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# Hepatotoxicity of Methanol Seed Extract of *Aframomum melegueta* [Roscoe] K. Schum. (Grains of paradise) in Sprague-Dawley Rats

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**Abstract** The hepatotoxic effects of the seeds of *Aframomum melegueta* (Grains of paradise), a spice were studied in Sprague-Dawley rats. Individual rat groups received sub-chronic exposure of the methanol seed extract at 300 mg/kg for 7, 14 and 21 days respectively. Liver toxicity was evaluated with assay of circulating serum aspartate aminotransferase (AST), alanine aminotransaminase (ALT), alkaline phosphatase (ALP), albumin, total bilirubin concentrations and histopathology of the liver of treated experimental rats. Serum levels of AST significantly ($p<0.05$) increased progressively in extract-treated rats compared to the control from day 7 till the termination of the study (day 21). However, serum ALT, ALP and total bilirubin levels of test rats were only significantly ($p<0.05$) elevated relative to the normal on days 14 and 21 of the investigation. The dose (300 mg/kg) of extract produced AST value of 55.8±3 $\mu L^{-1}$ while the control was 32.2±1.9 $\mu L^{-1}$; and ALT value became 16.8±1.1 $\mu L^{-1}$ when control was 8.6±1.1 $\mu L^{-1}$ on day 21; Total bilirubin was 1.4±0.1 $mgdL^{-1}$ relative to control value of 0.5±0.2 $mgdL^{-1}$. The serum albumin levels of extract-treated rats were however, comparable with that of the normal rats throughout the study period. Histopathology of the rat livers revealed mild focal necrosis of hepatocytes at day 7, moderate multifocal areas of hepatic necrosis at day 14 and severe, diffused necrosis of hepatocytes at day 21 of treatment with the extract. The results demonstrated that the methanol seed extract of *A. melegueta* was potent in inducing liver toxicity at the tested dose (300 mg/kg). Maximal caution should therefore be imbibed in prolonged excessive use of the plant seeds as spice in delicacies.

***Keywords:*** *Aframomum melegueta seeds, Wistar rats, toxicity, liver enzymes, albumin, bilirubin*

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## 1. Introduction

Medicinal plants contain diverse bioactive metabolites with useful therapeutic benefits and as precursors for a wide range of drug design. Herbal medication has been a popular practice for treatment of diseases and ailments since the ancient times. The roots, leaves, bark and seeds of plants were the principal sources of drugs used by the primitive man in the treatment of diseases (Meyer, 1962). The seeds of *A. melegueta* were well recognized by traditionalists in Mbaitoli Local Government Area, Imo State, Nigeria for healing effects in various health challenges including cough, stomach disorders, pile, abscesses, dysentery, rheumatism, fever, toothache and others. The seeds were reportedly valuable as remedy for stomach ache, snakebite, diarrhea, cardiovascular diseases, diabetes and inflammation (Ilic *et al.*, 2010; Akendengue and Louis, 1994). The seeds were also listed in Arab folk medicine with carminative properties in due to the ability to relieve hyperacidity and to increase appetite (Amal Abdulaziz, 2010).

*Aframomum melegueta* belongs to the ginger family (Zingiberaceae) and is commonly called Grains of paradise, Malagueta (chilli pepper), Guinea pepper, Alligator pepper, Guinea grains, and Negro pepper (Beichner, 1961). It is variously known locally as *ose oji, ataare and cittáá* in Nigeria, Fam-wisa, wisa in Ghana and *nengrekondre pepre* in Liberia (Odugbemi, 2008; Daniel, 2004). The plant is a perennial deciduous herb native to the tropics and grows at the swampy habitats of the West African coast. It has leafy stem that may be up to 1.5 m high. The leaves are simple, alternate and lanceolate with matured ones measuring as long as 40 cm in length and 12-15 cm wide. It produces trumpet-shaped, purple colored flowers which develop into 5 to 7 long pods and each containing as many as 300 reddish-brown seeds

(Dalziel, 1937). The seed of *A. melegueta* is used in different cultures. A small amount is given to an immediate new born baby to taste, serving as a welcome expression; the seeds are also offered as traditional wedding gift in *Yoruba* culture, Western Nigeria (Odugbemi, 2008). Alligator pepper is chewed with kolanut where the hot spicy taste reduces the bitter taste from kola nut in *Ibo* culture, Eastern Nigeria. It had been employed in divination and ordeal to determine the guilt among *Efik* people in Eastern Nigeria (Simons, 1956), religious (voodoo) rites in the Caribbean Islands (Voeks, 2013), to flavor alcoholic drinks (Harten, 1970) and as replacement for black pepper in European and American cuisines (Voeks, 2013).

The presence of the seeds of *A. melegueta* in the diet is considered to be responsible for the cardiovascular health of gorillas in the wild (Dybas and Ilya, 2007). The aqueous seed extract exhibited anti-inflammatory and peripheral analgesic activities (Umukoro and Ashorobi, 2001). It was used for medicinal purposes due to its anti-inflammatory and antimicrobial activities, to relieve dental pains, asthma and body weakness, enhance body activities and preservation of grains (Ukeh *et al.,* 2011). The sharp and peppery taste of the seeds is reportedly caused by the presence of aromatic ketones: 6-paradol, 6-gingerol and 6-shogaol as part of its content (Sugita *et al.,* 2013). Essential oils which are the dominating flavor components, is said to occur only in traces (Echo *et al.,* 2012).

The present study sought to evaluate the hepatotoxic effects of the methanol seed extract of *A. melegueta* seeds on Sprague-Dawley rats under prolonged exposure.

# 2. Materials and method

## 2.1. Collection of Plant Material and Extraction

Dried Alligator pepper (*A. melegueta*) fruits were purchased locally from *Ogige* market in Nsukka Local Government Area, Enugu State, Nigeria. The seeds (1800 g) were removed from their seats within the fruit, air dried and the dried seeds later powdered using an electric blender (Moulinex, China). The material was de-fatted with petroleum ether using a Soxhlet extractor at 40°C to remove impurities. The dried marc was further macerated with intermittent shaking in 80% methanol at room temperature for 48 h. It was filtered and the filtrate then dried in a rotary evaporator at 40°C.

## 2.2. Animals

Adult Sprague-Dawley rats (128-175 g) of both sexes obtained from the Laboratory Animal unit of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka (UNN) were used for the study. Animals were kept in stainless steel cages and had access to a predetermined mass of feed (Vital feed®, Nigeria Ltd.) but water was provided *ad libitum*. Animal room temperature and relative humidity were 19–22°C and 30–70%, respectively, and there was a 12 h light/dark cycle. The rats were allowed 14 days to acclimatize prior to commencement of experiments. The use of the animals conformed with internationally accepted standards for laboratory animal use and care as documented in the European Community guidelines, Council Directive, 1986 (86/609/EEC), revised in Directive 2010/63/EU. The experimental protocols were approved by the Ethics Committee for Animal Experimentation, UNN, in accordance with Nigerian Federal Government legislation on Animal care.

## 2.2. Acute Oral Toxicity Studies

Acute toxicity studies were conducted using the method described by Lorke (1983). Thirty (30) matured mice of both sexes were marked, weighed and randomly separated into 6 groups (A – F) of 5 mice each. Groups A–E were given varying oral doses (150, 300, 600, 1500 and 2000 mg/kg) of the methanol seed extract of *A. melegueta* respectively, while group F ($6^{th}$ group) received an equivalent volume (10 ml/kg) of distilled water. All treatments were given orally by gastric intubation. The mice were observed for signs suggestive of toxicity within 48 h and further monitored for two weeks for toxic effects.

## 2.3. Experimental Design

A total of 20 Sprague-Dawley rats were grouped into 4. Group 1 served as normal control and received only the vehicle (distilled water, 10 ml/kg) while Group 2, Group 3 and Group 4 received 300 mg/kg of the extract daily for 7, 14 and 21 days respectively. The dose selection was based on results of the preliminary studies and the planned human exposure in the potential nutritional supplement. The individual rat dose was calculated based on the most recent weekly body weight of each animal. Extract concentration was then adjusted each week to maintain the targeted dose level for each animal (i.e., 300 mg/kg/day) for the extract to be administered at a constant volume of 5 ml/kg. Each animal was drenched by oral gavage (using a stainless steel ball-tipped gavage needle attached to an appropriate syringe) daily at approximately the same time ± 5.0 min throughout the study period. The first day of administration was considered day 1 of the study.

## 2.4. Clinical Observations

All animals were observed twice daily for mortality. Cage-side observations were made daily during the study and any abnormal findings recorded. Detailed observations were recorded on day 1 (prior to administration of extract) and weekly thereafter on all animals. These observations were conducted both while handling the animal and with the animal placed in an open field. Observations included, but were not limited to: changes in skin, fur, eyes, and mucous membranes, occurrence of secretions and excretions and autonomic activity (e.g., lacrimation, piloerection, pupil size, unusual respiratory pattern). Changes in gait, posture and response to handling as well as the presence of colonic or tonic movements, stereotypes (e.g., excessive grooming, repetitive circling), or aberrant behavior (e.g., self-mutilation, walking backwards) were also recorded.

## 2.5. Body Weights and Feed Consumption

Individual body weights were recorded twice during the acclimation period, at study initiation (day 1), and weekly thereafter. Mean body weight gains were determined for each group at each interval and for the overall (days 7, 14 and 21) testing interval. The experimental rats were fed in

accordance with the normal feed consumption of rats at 10 g feed/100 g body weight/day (Hafez, 1970). The amount of feed consumed in each group was compared with that of the control. Animals were also weighed immediately prior to sacrifice (fasted body weight) for calculation of organ to body weight.

## 2.6. Biochemical Assay

At the end of the test period for each group, blood samples were collected through the ocular retrobulbar plexus of the rats into separate test tubes. Samples were allowed to clot at room temperature before they were centrifuged at 2,500 rpm for 10 min to separate the serum. Serum from individual animal was used for biochemical assays. The serum levels of ALT and AST were determined using the method of Reitman and Frankel (1957), ALP level by the method of King and King (1954), total bilirubin concentration by the method of Malloy *et al.* (1937) as modified by Tietz (1996) and serum albumin level by the method Kinsley and Frankel (1939).

## 2.7. Necropsy, Relative Organ Weights and Histopathology

### 2.7.1. Necropsy

At scheduled periods for each group on days 7, 14 and 21, rats were euthanized by exsanguination from the abdominal aorta under isoflurane anesthesia. Isoflurane (5–10 ml) was placed on gauze and administered to the rats enclosed in a bell jar. The animals were placed on the perforated surface above the gauze so they were never in contact with the liquid. The animals were monitored until anesthetized by checking for the absence of reflexes (pain response to pinch test) and that respiration was even and unlabored. Gross necropsy included an initial examination of external surfaces and orifices, as well as the cranial, thoracic and abdominal cavities and their contents. Rats were examined for gross lesions.

### 2.7.2. The relative organ Weights

The liver, kidneys, heart, spleen, and stomach of all sacrificed animals were weighed wet as soon as possible after dissection to avoid drying using a weighing balance (Metler, England). The relative organ weight of each rat was determined. Relative organ weight referred to the ratio of the weight of the organ to body weight.

### 2.7.3. Histopathology

Samples of the liver from the experimental animal groups were preserved in neutral buffered 10% formalin for minimum of 24 h. The tissues were dehydrated by washing in ascending grades of ethanol before clearing with xylene and embedding in paraffin wax. The samples were sectioned with a microtome, stained with Hematoxyline and Eosin (H and E) and mounted on Canada Balsalm. All sections were examined under light microscope (x10, x20 and x40) magnification. Photographs of the lesions were taken with an Olympus photo microscope for observation and documentation of histopathological lesions.

## 2.8. Statistical Analysis

All data collected were subjected to one-way analysis of variance (ANOVA) and Duncan New Multiple Range Test (DNMRT) as post hoc to separate the treatment means. Differences at $p<0.05$ were considered significant.

# 3. Results

## 3.1. Description of the Extract

The methanol extract of *A. melegueta* seeds was dark brown in color and with a peculiar fragrance. The extraction process gave a yield of 32.76% w/w.

## 3.2. Acute Toxicity Test

Acute toxicity studies revealed no extract-induced mortality or overt serious clinical manifestation even at the highest test dose of 2000 mg/kg. However, transient restlessness was observed in animals treated with the extract doses above 600 mg/kg.

## 3.3. Clinical Signs

There were no extract related mortalities during the prolonged (21 days) study period, however one female died on test day 6 as a result of an intubation error. Transient clinical signs, most prevalent on day 14 included soft fecal droppings (2/5 males; 1/5 females) and uro-genital staining (1/5 males). In addition, one female had ocular discharge on test day 21.

## 3.4. Body Weights and Feed Consumption

There was a significant ($p<0.05$) and time dependent decrease in the mean body weight of all rats that received the extract (300 mg/kg) relative to normal ones. The 12.5% lose in weight of the experimental rats on day 21 was most remarkable compared to 5.4 and 6.4% weight loses on days 7 and 14 respectively (Table 1). The mean daily feed consumption of the test rats was however, comparable with that of the control value within the overall duration of study (21 days).

**Table 1. Effects of *A. melegueta* methanol seed extract (300 mg/kg) on rat body weight**

| Mean weights (g) | | | |
|---|---|---|---|
| Duration | Initial | Final | Mean difference (± S.E.) |
| Normal (control) | 180.2 | 188.6 | 8.4±1.6 (4.7%) + |
| 7 days | 218.6 | 206.9 | 11.7±1.2 (5.4%)-* |
| 14 days | 198.9 | 186.2 | 12.7± 0.8 (6.4%)-* |
| 21 days | 208.2 | 182.2 | 26.0±2.2 (12.5%)-* |

[+] Weight gain compared to test groups; [-]*Significant weight lose compared to control value at $p<0.05$.

## 3.5. Biochemical Analyses

The administration of the extract (300 mg/kg) caused elevation in AST, ALT, ALP and total bilirubin levels of test rats. The observed increases in the rat serum AST were progressive and significant ($p<0.05$) right from day 7 till the end of the study at day 21 but ALT, ALP and total bilirubin values became only significantly ($p<0.05$)

increased compared to the normal from day 14 to the termination of the investigation. There were no significant (p>0.05) variations in the serum albumin concentration of rats that were treated with the extract relative to the control (Table 2).

**Table 2. Serum enzymes, albumin and total bilirubin of rats under sub-chronic treatment with *A. melegueta* seed extract (300 mg/kg)**

| Duration | AST (µL-1) | ALT (µL-1) | ALP (µL-1) | albumin (mgdL-1) | Total bilirubin (mgdL-1) |
|---|---|---|---|---|---|
| Normal (control) | 32.2±1.9 | 8.6±1.1 | 25.2±1.7 | 0.8±0.2 | 0.5±0.2 |
| 7 days | 47.6±2.5* | 12.4±1.0 | 29.0±1.1 | 0.7± 0.1 | 0.7±0.1 |
| 14 days | 54.4±1.7* | 13.2±1.2* | 34.3±1.2* | 0.6±0.3 | 1.1± 0.2* |
| 21 days | 55.8±3.0 * | 16.8±1.1* | 37.5±1.4* | 0.5± 0.2 | 1.4±0.1* |

*Significant at p<0.05; AST= aspartate aminotransferase; ALT= alanine aminotransaminase; ALP= alkaline phosphatase

## 3.6. Necropsy

No gross abnormalities of toxicological significance were noted for any of the euthanized animals during necropsies at the conclusion of the 21-day observation period. The organs of the experimental animals that received the extract could not easily be differentiated visibly for any lesion deviating from those of the control appearance.

## 3.7. Effect of the Extract on Rat Body Weight and Mean Relative Organ Weights

The methanol extract of *A. meleguata* (300 mg/kg) induced no observable changes in the absolute and relative weight of the organs (spleen, kidney, heart and stomach) of test rats that contrasted the normal with the exception of the liver. There were significant (p<0.05) progressive decreases in the mean relative liver weights of extract-treated rats compared to control on days 14 and 21 of the study. The mean relative liver weight of control rats was 25.3 $x10^{-3}$ g ±0.9 but that of test rats on days 14 and 21 were 20.01 $x10^{-3}$ g ±0.4 and 1.8±0.5 $x10^{-3}$ g respectively (Table 3).

**Table 3. Effects of *Aframomum melegueta* methanol seed extract on relative organ weights**

| Weights x10 -3 g (mean ± S.E.) | | | | | |
|---|---|---|---|---|---|
| Duration | Liver | Spleen | Kidney | Heart | Stomach |
| Normal (control) | 25.3±0.9 | 5.9±0.1 | 5.5±0.6 | 4.1± 0.2 | 6.4±0.2 |
| 7 days | 24.7±0.6 | 4.6±0.3 | 5.5±0.2 | 3.7± 0.2 | 7.2±0.1 |
| 14 days | 20.0±0.4* | 6.2±0.2 | 4.3±0.3 | 3.9± 0.5 | 0.85±0.2 |
| 21 days | 1.8±0.5* | 4.8±0.4 | 4.8±0.7 | 4.0 ± 0.2 | 0.53±0.1 |

*significant difference between each group and normal control at p<0.05.

## 3.8. Histopathology of the Experimental Rat Livers

Histopathological study of the livers of rats treated with the methanol seed extract of *A. melegueta* at 300 mg/kg for 7 days (group I), 14 days (group II) and 21 days (group III) was carried out.

Group I (Rats treated with the methanol seed extract (300 mg/kg) for 7 days): The liver of the rats had mild focal necrosis of hepatocytes (Plate I).

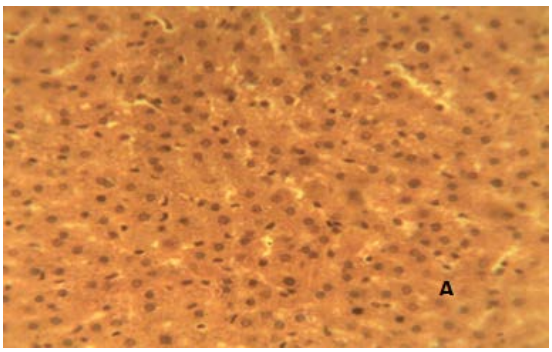


**Plate I.** Section of the liver of a rat treated with methanol seed extract (300 mg/kg) for 7 days. Note the mild focal necrosis of the hepatocytes (A). H and E stain. x400

Group II (Rats treated with the methanol seed extract (300 mg/kg) for 14 days): The liver had moderate multifocal areas of necrosis of the hepatocytes (Plate II).

Group III (Rats treated with the methanol seed extract (300 mg/kg) for 21 days): The rat liver was congested and had severe, diffused necrosis of the hepatocytes (Plate III).

Group IV (Normal rats): The micrograph of the liver showed no damage to liver cells. Hepatocytes were seen arranged in trabeculae separated by sinusoids containing Kupffer cells. Hepatocytes were regular and contained spheroidal nuclei with distinctly marked nucleoli (Plate IV).

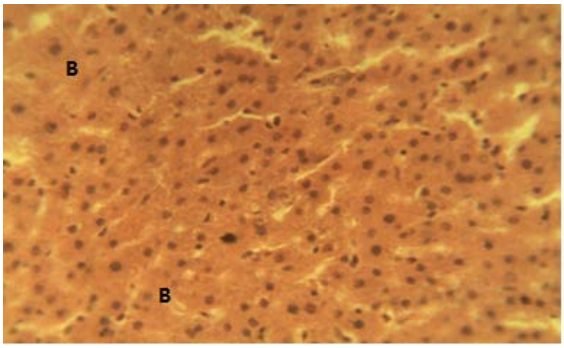


**Plate II.** Section of the liver of a rat treated with methanol seed extract (300 mg/kg) for 14 days. Note the moderate multifocal areas of necrosis of the hepatocytes (B). H and E stain. x400

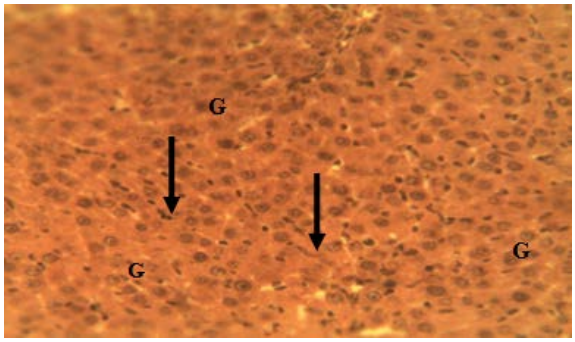


**Plate III.** Section of the liver of a rat treated with methanol seed extract (300 mg/kg) for 21 days. Note the congestion (arrow heads) and severe diffuse necrosis of the hepatocytes (G). H and E stain. x400

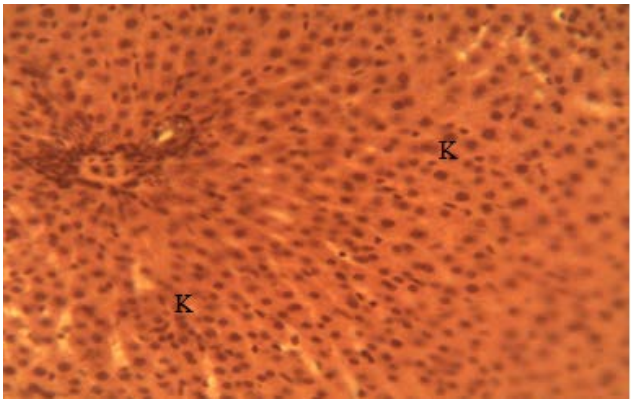


**Plate IV.** Normal rat liver. K = normal hepatocytes; H and E stain. x400

## 4. Discussion

The crude methanol seed extract of *A. melegueta* was tolerated by experimental rats since the highest test dose (2000 mg/kg) did not cause mortality or overt clinical pathology in the animals with 48 h duration of the study. The extract (300 mg/kg) induced a significant ($p<0.05$) decrease in the mean body weight of all the rats treated, and this manifested in the gradual weight loses of 5.4, 6.4 and 12.5% compared to the normal rats at 7, 14 and 21 days respectively. It is logical for the animals to experience weight loses in situations of hepatotoxicity. Liver is a vital organ in the body and it carries on numerous important steps in the metabolism of all three kinds of foods: proteins, fats and carbohydrates, and also produces plasma proteins as well as vital enzymes (Thibodeau and Patton, 1999). Toxic injury to hepatocytes impairs the routine functions of the liver and induces defective utilization of energy sources which could invariably lead to weight lose in animals. Prolonged aflatoxin toxicity have been reported to lower egg production in poultry, suppressed immune responses, decreased body weight and milk yield in different animal species (Robens and Richard, 1992).

Treatment of rats with the extract (300 mg/kg) caused significant ($p<0.05$) increases in serum AST, ALT, ALP and total bilirubin levels but unchanged albumin values of test rats relative to the control, particularly on days 14-21 of the study (Table 2). Hepatic cells contain higher concentrations of AST and ALT in the cytoplasm and AST in particular exists in the mitochondria (Wells, 1988; Jan shin, 2003). Damage to hepatic cells induces leakage of plasma to cause an increased level of hepato-specific enzymes in serum (Tolman and Rej, 1999). The measurement of AST, ALT and ALP levels serve as a means for indirect assessment of liver function. The elevation in total bilirubin level of test rats buttressed the extent of damage done to the liver tissue; hepatocytes were no longer in position to effectively conjugate bilirubin for excretion. However, this study was limited to sub-chronic treatment with the extract, hepatotoxicity had not reached a critical stage, and some hepatocytes remained viable in carrying out biosynthesis of albumin. Hence there was no alteration in albumin levels of treated and control rats.

The different organs (heart, stomach, spleen and kidney) examined post-treatment with the extract showed comparable weights with the control except liver. The mean relative liver weight of test rats significantly ($p<0.05$) decreased from the value of 25.3 $x10^{-3}$ g ±0.9 seen in the control, to 20.01 $x10^{-3}$ g ±0.4 and 1.8±0.5 $x10^{-3}$ g on days 14 and 21 were respectively (Table 3). The weight loss in liver tissue may be related to the toxic effect of the extract. Certain toxic substances, e.g. oxamyl used in pesticides are known to decrease various organ weights in rat (Cohen, 1986).

The histo-morphology of the liver from treated rats showed that, 300 mg/kg of *A. melegueta* extract induced a time dependent progressive, degenerative lesions on hepatic tissue. These involved mild focal necrosis of hepatocytes at day 7 of treatment, moderate multifocal necrosis of hepatocytes at day 14 and then severe diffused necrosis of hepatocytes at termination of the study at day 21 (Plates I, II and III). The mechanism in which the extract damaged the rat liver was not fully understood but some hepatotoxic drugs produce their effects through toxic metabolites and immunological reaction (Krishna *et al.*, 2009). Some of the drugs that damage liver include amiodarone, methotrexate, chlorpromazine, acetaminophen, pirprophen, amineptin, tetracycline, valproate (Lewis and Schiff, 1988; Yeung *et al.*, 1993; Krishna *et al.*, 2009) and many more.

In conclusion, the findings suggest that the methanol extract of *A. melegueta* seeds is potentially hepatotoxic at 300 mg/kg under prolonged ingestion at high dose. This is supported by the remarkable decrease in the mean body weight of all extract-treated rats, elevation in serum AST, ALT, ALP and total bilirubin, decrease in the mean relative liver weight of test rats compared to the control and histopathological lesions on treated rat livers. Further studies are however, ongoing to isolate the hepatotoxic principle(s) in the extract and to determine the mechanism(s) of action.

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