on familiarity and habit, of course, but also their easy of administration (as once-daily tablets, in many cases), reliable effectiveness for recently diagnosed patients, lack of symptomatic side effects other than hypoglycemia, and low cost (Riddle, 2003).

1.6.2 **Mechanism of action**

The drug works by blocking ATP-sensitive potassium channels in pancreatic beta cells (Schmid-Antomarchi *et al*., 1987; Serrano-Martín *et al*., 2006). This inhibition causes cell membrane depolarization, which opens voltage-dependent calcium
channels, which causes an increase in intracellular calcium in the beta cell, leads to stimulates insulin release.

Six patients with type 2 diabetes underwent detailed metabolic studies before and after a minimum of 3 month's Glibenclamide therapy. Treatment was associated with a small but significant increase in body weight. Despite improvements in almost all the measured parameters of glucose homeostasis include plasma glucose, glycosylated hemoglobin (HbA1), hepatic glucose production and insulin-mediated glucose disposal (Baynes et al., 1993).
CHAPTER TWO
MATERIALS AND METHODS

2.1 Experimental details

These experiments were carried out to investigate the hypoglycemic effect of *Nigella sativa* seeds and how glucose metabolism is modulated in Wister albino female rats fed the black seeds.

2.1.1 Experimental animals

Thirty female Wister albino rats weighing 65g to 115g were used in this experimental study. They were randomly picked up from the experimental breeding station at Department of Preventive Medicine, Faculty of Veterinary Medicine. The rats were initially fed a standard basal laboratory rat diet for 7 days to be acclimatized to the laboratory environment. All animals were maintained under standard conditions (temperature 27-30°C, 12 h natural light and 12 h darkness); they were housed identically in stainless cages and were supplied with water and rodent chow ad libitum.

2.1.2 The basal rat diet

The rats were given a basal rat diet of the following composition:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat flour</td>
<td>690g</td>
</tr>
<tr>
<td>Meat powder</td>
<td>165g</td>
</tr>
<tr>
<td>Oil</td>
<td>120g</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>4g</td>
</tr>
</tbody>
</table>

15
Nigella sativa seeds were obtained from the South western Ethiopia. The viability of N. sativa seeds was confirmed by germination test according to Ista, (1976). The rats were divided into five groups. For each group the calculated treatment was mixed with 2g of the basal diet for each rat and fed to the animals first. Then the rest of the measured daily requirements for each rat, 6g from the basal rat diet, were supplied to the experimental animals.

2.1.3 Equipments

- Test tubes.
- Racks.
- Gloves.
- Capillary tubes.
- Pipettes.
- Tips.
- Centrifuge.
- Spectrophotometer.
- Florid Oxalate Blood Collection tube.
- EDTA Blood Collection tubes.
- Plane Blood Collection tubes.
- Eppendorf tubes.

2.2 Methods

2.2.1 Germination test

N. sativa seeds were obtained from Omdurman Market. The viability of N. sativa seeds was confirmed by germination test according to Ista (International Seed Testing Agency), 1976. Germination test was performed as follows:
1- 100 seeds were selected randomly and divided into four groups, 25 seeds in each one.

2- Each group of the seeds was placed in a dish containing a wet filter paper and incubated in incubator at 20°C for seven days.

3- On day four, the normal seedling that developed was count.

4- On day seven, the final count of the germination seeds was done.

5- All four set of germinated seeds should have a germination rate of at least 85%.

6- A germination rate of 85% is 85 normal seedlings of 100 seeds.

The seeds tested showed germination rate more than 85% and considered viable.

The seeds identification was done by Mr. Hamza Tag-Elsir Osman, Department of Botany and Agricultural Biotechnology, Faculty of Agriculture, University of Khartoum.

2.2.2 Experimental procedure

The Wister albino rats were divided into five groups A, B, C, D, and E, each containing six rats. They were fed 8g normal basal rat’s diet per day for each rat.

The control group (A) was fed normal basal rats diet, while the treated groups (B, C, D and E) were fed normal rat’s diet plus the following treatments calculated as part from the 8 g of the basal diet for each rat.

Group (B): received Glibenclamide (Produced by CP Pharmaceuticals Ltd Wrexham UK) 10mg/kg body weight.

Group (C): received *N. sativa* seeds 50mg/day for each rat (El-Daly, 1998).

Group (D): received *N. sativa* seeds 50mg/day for each rat + glucose 2g/kg body weight.

Group (E): received glucose 2g/kg body weight.

Blood samples were collected from at the zero, 7th, 14th, 21th and 28th days.
2.2.3 Blood samplings

After 1 h of feeding 2g of experimental diet mixed with treatment, at zero time then after 1, 2, 3 and 4 weeks after treatment, 1 ml of blood sample was collected from the orbital venous plexus by plain capillary tubes into fluorinated test tubes (to avoid glycolysis by RBCs) for blood glucose determination. Plasma separated spontaneously after centrifugation at 5000 rpm for 10 min, and then blood glucose concentration was measured. Also at 14th and 28th days of experiment, blood samples were collected in plain containers for insulin concentration. Serum was separated and stored in clean labeled aliquots at -20°C until analyzed. For Alanine aminotransferase (ALT) activity blood samples were collected at the 30th day of the experiment from the orbital plexus by plain capillary tubes in fluoride containers. Plasma was stored in clean labeled aliquots at -20°C until analyzed for ALT activity. At day 30, Blood samples were also collected in EDTA tubes for Hemoglobin determination and glucose-6-phosphate dehydrogenase activity (G6PD). Plasma was separated by centrifugation at 5000 rpm for 10 min for G6PD determination.

2.2.4 The body weight

Rats were weighed at the beginning and the end of the experiment.

2.2.5 Histopathological examinations

At the end of the experimental period, the rats were slaughtered and specimens of liver and pancreas were taken immediately and fixed in 10% formaline for histopathology.

2.3 Analytical methods

2.3.1 Determination of plasma glucose
Glucose concentration was determined according to the method described by Trinder, (1969) and Teuscher et al., (1971).

Principle

The oxidation of glucose is catalysed by glucose oxidase (GOD). The resultant hydrogen peroxide ($H_2O_2$) is oxidatively coupled with 4-aminophenazone and phenol in the presence of peroxidase (POD) to yield a red quinoneimine dye, the concentration of which at 546 nm is proportional to the concentration of glucose.

Alpha-D-glucose $\xrightarrow{\text{Mutarotase}}$ beta-D-glucose.

Beta-D-glucose + $H_2O + O_2$ $\xrightarrow{\text{GOD}}$ D-gluconic acid + $H_2O_2$

$H_2O_2 + 4$-aminophenazone + phenol $\xrightarrow{\text{POD}}$ quinoneimine + $4H_2O$

Reagent composition

1- Phosphate buffer (PH 7.5) 0.1mol/l
2- Aminoantipyrine 0.25mmol/l
3- Phenol 0.75mmol/l
4- Glucose oxidase 15KU/I
5- Peroxidase 1.5KU/I
6- Mutarotase 2KU/I
7- Glucose standard 100mg/dl or 5.5mmol/l

Procedure

1- Three test tubes were labeled as blank, standard and test.
2- 1 ml of working reagent was pipette into each one. 0.01 ml distilled water was pipetted into the blank tube, 0.01ml of glucose standard was pipetted into the standard tube, and 0.01ml plasma was pipetted into test tube.
3- Tubes were mixed and incubated for 10 min at 20 – 25°C.
4- 1ml content of each tube was delivered into cuvette and the absorbance of the sample and standard were read against the blank at 500 nm using spectrophotometer.

Calculations

\[
\text{Glucose (mg/dl)} = \frac{A_{\text{sample}}}{A_{\text{standard}}} \times \text{concentration of standard}
\]

2.3.2 Determination of glucose-6-phosphate dehydrogenase activity (G6PD)
The determination of G6PD activity was done according to the method of Rodak, (1995).

Principle
G6PD in the RBCs is released by a lysing agent present in the reagent. The G6PD released catalyzes the oxidation of glucose-6-phosphate with the reduction of NADP to NADPH. The rate of reduction of NADP to NADPH is measured as increase in absorbance, which is proportional to the G6PD activity in the sample.

\[
\text{Glucose-6-phosphate} + \text{NADP} \xrightarrow{\text{G6PD}} \text{Gluconate-6-phosphate} + \text{NADPH} + H^+
\]

Procedure
1- 1ml of G6PD working reagent was pipetted into cuvette.
2- 0.01ml of whole blood with EDTA was added. The cuvette content was mixed well and incubated for 5 min at 30°C.
3- 2ml of starter reagent was added, mixed well and incubated for another 5 min at 30°C.
4- The initial absorbance A was read and the absorbance reading was repeated after every 1, 2 and 3 min.

Calculations

\[
\triangle A/\text{min} = \frac{A_2 - A_1}{5}
\]
G6PD activity (U/g Hb) = \( \triangle A \times \frac{4778}{\text{Hb (g/dl)}} \)

2.3.3 Determination of insulin

The determination of serum insulin was done according to Midgley et al., (1969) using insulin Radioimmunoassay kit (RIA) IMK-414 (Beijing China, CIAE 1995).

**Principle**

The radioimmunoassay method in this test depends upon the competition between iodine-125 labeled insulin and insulin in the sample (or in standard) for the limited number of binding site on insulin specific antibody. After incubation for fixed time, separation of bound from free is achieved by the PEG-accelerated double-antibody procedure. The tube is then counted in a gamma counter, the counts being inversely related to the amount of insulin present in the sample. By measuring the proportion of iodine-125 labeled insulin bound in the presence of varying known amount of insulin standards, the concentration of insulin in known samples can be interplated.

**Assay protocol**

1- Assay tubes were labeled and arranged in the assay rack.
2- 100 µl, 200 µl aliquots of buffer were pipetted into insulin standard (0), NSB tube respectively.
3- 100 µl of insulin antibody solution were pipette into all tubes except total and NSB tubes.
4- 100 µl of iodine – 125 labeled insulin solutions were pipetted to all tubes.
5- The tubes were mixed thoroughly and incubated at 37°c for 2 hours, the total count tubes were been set aside at this stage.
6- 500 µl of separating agent solution were pipetted to all tubes except total count tubes. Then tubes were mixed thoroughly and placed at 37°c for 15 min.
7- All tubes were centrifuged at 1500xg for 15min except total count tubes, and the supernatants were decanted carefully.  
8- All tubes were counted in a gamma counter.

**Calculations**

\[ \text{NSB}\% = \frac{\text{NSB} - \text{Back grand cpm}}{\text{Total counts cpm} - \text{Back grand cpm}} \times 100 \]

\[ \text{Maximum binding B}\_o\% = \frac{\text{zero standard cpm} - \text{NSB cpm}}{\text{Total counts cpm} - \text{Back grand cpm}} \times 100 \]

\[ \text{B}\% = \frac{\text{standard (or sample) cpm} - \text{NSB cpm}}{\text{zero standard cpm} - \text{NSB cpm}} \times 100 \]

\[ \text{B}_{o} = \frac{\text{zero standard cpm} - \text{NSB cpm}}{\text{zero standard cpm} - \text{NSB cpm}} \]

**2.3.4 Determination of Alanine aminotransferase (ALT)**

The determination of serum ALT activity was done according to the method of Gella *et al.* (1985) and Young, (1997).

**Principle**

Alanine aminotransferase (ALT or GPT) catalyzes the transfer of the amino group from alanine to 2-oxoglutarate, forming pyruvate and glutamate. The catalytic concentration is determined from the rate of decrease of NADH, measured at 340 nm; by means of the lactate dehydrogenase (LDH) coupled reaction.

\[ \text{Alanine} + 2\text{-oxoglutarate} \xrightarrow{\text{ALT}} \text{pyruvate} + \text{Glutamate} \]
\[ \text{Pyruvate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{LDH}} \text{Lactate} + \text{NAD}^+ \]

**Reagents composition**

Reagent (A): Tris 150 mmol/l, L-alanine 750 mmol/l, Lactate dehydrogenase >1350U/L, PH 7.3.

Reagent (B): NADH 1.3 mmol/l, 2-oxoglutarate 75 mmol/l, sodium hydroxide 148 mmol/l, sodium azide 9.5 g/l.

**Procedure**
1- Working reagent and the instrument were brought to reaction temperature.
2- 1.0 ml of working reagent and 100 µl of sample were pipetted into a cuvette at 30°C, mixed and inserted into the photometer.
3- Initial absorbance was recorded after 1 min and absorbance was recorded at 1 min intervals thereafter for 3 min.
4- The difference between the consecutive absorbance and the average absorbance difference per min (A/min) were calculated.

**Calculations**

\[
\text{ALT concentration (U/L)} = \frac{\Delta A_{\text{min}} \times V_t \times 10^6}{E \times I \times V_s}
\]

Whereas:

\(V_t\): equal 1.1 at 30°C.

\(E\): the molar absorbance of NADH at 340 nm is 6300.

\(I\): 1 cm light bath.

\(V_s\): equal 0.1 at 30°C.

**2.3.5 Hemoglobin estimation**

Haemoglobin (Hb) concentration was determined by cyanmethaemoglobin method as describe by Van Kampen and Zijlstra (1961).

**Principle**

Ferrous ions of Hb are oxidized to the ferric state by potassium ferricyanide to form methemoglobin, which reacts with cyanide to form cyanmethemoglobin that can be measured colorimetrically.

**Reagents**

**Cyanide reagent (Drabkin’s solution)**
The reagent was prepared by dissolving 0.2g potassium cyanide, 0.05g of ferricyanide and 0.14g of potassium hydrogen diorthophosphate in 1 litre of distilled water.

**Standard Hb solution**

One ml of human Hb standard (Biosystem-Spain), with a concentration of 14.6 g/dl was used as standard.

**Procedure**

1- Dry clean test tubes were prepared for sample and standard.
2- To each tube, 4 ml of cyanide reagent were added. Then 0.02 ml of blood sample and Hb standard solution were added to the samples and standard tube, respectively.
3- The tubes were allowed to stand for 15 min, and then the optical density (O.D) was read at 540 nm in the colorimeter using cyanide reagent as blank.

**Calculation**

\[
\text{Hb concentration (g/dl)} = \frac{\text{O.D sample}}{\text{O.D standard}} \times 14.6
\]

**2.4 Oral glucose tolerance test (OGTT)**

Oral glucose tolerance test was performed according to the method of Du Vigneand and Karr, (1925).

Rats were fasted for 16 hour before being subjected to OGTT by intragastric gavages with glucose solution to achieve a glucose load of 2 g/kg body weight.

Blood samples were collected from the orbital venous plexus by plain capillary tubes at 0, 30, 60, 90 and 120 min and blood glucose was determined.
2.5 Histopathological methods
Rats were killed at the end of the experiment. The liver and pancreas were harvested, cut into a small pieces and were fixed in 10% formalin. They were embedded in paraffin and were cut into 5 µm thickness in microtome. These sections were collected in slides and then stained with Hematoxylin and Eosin before observation under light microscopy (Steward, 1960).

2.6 Statistical analysis
Data collected in various experiments were subjected to appropriate general linear model (GLM) procedure of the statistical analysis using the SAS package. The SAS was used to perform analysis of variance (ANOVA) and mean separations were performed using Ryan-Einot-Gabriel-Welsch Multiple F test (REGWQ) (Day and Quinn, 1989).
CHAPTER THREE
RESULTS

The present study was carried out to investigate the hypoglycemic effects of *Nigella sativa* seeds and to show how glucose metabolism is modulated in normal Wister albino female rats. The results were included estimation of serum glucose, serum insulin and Oral Glucose Tolerance Test. Activities of glucose-6-phosphate dehydrogenase and Alanine aminotransferase enzymes were assayed. Hemoglobin concentration and animal body weight were also measured. In addition to the histopathological changes of the liver and pancreas were examined.

3.1 The effects of feeding *N. sativa* seeds on blood glucose concentration

The effect of the treatments on glucose concentration is presented in table (1) and (2).

The Glibenclamide treated group (B) demonstrated a highly significant decrease (p<0.001) in the serum glucose level compared to the control group (A) at 7 and 21 days of the experiment. A significant (p<0.01) decrease was also seen at 14 and 28 days compared to the control group. The total mean of glucose level of the different days is significantly (p<0.001) decreased compared to the total mean of the control group. The *N. sativa* treated group (C) showed a significant (p<0.01) decrease in plasma glucose level compared to the control group at all intervals. The total mean of glucose of the different days showed a significant decrease in glucose level in group (C) compared to the control group. *N. sativa* + glucose treated group (D) revealed a significant decrease (p<0.001) in serum glucose level compared to the control group after 7 days of treatment, but no significant difference was observed after 14, 21 and 28 days of treatment compared to the control group. The total mean of glucose of the different days showed a significant decrease in glucose level in group (D) compared to the control group. On the other hand treatment with glucose
in group (E) showed a significant (p<0.001) decreased in serum glucose level after 7 days of treatment and showed no significant difference after 14 to 28 days of treatment compared to the control group. The total mean of glucose at different days showed no significant decrease in glucose level in group (E) compared to the control group (table 1 and 2).

Table (1): The changes in the serum glucose concentration (mg/dl) among the different groups of rats at different time intervals

<table>
<thead>
<tr>
<th>Group Time</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
<th>Group E</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 day</td>
<td>67.750±4.49a</td>
<td>65.340±7.61a</td>
<td>52.350±6.16a</td>
<td>57.950±5.09a</td>
<td>67.983±2.00a</td>
</tr>
<tr>
<td>7 days</td>
<td>93.940±3.28a</td>
<td>26.367±4.81c</td>
<td>50.250±9.50b</td>
<td>50.880±3.98b</td>
<td>51.975±3.03b</td>
</tr>
<tr>
<td>14 days</td>
<td>47.740±4.53b</td>
<td>21.950±2.75b</td>
<td>26.050±3.68b</td>
<td>48.325±5.49a</td>
<td>48.767±8.12a</td>
</tr>
<tr>
<td>21 days</td>
<td>73.425±6.05a</td>
<td>21.220±3.51c</td>
<td>47.080±7.08b</td>
<td>67.420±3.46a</td>
<td>81.800±4.38a</td>
</tr>
<tr>
<td>28 days</td>
<td>35.675±2.77a</td>
<td>20.340±1.96c</td>
<td>27.300±3.47b</td>
<td>33.920±4.22a</td>
<td>34.175±2.94a</td>
</tr>
</tbody>
</table>

Mean ± SE within the same row with different superscript small letters are significantly different at (p<0.01) based on ANOVA.

Group A: control group (received normal diet).
Group B: Glibenclamide treated group.
Group C: *N. sativa* treated group.
Group D: *N. sativa* + glucose treated group.
Group E: glucose treated group.
3.2 Blood insulin level

The effect of the four treatments on the level of the insulin is presented in table (2) and fig. (1).

There is no significant different in the level of serum insulin in groups B, C, D and E after 14 days treatment compared to the control group A. whereas, there is a significant (p<0.01) increase in the level of serum insulin in groups B, C, D and E after 28 days treatment compared to the control group A. In groups B, C and E there is a significant increase in insulin level after 28 days compared to the level after 14 days of treatment. There is insignificant increase in insulin level at 28 days of treatment in group D compared to day 14.

Table (2): The changes in the serum insulin concentration (µIU/ml) in the treated and control groups of rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Time</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
<th>Group E</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>5.875±0.66&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.775±1.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.520±0.82&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.525±1.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.725±0.84&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>14 days</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>28 days</td>
<td>5.833±1.97&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.460±1.24&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>10.480±1.47&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>10.660±1.74&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>15.700±.43&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Mean ± SE within the same row with different superscript small letters are significantly different at (p<0.01) based on ANOVA.

Group A: control group (received normal diet).
Group B: Glibenclamide treated group.
Group C: *N. sativa* treated group.
Group D: *N. sativa* + glucose treated group.
Group E: glucose treated group.
Fig. (1): The effect of *N. sativa* seeds on serum insulin level (µIU/ml) day 14\(^{th}\) and 28\(^{th}\)

Bars having different small superscript letters are significantly different at (p<0.01) based on ANOVA.

Group A: control group (received normal diet).

Group B: Glibenclamide treated group.

Group C: *N. sativa* treated group.

Group D: *N. sativa* + glucose treated group.

Group E: glucose treated group.
3.3 Oral glucose tolerance test (OGTT)

Results of OGTT carried out for the control and different experimental groups after the end of the experimental period are showed in fig. (2).

After the application of the oral dose of glucose in group (A) the control the blood glucose increased significantly (p<0.05) after 30 min but for groups (D and E) the significant increase (P<0.05) was seen after 60 min (with very high score in group E) and for group (B and C) was seen after 90 min. The blood glucose level returned to the fasting levels after 2 h for all groups except groups (B and C) where it was still significantly (P<0.05) higher than the fasting level.

**Fig. (2): The effect of *N. sativa* seeds on oral glucose tolerance test**

Values are mean ± SE of each group. (p<0.05) based on ANOVA.

Group A: control group (received normal diet).

Group B: Glibenclamide treated group.

Group C: *N. sativa* treated group.

Group D: *N. sativa* + glucose treated group.

Group E: glucose treated group.
3.4 Glucose-6-phosphate dehydrogenase activity (G6PD)

The effect of feeding *N. sativa* to normal albino rats for four weeks is presented in table (4). The activity of G6PD showed no significant change in groups B, C, D and E. only numerically lower levels were observed in groups B and C and higher levels were observed in groups D and E compared to the control group (A) after 28 days of the experiment.

3.5 Alanine aminotransferase activity (ALT)

The levels of ALT activity in the different treated groups are presented in table (4). The serum ALT activity at the end of the experiment is significantly (p<0.001) decreased in group B compared to the control group (A). Whereas a significant increase in serum ALT activity was seen in group C compared to the control group. ALT activity is not significantly different in group D compared to the control group. In addition, there is a significant (p<0.001) decrease in ALT activity in group E compared to the control group (fig.3)
Fig. (3): The effects of *N. sativa* seeds on Alanine aminotransferase activity (U/L) in the different treated groups of rats

Bars having different small superscript letters are significantly different at (p<0.001) based on ANOVA.

Group A: control group (received normal diet).
Group B: Glibenclamide treated group.
Group C: *N. sativa* treated group.
Group D: *N. sativa* + glucose treated group.
Group E: glucose treated group.

3.6 The effects of *N. sativa* seeds on Hemoglobin concentration

The levels of Hemoglobin concentration in the different groups is presented in table (2).

There is no significantly different between group B, C, D, and E and control group (A) in hemoglobin concentration after 28 days of the experiments.
3.7 The effects of *N. sativa* seeds on body weight

Changes in body weight in the different groups of rats are presented in table (3) and (4).

There is no significant different between group B, C, D, and E and control group (A) with respect to body weight at 0 and 30 days of the experiment.

**Table (3): The effects of seeds on body weight (g)**

<table>
<thead>
<tr>
<th>Time</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
<th>Group E</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 day</td>
<td>93.4±4.86 a</td>
<td>94.2±5.49 a</td>
<td>92.5±4.83 a</td>
<td>89.0±6.56 a</td>
<td>95.3±5.75 a</td>
</tr>
<tr>
<td>30 days</td>
<td>106.2±4.04 a</td>
<td>101.8±7.37 a</td>
<td>103.5±5.86 a</td>
<td>107.4±7.61 a</td>
<td>116.3±4.21 a</td>
</tr>
</tbody>
</table>

Mean ± SE within the same row with different superscript small letters are significantly different at (p<0.05) based on ANOVA.

Group A: control group (received normal diet).

Group B: Glibenclamide treated group.

Group C: *N. sativa* treated group.

Group D: *N. sativa* + glucose treated group.

Group E: glucose treated group.
Table (4): Biochemical and Hemoglobin concentration changes in rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
<th>Group E</th>
</tr>
</thead>
<tbody>
<tr>
<td>G6PD (U/g Hb)</td>
<td></td>
<td>9.88±1.34&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.40±1.71&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.28±0.93&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.44±2.61&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.40±3.54&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td></td>
<td>41.33±8.27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23.83±1.47&lt;sup&gt;b&lt;/sup&gt;</td>
<td>53.83±2.33&lt;sup&gt;c&lt;/sup&gt;</td>
<td>38.70±2.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.80±1.82&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>TMG (mg/dl)</td>
<td></td>
<td>63.89±4.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>30.53±4.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>41.30±3.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>52.08±2.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>57.21±3.9&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>TMI (µIU/ml)</td>
<td></td>
<td>5.85±0.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.38±1.4&lt;sup&gt;a&lt;/sup&gt;&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.0±1.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.27±1.2&lt;sup&gt;a&lt;/sup&gt;&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.71±1.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td></td>
<td>11.92±0.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.80±0.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.43±1.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.78±1.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.50±1.05&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>TMBW (g)</td>
<td></td>
<td>99.80±3.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>98.0±4.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>98.0±3.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>98.20±5.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>105.75±5.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Mean ± SE within the same row with different superscript small letters are significantly different at (p<0.05) based on ANOVA.

Group A: control group (received normal diet).
Group B: Glibenclamide treated group.
Group C: *N. sativa* treated group.
Group D: *N. sativa* + glucose treated group.
Group E: glucose treated group.

**Abbreviations:** G6PD, glucose-6-phosphate dehydrogenase; ALT, alanine aminotransferase; TMG, total mean of glucose; TMI, total mean of insulin; Hb, hemoglobin; TMBW, total mean of body weight.
3.8 Histopathological findings

The histological evaluation of pancreas in group B, C, D and E revealed normal and healthy microscopic images fully comparable to control group A. The histology of liver was normal in group C and D compared to the control group A (fig. 4, 6 and 7). There is a focal infiltration of mononuclear cells especially around blood vessels in group B (fig. 5). There is a focal infiltration of mononuclear cells especially around blood vessels, some hepatocytes exhibit fatty degeneration and focal areas of necrosis in glucose treated group E (fig. 8).
Fig. (4): liver from rats received normal basal rat’s diet. Notice no changes. (H and E X 400).
Fig. (5): liver from rats received Glibenclamide (10mg/kg body weight) on feed. Notice a focal infiltration of mononuclear cells especially around vessels. (H and E X 400).
Fig. (6): liver from rats received *N. sativa* (50mg/day) on feed. Notice no changes. (H and E X 400).
Fig. (7): liver from rats received *N. sativa* (50mg/day) +glucose (2g/kg body weight) on feed. Notice no changes. (H and E X 400).
Fig. (8): liver from rats received 2g/kg body weight glucose on feed. Notice that some hepatocytes exhibit fatty degeneration and focal areas of necrosis. (H and E X 400).
CHAPTER FOUR
DISCUSSION

*Nigella sativa* (*N. sativa*) is widely traditionally used as hypoglycemic plant in the Middle East, Northern Africa and Asia for treatment of various diseases. Many studies proved that *N. sativa* has hypoglycemic effects (Uddin *et al*., 2002; Uddin *et al*., 2005). The present study deals with its hypoglycemic effect and how this can modulate glucose metabolism. Wister albino female rats were used as experimental animals.

Normal glucose homeostasis represents the balance between intake (glucose absorption from the gut), tissue utilization (glycolysis, pentose phosphate pathway, tricarboxylic acid cycle, glycogen synthesis) and endogenous production (glycogenolysis and gluconeogenesis) (Meyer *et al*., 2002). By this way, the body tries to keep a constant glucose supply for cells by maintaining a constant blood glucose level.

4.1 The effects of feeding *N. sativa* seeds on serum blood glucose concentration

Glucose is the most important carbohydrate in mammalian biochemistry because nearly all carbohydrates in food are converted to glucose for further metabolism. Blood glucose is derived from the diet, gluconeogenesis and from glycogenolysis.

In the present study blood glucose levels were decreased significantly in group treated with Glibenclamide treated group B, *N. sativa* treated group C and *N. sativa* + glucose treated group D compared to the control group A. There was insignificant decreased in blood glucose level in glucose treated group E.

Similar observations were obtained by Al-Awadi *et al*. (1985), who reported that plant mixture extract comprising of *N. sativa*, Myrrh, *Gum olibanum*, *Gum asafetida* have a blood glucose lowering effect. It stated that the *N. sativa* induced increases in serum insulin concentration in rats which was accompanied by a
decrease in blood glucose level (Al-Awadi et al., 1985). Also Zaoui et al. (2002) suggested that the daily gavages of hexane extract of *N. sativa* seeds in Wister-kyoto rats for 12 weeks had reduced blood glucose level. Also *N. sativa* was studied by Alhader et al. (1993) and Sinada, (2007) showed a significant hypoglycemic effect.

The results obtained in the present study were also agree with El-Daly, (1994) who indicated that blood glucose level significantly decreases in a group treated with *N. sativa* for 7 and 14 days. Also significant increases in serum insulin level were observed after treatment of *N. sativa* and there was significant decrease in blood glucose level in a study by Farah, (2002). Other data showed that the hypoglycemic effect of *N. sativa* occurred by oral treatment (20 mg/kg) *N. sativa* resulted, at least partly, from stimulatory effect on B-cell function with consequent increase in serum insulin level. Similarly (Eskander et al., 1995) and (El-shabrawy and Nada, 1996) were reported the hypoglycemic effect of *N. sativa* in combination with other herbs in alloxan-induced diabetic rats.

*N. sativa* seeds are known to reduce blood glucose level in different animal species (Haddad et al., 2003). These results were in line with the Kamal, (2006), who reported that oral administration of water extract, *N. sativa* (30 ml/kg body weight) significantly decreased serum glucose level in diabetic rats from 19.8 mmol to 9.7 mmol and increased serum insulin level from 0.5 mIU/l to 0.7 mIU/l. It is also agree with Abdelmeguid *et al.* (2010), as they reported that *N. sativa* extract, oil and thymoquinone significantly decreased the elevated serum glucose level and significantly increased the serum insulin levels in streptozotocain induced diabetic rats. He suggested that the hypoglycemic effect observed could be due to amelioration of B-cells thus leading to the increased insulin level.

The mechanism of glucose reduction was reported by Kamal, (2006) who suggested that *N. sativa* seeds induce preserving pancreatic B-cell integrity, thus
attributing to increased glucose metabolism, by increasing the serum insulin. On the other hand, Farah et al. (2004) suggested that the reduction of blood glucose from 391 mg/dl before treatment to 197 mg/dl after treatment in hamster is due significantly to the low hepatic glucose production from gluconeogenic precursors.

4.2 The effects of feeding *N. sativa* seeds on insulin level

Insulin is a hormone that affects the carbohydrates metabolism by enhancing the rate of glucose metabolism, decreasing blood glucose concentration and increasing glycogen storage in tissues.

In the present study, there was no significant difference in the insulin level in Glibenclamide treated group B, *N. sativa* treated group C, *N. sativa* + glucose treated group D and glucose treated group E compared to control group A at day 14th of the experiment. Whereas, there were a significant (p<0.01) increased in insulin level in group B, C, D and E after 28 days of the experiment compared to the control group. In group B, C and E there is a significant increase in insulin level after 28 days compared to 14 days of treatment. Whoever, there is insignificant increase in insulin level at 28 days of treatment in group D compared to day 14.

The findings could agree with Al-Daly, (1994), who reported significant increase in serum insulin level after administrating aqueous extract of *N. sativa* seeds orally under light ether anesthesia for 7 and 14 days to rats. Similarly, Halit et al. (2002) reported that *N. sativa* treatment had increased the lowered insulin levels in rats. Moreover, Rchid et al. (2005) and Sinada, (2007) reported that using of different doses of *N. sativa* had caused significant increase in serum insulin levels.

Gyton, (1966), presented the feedback mechanism between glucose and insulin, when glucose concentration becomes elevated, the excess glucose acts directly on the islets of langerhans to increase their secretion of insulin. Almost immediately, more insulin become available to cause glucose transport into most cells of the body, and this reduces the blood glucose level back towards normal. Goodner and
Porte (1972) reported that when beta cells are stimulated the insulin is discharged; this is then followed by a refractory period in which responses to other wise potent stimuli then promotes a second sustained secretion.

The increase in serum insulin level was observed by Farah, (2002), who suggested that, the increase in serum insulin levels resulted from stimulatory effect of \textit{N. sativa} on B- cell function, which indicated that \textit{N. sativa} has insulinotropic properties. In contrast, Al-Hader \textit{et al.} (1993) reported that the volatile oil of \textit{N. sativa} had produced a significant hypoglycemic effect on normal and alloxan-induced diabetic rabbits without changes in insulin levels.

4.3 \textbf{The effects of feeding \textit{N. sativa} seeds on oral glucose tolerance test}

After the oral dose of glucose in normal control group the blood glucose significantly (p<0.05) increase at 30 and 60 min and then decreased at 90 min and reached the fasting levels at 2 h. on the other hand blood glucose in Glibenclamide treated group a significant (p<0.05) increase after 30, 60 and 90 min and insignificant decreased after 2 h. Treatment with \textit{N. sativa} showed insignificant increase in serum glucose level after 30 min, a significant (p<0.05) increase in the serum glucose level after 60 min and showed a significant (p<0.05) decrease in the serum glucose level after 90 and 120 min of oral glucose dose to near normal. These results showed that similar behavior was seen in the groups that received Glibenclamide or only \textit{N. sativa}. Treatment with \textit{N. sativa} + glucose showed insignificant increase in serum glucose level after 30 min, a significant (p<0.05) increase in the serum glucose level after 60 and 90 min and showed a significant (p<0.05) decrease in the serum glucose level after 120 min of oral glucose dose. There is no significant different in glucose concentration after 120 min compared to 0 min. Treatment with glucose showed a significant (p<0.05) increase in serum glucose level after 30 and 60 min, a significant (p<0.05) decrease in the serum glucose level after 90 and 120 min of oral glucose dose.
These results therefore confirm the reduction of intestinal transport in vivo and are consistent with increased insulin sensitivity observed in previous studies (Le et al., 2004). They are also in agreement with results Al-Awadi et al., (1985) who obtained an improved OGTT response in normal and streptozotocin rats treated with a mixture of plants containing *N. sativa*. Indeed, in subsequent studies, the same group failed to observe an improvement of fasting blood glucose or OGTT response with *N. sativa* alone administered again at 0.5 g/kg for 1 week (Al-Awadi and Gumaa, 1987).

Bouchra et al., 2008 reported that an OGTT challenges were carried out acutely at the beginning of the study, 2h after the first *N. sativa* gavages, and chronically at the end of 6-week treatment regimen. *N. sativa* was without significant effect after acute administration but reduced the overall OGTT response after chronic treatment.

### 4.4 The effects of feeding *N. sativa* seeds on glucose-6-phosphate dehydrogenase activity

Liver plays a central role in blood sugar homeostasis. In diabetic state the activities of hexokinase and glucose-6-phosphate dehydrogenase are decreased which due to the total absence or insufficiency of insulin (Chandrasekaran and Leelavinothan, 2009). Thymoquinone up regulates the activities of both these enzymes in hepatic tissues through insulin release and thereby it enhances the utilization of glucose for cellular biosynthesis, which is marked by the significant decrease in plasma glucose level (Chandrasekaran and Leelavinothan, 2009).

The activity of G6PD is insignificantly changed in group B, C, D and E compared to the control group (A) after 28 days of the experiment. The results were agree with Chandrasekaran and Leelavinothan, (2009) who reported that an oral administration of Thymoquinone (major constituent of *N. sativa* seeds) for 6 weeks,
did not affect the G6PD activity in normal rats, but it significantly decreased in diabetic rats.

4.5 The effects of feeding *N. sativa* seeds on Alanine transaminase (ALT) activity

ALT is a cytoplasmic enzyme found in very high concentration in the liver. The level of ALT in the blood is used as indicator for hepatocytes integrity; disrupt hepatic parenchyma cells, with necrosis or the altered membrane permeability, will lead to the leakage of the enzyme to the blood stream (Cornelius and Kaneko, 1986). In the present study the level of serum ALT showed a significant (p<0.001) decreased in Glibenclamide treated group and Glucose treated group compared to the control group. However, there is a significant (p<0.001) increase in serum ALT activity in *N. sativa* treated group compared to the control group. There is no significant change in ALT activity *N. sativa* + glucose treated group compared to the control group. The present study suggested that, 50 mg/day of *N. sativa* seeds for four weeks may slightly affect liver integrity.

The results were in line with Tennekoon, *et al.* (1991) who reported the increase in serum ALT in rats consuming aqueous extract of *N. sativa* (250g/1000ml distilled water) for consecutive 14 days when compared to the control group. Also Elhag, (1998) who reported that serum ALT activity is slightly increased in rabbits fed 10% *N. sativa* seeds for two weeks and maintained level for the rest of the experimental period. The oral administration of *N. sativa* led to marked and significant increases in ALT enzyme activity of serum after 7 and 14 days post-treatment (Al-Daly, 1994).

4.6 The effects of feeding *N. sativa* seeds on blood hemoglobin level

There is no significant (p<0.05) difference between groups B, C, D, and E and control group (A) in Hemoglobin concentration at 28 days of the experiments.
Similarly Chandrasekaran and Leelavinothan, (2009) reported that oral administration of thymoquinone in normal rat did not affect the hemoglobin level after 45 days of experiment compared to the control group.

4.7 The effects of feeding *N. sativa* seeds on body weight

In the present study initial and final body weight of experimental animals were measured. There is no significant different between all treated groups (B, C, D and E) compared to the control group with respect to the body weight at the end of the experimental period, only group E showed a higher body weight compared to other groups.

Similarly Brake, (2004), reported that administration of *N. sativa* seeds did not causes any differences between the control and treated animals. Also Alhomidan *et al.* (2002), reported that, feeding 20 and 100g/kg body weight of *N. sativa* seeds to 7day old broiler chicks did not affect growth. The results were in line with Chandrasekaran and Leelavinothan, (2009); they reported that oral administration of thymoquinone in normal rat did not affect the body weight after 45 days of experiment compared to the control group.

Similarly Sinada, (2007) has recently reported that the live weight gain in broiler chicks was not affected by crushed *N. sativa* seeds mixed with commercial ration compared to the control group. Zaoui *et al.* (2002) found that the fixed oil of *N. sativa* slow growth rate of normal rats.

4.8 Histopathological findings

Histopathological examination of pancreas showed no evidence of degeneration changes in the islets of langerhans in all treated groups for four weeks.

However, histopathological examination of livers showed no evidence of degenerative changes in hepatocytes in animals treated with *N. sativa* for four weeks and animals treated with *N. sativa* + glucose compared to the control group. There is a focal infiltration of mononuclear cells especially around blood vessels in
group B. There is a focal infiltration of mononuclear cells especially around blood vessels, some hepatocytes exhibited fatty degeneration and focal areas of necrosis in group E.

Similarly Al-Daly, 1994 reported that oral administration of *N. sativa* extract did not have a superimposing effect. Focal infiltration of mononuclear cells especially around blood vessels in group B it may be due to defensive role against degenerative changes in the hepatocytes. Arseculeratna *et al.*, (1981, 1985) have previously reported that some plant toxins may damage the hepatic cells at the molecular level without causing overt histopathological changes.

Bouchra *et al.*, 2008 reported that the histological study of kidney, pancreas and liver samples after chronic administration of *N. sativa* aqueous extract over 6 weeks showed these tissues to be normal and healthy. This is also consistent with the high safety margin observed in rats and mice with *N. sativa* fixed oil (Zaoui *et al.*, 2002).
CONCLUSIONS

In the present study it was found that daily oral administration of *Nigella sativa* seeds, supplemented to the basal rat diet, for four weeks resulted in a significant reduction in plasma glucose concentration and significant increase insulin level. Suggested that this seeds could be used as a hypoglycemic agent to reduced blood glucose level. There was no histopathological change in *N. sativa* treated group but there was a significant increase in ALT activity, it may be due to the hepatocytes damage at the molecular level. Also the live weight gain, G6PDH activity and hemoglobin concentration in treated groups were not affected by dietary treatment compared to the control group.

RECOMMENDATIONS

There are several lines of research arising from this work which should be pursued.

- All key enzymes in glucose metabolism should be studied.

  Enzymes of glycolysis eg. hexokinase or glucokinase, phosphofructokinase-1 and pyruvate kinase that cause decreases in blood glucose concentration.

  Glycogen synthase and glycogen phosphorylase enzymes that decreases and increases blood glucose level respectively.

  Gluconeogenesis pathway enzymes eg. glucose-6-phosphatase, fructose-1, 6-bisphosphatase, phosphoenol pyruvate carboxykinase and pyruvate carboxylase which increase blood glucose level.

- Results provide convincing evidence that intestinal glucose transport inhibition represents an important component through which *Nigella sativa*
seeds can reduce blood glucose in this context. Future studies will need to elucidate the active principles as well as molecular sites of action responsible for the effect of *Nigella sativa* seed on the intestinal absorption.
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