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Full Length Research

Evaluation of oral toxicity level of ethyl acetate extract, from garlic (allium sativum) in onorrh dawleys rats as per OECD guidelines 423.

[*1]Njue L. G, [2]Ombui J.N.[2] Kanja L.W. [3]Gathumbi J.K. [2]Nduhiu J.G.

[*1] Department of Public Health Pharmacology and Toxicology, University of Nairobi, P. O. Box 29053, 00625, Kangemi, Nairobi;
[2] Department of Public Health Pharmacology and Toxicology, University of Nairobi, P. O. Box 29053, 00625, Kangemi, Nairobi.
[3] Department of Veterinary Pathology, Microbiology and Parasitology, University of Nairobi, P. O. Box 29053, 00625, Kangemi, Nairobi.Kenya.

Correspondence Author's E mail: wamakesh@yahoo.com.

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This study was undertaken to evaluate toxicological effects of garlic ethyl acetate extract and subsequently determine the LD_{50} to establish the safety of the extract in Sprague Dawleys' Rats as per OECD guidelines 423. All rats, three per set, were sequentially administered with garlic ethyl acetate extract, first in a single dose of 300mg/kg, 2000mg/kg and 5000mg/kg of body weight, in that order respectively, to determine acute toxicity. Extra virgin oil acted as a control. The following parameters were observed keenly for any changes after 30min, 4h, 24h, 48h, 1wk and 2wks for each dose: body weight, skin color, eyes, Mucous membrane, salivation, lethargy, sleep, coma, convulsion, tremor, diarrhea, morbidity and mortality. All animals were subjected to gross necropsy and microscopic examination using standard pathological procedures and the following parts were observed; parts of the liver, lungs, heart, kidney, spleen and intestines (GIT).Dose increase from 300mg/kg, 2000mg/kg and 5000mg/kg body weight did not result to any death of the rats. No significant change was observed in all parameters used for gross necropsy and pathological change. Garlic contains compounds that are antibacterial, antiviral and antifungal. Garlic ethyl acetate extract is safe for human consumption.

Keywords: Garlic, Ethyl acetate, Extract, Rats, Acute, Toxicity

INTRODUCTION

About 3.4 billion people in the developing world depend on plant-based traditional medicines. According to the World Health Organization, WHO (1999) a medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemo-pharmaceutical semi synthesis. Such a plant will have its parts including leaves, roots, rhizomes, stems, barks, flowers, fruits, grains or seeds, employed in the control or treatment of a disease conditions such asinfectious pathologies in the respiratory system, urinary tract, gastrointestinal and biliary systems, as well as on the skin due to the phytochemicals in them, that are medically active (Adekunle and Adekunle, 2009; Liu, 2004; Nweze et al., 2004; Doughari et al., 2009).

Phytochemicals have been isolated and characterized from spices such as garlic and turmeric, fruits such as grapes and apples, vegetables such as broccoli and onion, beverages such as green tea and red wine, as well as many other sources (Doughari and Obidah, 2008; Doughari et al., 2009).Species such as garlic (*Allium sativum*), lemon balm (*Melissa officinalis*), and tee tree (*Melaleucaalternifolia*) are described as broad-spectrum antimicrobial agents (Heinrich et al., 2004). Several ailments including fever, asthma, constipation, esophageal cancer and hypertension have been treated with traditional medicinal plants (Grieve, 2006).Natural products have been an integral part of the ancient traditional medicine systems, such as; Chinese, Ayurvedic and Egyptian (Sarker and Nahar, 2007). Over

the years plants have assumed a very central stage in modern civilization amongst scientist in search for alternative sources of drugs.

Medicinal plants are increasingly gaining acceptance even among the literates in urban Settlements, probably due to the increasing inefficacy of many modern drugs used for the control of many infections such as typhoid fever, onorrhea, and tuberculosis as well as increase in resistance by several bacteria to various antibiotics and the increasing cost ofprescription drugs, for the maintenance of personal health (Van den Bogaard et al., 2000; Smolinski et al., 2003).

Garlic (*Allium sativum*) has long been used both for flavoring and for the potential benefits of preventing and curing ailments in many cultures (Hellen, 2005;Rivlin, 2001).Epidemiological, clinical, and preclinical studies have shown the close relation between dietary habits, including garlic intake, and the occurrence of disease such as gastrointestinal discomfort, sweating, dizziness, allergic reactions, bleeding, and menstrual irregularities (Garty, 2000). Garlic has been investigated extensively for health benefits, resulting in more than 1000 publications over the last decade alone, and it is considered one of the best disease-preventive foods (Doughari et al., 2009), based on its potent and varied effects. Garlic has been processed by various methods such as use of organic solvents like methanol, dichloromethane, hexane, and water Harris, 1995).However, some studies shed doubt on garlic's benefits (Garty, 2000), and careful examination of such research can help clarify the pros and cons of processing garlic by different methods. There are various commercially available garlic preparations in form of beverage, spices, and capsulated drugs including herbal remedies. Although many garlic preparations are commercially available, confusion remains because of the inconsistency of clinical-study results and the lack of scientific studies on individual products (Harris, 1995).

Unfortunately, rapid explosion in human population has made it almost impossible for modern health facilities to meet health demands all over the world, thus putting more demands on the use of natural herbal health remedies. However, the use of herbal remedies should be based on scientific origin; otherwise they would be useless and unsafe. Furthermore, the irrational use of these herbal products may cause serious toxicity for humans. Unfortunately, many people underestimate the toxicity of natural products and do not realize that these agents could be as toxic as more than synthetic drugs. An example for a toxic herbal product are the leaves of Digitalis *purpured* used to treat heart diseases which show severe systemic toxicity if taken orally (Tripathi, 2008).

Toxicology is the important aspect of pharmacology that deals with the adverse effects of bio active substance on living organisms prior to the use as drug or chemical in clinical use(Aneela et al., 2011). According to the OECD guidelines, in order to establish the safety and efficiency of a new drug, toxicological studies are very essential in animals like rat,mice, guinea, pigs, rabbits and monkeys. Toxicological studies help to make decision whether a new drug should be adopted for clinical use. Depending on the duration of drug exposure to animals, there are three types of toxicological studies namely; acute, sub acute and chronic toxicological studies.

In acute toxicity studies, a single dose of a large quantity of the drug is given to determine immediate toxic effects. Acute toxicity studies are commonly used to determine LD_{50} of drug or chemicals and natural products. In sub-acute toxicity studies, repeated doses of drug are given in sub-lethal quantity for a period of 15-20 days. Sub- acute toxicity studies are used to determine effect of drug on biochemical and pathological parameters of organs and tissues. In chronic toxicity studies drug is given in different doses for a period of 90 days to over an year to determine carcinogenic and mutagenic potentials of a drug (Lipnick et al., 1995).

Limit test and main tests are types of acute oral toxicity tests. The limit test is primarily used in situations where the experimenter has information indicating that the test material is likely to be nontoxic that is, having toxicity only above regulatory limit doses. Information about the toxicity of the test material can be gained from knowledge about similar tested compounds or similar tested mixtures or products, taking into consideration the identity and percentage of components known to be of toxicological significance. However, in situations where there is little or no information about its toxicity, or in which the test material is expected to be toxic, the main test should be performed (OECD, 2001). Limit test at one dose level of 300 mg/kg body weight may be carried out with six animals (three animals per step). Exceptionally a limit test at one dose level of 5000 mg/kg may be carried out with three animals. If test substance-related mortality is produced, further testing at the next lower level may need to be carried out. When there is no information on a substance to be tested, for animal welfare reasons it is recommended to use the starting dose of 300 mg/kg body weight. This is what was used in my limit doze.

This study was undertaken to determine the toxic effect of ethyl acetate extract from garlic (*Allium sativum*) in Sprague Dawley's Rats (females). At the dosage of 300mg/kg, 2000mg/kg and 5000mg/kg body weight of an animal for a period of 14 days according to the OECD 423 guidelines.

MATERIAL AND METHODS

Preparation of Garlic Cloves

Garlic cultivar used was Italian, a selection of Creole from Italy (Singh, 1983). Fresh garlic was collected from

Nganoini farm Laikipia County in Kenya and was used not more than four months old.Garlic bulbs were washed with water in a basin to remove any soil from the surface. After rinsing the bulbs with distilled water, they were dried, peeledand then placed in another clean basin. The garlic cloves were then placed on an aluminum foil ready for weighing.

Extraction of garlic with ethyl acetate

One hundred grams of the peeled garlic cloves were weighed on a clean aluminium foil using a weighing balance (Mettler pm 4600, Deltarange, Zurich). They were then put in an electric blender (Ohms, Internationalfzc,China) and 125 ml of ethyl acetate (AR) was added. The mixture was homogenized by blending to a pasteand put in a flat bottomed flask (1000ml) using a glass funnel and then covered with an aluminium foil. The content was kept in a dark cabinet for 24 h.Shaking was done in the morning and in the evening to mix all the flask contents. The contents was then sterilized by filtering using Whatman's paper No.1 pore size 0.45µm according to (Saravanan et al., 2010). The resulting filtrate was evaporated using rotary evaporator (Rotor Vapour Pump, Laboratoriums-Technic Ag, Buchi) at 50°C to remove ethyl acetate**.** This process yielded 710 g of extract.

The prepared crude extract was put in a beaker for fractionation with solvents. It was dissolved in 4 litres of water and mixed thoroughly. A portion of 100 ml of the mixture was measured and mixed with 300 ml of ethyl acetate. Mixing was done in a 2 litres separation funnel by shaking to separate the two phases as ethyl acetate being immiscible with water. Compounds soluble in the upper ethyl acetate phase (ethyl acetate being lighter than water) were collected and the lower aqueous phase was extracted thrice with ethyl acetate. All fractions of ethyl acetate were combined and poured into round bottomed flask of rotary evaporator and ethyl acetate was isolated from the fraction leaving behind semisolid ethyl acetate fraction. The total concentrate was air dried for one week, stored in the glass vials, then freeze dried at -20°C to confirm total solvent removal (Padìas and Anne 2011). This process yielded 10.76 g of the ethyl acetate crude extract.

Experimental Animals

A total of 18 healthy young and nulliporous, non pregnant Sprague Dawleys female Rats weighing from 100-153mg of 8-10 weeks old were selected. Tests to determine the LD50 values usually indicate that there is little difference in sensitivity between sexes, but generally females are found slightly more sensitive (OECD, 2001).The rats were then kept in clean polypropylene cages, covered with cleanwood shavings, for 5 days prior to start of the experiments to allow for acclimatization with the laboratory conditions.

Housing and feeding conditions

The temperature in the experimental animal rooms was kept between 21ºC and 23ºC with relative humidity ranging from 60-70%. Lighting conditions were set such that there were 12 hours light and 12 hours dark. For feeding, 30g of conventional standard Mice pellets were given to each group of 3 rats per day, with an unlimited supply of drinking water. The rats were group-caged in threes, by dose of both the extract (ethyl acetate extract) and the controls (extra virgin oil) (Bertolli Bal 1885, Italy). Volume given in each case depended on the dose rate, weight of the rats and the concentration required per kg

Allocation of animals to various groups

The rats were allocated in cages, in groups of three according to annex 2c (OECD, 2001), which recommends a starting dose of 300mg/kg body weight for the extract whose toxicity is not known, for both the extract and control. It shows that after administration of the dose, if 2/3 of the animals survive then the next higher dose (2000mg/kg body weight) should be used.Three groups of rats were given Extra virgin oil (Bertolli Bal 1885, Italy) as a control.

Blending in Olive

Garlic extract was blend with extra virgin oil (Bertolli Bal 1885, Italy) to make it easily transported to the stomach prior to dosing. The extra virgin oil acted as a control.

Dosing procedure:

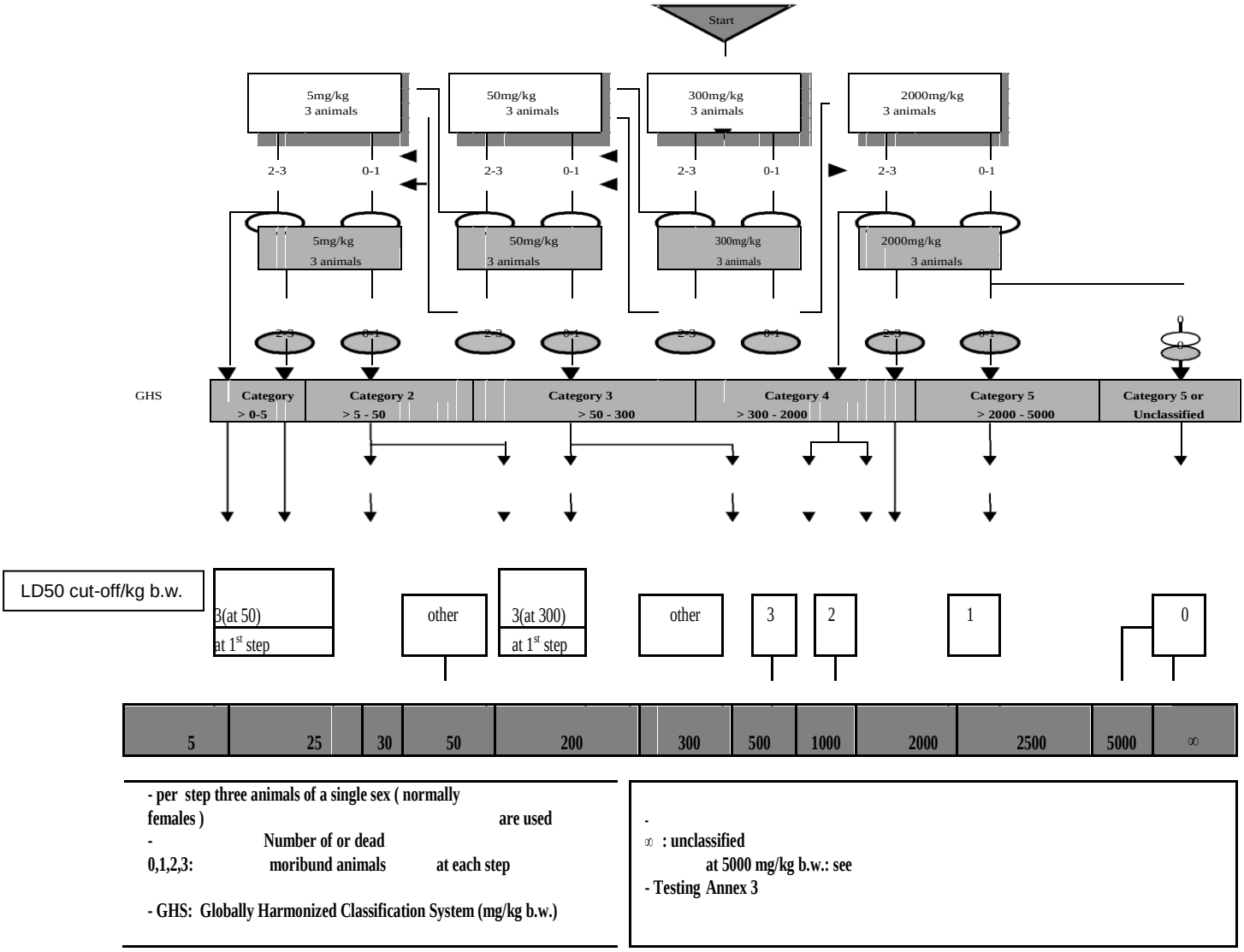
Prior to dosing, the rats were fasted for 12h and weighed. Following the period of fasting, the body weights of rats were determined using weighing balance (Mettler pm 4600,Deltarange, Zurich).The first doses were calculated using 300mg/kg. The doses both garlic extract and controls were calculated according to the test body weight. The concentration used was calculated such that the volume to be given was less than 1ml.(OECD, 2001).The following formula was used:

$$\text{Volume given} = \frac{\text{Dose rate} \times \text{weight of the rats (kg)}}{\text{Concentration mg/kg}}$$

(OECD, 2001).

The doses were calculated as per the weight of the individual animal in each dose rate of 300mg/kg, 2000mg/kg and 5000mg/kg body weight (tables 2, 3 and 4) respectively. The first dose used was of 300mg/kg body weight of an animal at 50mg/ml of virgin olive oil.

Annex 2c: Test procedure with a starting dose of 300 mg/kg body weight



For the rats fed with garlic extract, Serial numbers 1, 2 and 3 were given 33.902mg/0.678ml, 34.31mg/0.686ml, and 36.90mg/0.738ml of virgin oil respectively (Table 2). For controls the doses used for serial numbers 4, 5 and 6 were 0.689ml, 0.673ml and 0.602ml of virgin oil (table 2) respectively. The second dose of 2000mg/kg body weight was used at 500 mg/ml of virgin olive oil, for rats fed with garlic extract serial numbers 1, 2 and 3. These were 303.62mg/0.607ml, 303.24mg/0.606ml and 266.52mg/0.533ml of virgin oil (table 3). For the controls serial numbers 4, 5 and 6 were 0.611ml of virgin oil, 0.543ml of virgin oil and 0.565ml of virgin oil (table 3). The third dose of 5000mg/kg body weight of an animal used at 1000mg/ml of Virgin Olive oil, for rats fed with garlic extract serial numbers 1, 2 and 3 were 610.70mg/0.61ml, 667.85mg/0.67ml and 633.70mg/0.63ml of virgin oil (table 4)l. For controls serial numbers 4, 5 and 6 were 0.64ml, 0.56ml and 0.60ml of virgin oil (table 4).

Administration of Doses

The test substances were administered orally in a single dose by gavage using a stomach tube. After oral administration of the extract the animals were observed individually after 30 minutes, 4h, 24h, 48h, 1wk and 2wks respectively and the following parameters were observed and recorded: changes in skin and fur, eyes, mucus membrane, salivation, lethargy, sleepy, coma, convulsion, tremor, diarrhoea, morbidity, mortality and body weights (Tables 2, 3. 4 and 5) Annex 2c.

Table 1: Effect of garlic extract on S.D. Rats at 300mg/kg, 2000mg/kg and 5000mg/kg body weight and their acute toxicity study

Effect of garlic extract on S.D. Rats at 300mg/kg, body weight and their acute toxicity study				
Tests	**df**	**p- value**	**Mean weight value**	**Significance**
Extract	76.23	< 0.001	130.95	There was significant difference
Control	32.33	< 0.001	130.9	There was significant difference
Effect of garlic extract on S.D. Rats at 2000mg/kg, body weight and their acute toxicity study				
Extract	29.1	< 0.001	155.26	There was significant difference
Control	15.21	< 0.001	155.3	There was significant difference
Effect of garlic extract on S.D. Rats at 5000mg/kg, body weight and their acute toxicity study				
Extract	23.48	< 0.001	130.9	There was significant difference
Control	12.96	< 0.001	131.7	There was significant difference

Least significant value used was at 5%

Table 2: Effect of garlic extract (E) and Extra virgin oil as control (C) on S.D. Rats at 300mg/kg body weight and their acute toxicity study

Serial no.	**Experimental animals**	**Body weight in g**	**Extract used 300mg/kg**	**Dose prepared in virgin oil at 50mg/ml**
1	AE1	113.03	33.902mg	33.902mg/0.678ml of virgin oil
2	AE2	114.37	34.31mg	34.31mg/0.686ml of virgin oil
3	AE3	123.01	36.90mg	36.90mg/0.738ml of virgin oil
4	AC1	114.88	34.46mg	0.689ml of virgin oil
5	AC2	112.17	33.65mg	0.673ml of virgin oil
6	AC3	123.01	30.13mg	0.602ml of virgin oil

Table 3 : Effect of garlic extract (E) and Extra virgin oil as control (C) on S.D. Rats at 2000mg/kg body weight and their acute toxicity study

Serial no.	**Experimental animals**	**Body weight in g**	**Extract used 2000mg/kg**	**Dose prepared in virgin oil at 500mg/ml**
1	BE1	151.81	303.62mg	303.62mg/0.607ml of virgin oil
2	BE2	151.12	303.24mg	303.24mg/0.606ml of virgin oil
3	BE3	133.26	266.52mg	266.52mg/0.533ml of virgin oil
4	BC1	152.76	305.52mg	0.611ml of virgin oil
5	BC2	135.94	271.90mg	0.543ml of virgin oil
6	BC3	141.35	282.70mg	0.565ml of virgin oil

Table 4: Effect of garlic extract (E) and Extra virgin oil as control (C) on S.D. Rats at 5000mg/kg body weight and their acute toxicity study

Serial no.	**Experimental animals**	**Body weight in g**	**Extract used 5000mg/kg**	**Dose prepared in virgin oil at 1000mg/ml**
1	CE1	122.14	610.70mg	610.70mg/0.61ml of virgin oil
2	CE2	133.57	667.85mg	667.85mg/0.67ml of virgin oil
3	CE3	126.74	633.70mg	633.70mg/0.63ml of virgin oil
4	CC1	128.05	640.25mg	0.64ml of virgin oil
5	CC2	111.37	556.85mg	0.56ml of virgin oil
6	CC3	120.39	601.95mg	0.60ml of virgin oil

Pathological examination

All experimental animals were observed for a period of 2 weeks for any changes in the following parts: skin and fur, eyes, mucous membrane, salivation, lethargy, sleep, coma, convulsion, tremor, diarrhea, morbidity and mortality tables (6, 7 and 8) and no significant changes were observed, all were normal.

Postmortem was carried in all the animals as they did not die for pathological observation. Both gross necropsy and Microscopic observations were made on parts of the lungs, liver spleen kidney, heart and intestines (GIT) tables (8, 9 and 10), and were found to be normal.

All the rats were then euthanized by the 'Drop method' using halothane according to AVMA (2013). The rats were placed in a glass chamber together with a piece of cotton gauge to which a drop of halothane had been added. Death was confirmed by adjunctive physical method, lack of heartbeat, respiration and corneal reflex. Dead animals were dissected and examined for any

Table 5: Observation of D. S. rats (Group A), at 300MG/KG body weight after tests administration

Observation	30min		4h		24h		48h		1wk		2wks	
	E	C	E	C	E	C	E	C	E	C	E	C
Skin& Fur	N	N	N	N	N	N	N	N	N	N	N	N
Eyes	N	N	N	N	N	N	N	N	N	N	N	N
Mucus membrane	N	N	N	N	N	N	N	N	N	N	N	N
Salivation	2/3	N	N	N	N	N	N	N	N	N	N	N
Lethargy	N	N	N	N	N	N	N	N	N	N	N	N
Sleepy	ALL	N	ALL	N	N	N	N	N	N	N	N	N
Coma	N	N	N	N	N	N	N	N	N	N	N	N
Convulsion	N	N	N	N	N	N	N	N	N	N	N	N
Tremor	N	N	N	N	N	N	N	N	N	N	N	N
Diarrhoea	N	N	N	N	N	N	N	N	N	N	N	N
Morbidity	N	N	N	N	N	N	N	N	N	N	N	N
Mortality	N	N	N	N	N	N	N	N	N	N	N	N

E = Extract **C** = Control **N**= Normal

Table 6: Observation of D. S. Rats (Group B), at 2000MG/KG body weight after tests administration

Observation	30min		4h		24h		48h		1wk		2wks	
	E	C	E	C	E	C	E	C	E	C	E	C
Skin& Fur	N	N	N	N	N	N	N	N	N	N	N	N
Eyes	N	N	N	N	N	N	N	N	N	N	N	N
Mucus membrane	N	N	N	N	N	N	N	N	N	N	N	N
Salivation	ALL	N	N	N	N	N	N	N	N	N	N	N
Lethargy	N	N	N	N	N	N	N	N	N	N	N	N
Sleepy	ALL	N	ALL	N	N	N	N	N	N	N	N	N
Coma	N	N	N	N	N	N	N	N	N	N	N	N
Convulsion	N	N	N	N	N	N	N	N	N	N	N	N
Tremor	N	N	N	N	N	N	N	N	N	N	N	N
Diarrhoea	N	N	N	N	N	N	N	N	N	N	N	N
Morbidity	N	N	N	N	N	N	N	N	N	N	N	N
Mortality	N	N	N	N	N	N	N	N	N	N	N	N

E = Extract **C** = Control **N**= Normal

gross pathological lesions. Parts of the liver, lungs, heart, spleen, GIT and kidney were collected and fixed in10% formalin for 7 days. Formalin fixed tissues were processed and histopathological examination done according to(OECD, 2008). The histopathological processing involved dehydration using graded alcohol (Ethanol) with the following increasing order; 70% (2h), 80% (1h), 90% (1h), 96 (1h), 100% (1h) and100% (1h). Clearing/de-alcohlization followed in Xylene for 3h (1h at a time).Infilteration of the tissues was done using Molten paraffin wax was done in glass beakers in the oven (Memmert 854, Schwabach,Germany) set at 60°C for 2h. Embedding was done on papers, supported by embedding molds at the edges, where the molten paraffin wax was placed on a hot plate. For microtomy, the separated solid tissues were fixed on wooden blocks by heating and then labeled. Rotary microtome (LeitzWetzlar, Germany) wasused to trim off excess work. Sectioning was carried out using the microtome set, at 5µm. The sections cut were flattened on a water bath set at 45°C for 1min. to remove creases.

A microscope slide spread with an adhesive (egg albumen) ,was dipped in the water bath at an angle of 90% perpendicular to the section. This allowed the section to adhere on the slide, then removed and air dried. The slide was then put in an oven set at 60°C for 2h.

H and E Staining;(paraffin wax section): Dewaxing was done using xylene twice for 5mim (5min. in each case). This was followed by hydration of sections for 5min. in the following order; 100% absolute alcohol (5min), 96% (5min), 90% (5min), 80% (5min), 70% (5min), 50% (5min) and water. Staining was then done using Mayer's Heamatoxylin for 10 min. then Eosin added for 5min.

The slides were then dehydrated as follows 50% (5min) 70% (5min), 80% (5min), 90% (5min), 96% (5min), 100% absolute alcohol (5min). To clear, xylene was used, and then the slide was mounted with a cover slip using Canada balsam, then observed with a microscope. Tissue slides were examined for any microscopic lesions using a light microscope (Leica DM 500, Germany).

Microscopic observations were made on parts of the lungs, liver spleen kidney, heart and intestines (GIT) tables (8, 9 a

Table 7: Observation of D. S. rats (Group B), at 5000MG/KG body weight after tests administration

Observation	30min		4h		24h		48h		1wk		2wks	
	E	C	E	C	E	C	E	C	E	C	E	C
Skin& Fur	N	N	N	N	N	N	N	N	N	N	N	N
Eyes	N	N	N	N	N	N	N	N	N	N	N	N
Mucus membrane	N	N	N	N	N	N	N	N	N	N	N	N
Salivation	2/3	N	N	N	N	N	N	N	N	N	N	N
Lethargy	N	N	N	N	N	N	N	N	N	N	N	N
Sleepy	N	N	N	N	N	N	N	N	N	N	N	N
Coma	N	N	N	N	N	N	N	N	N	N	N	N
Convulsion	N	N	N	N	N	N	N	N	N	N	N	N
Tremor	N	N	N	N	N	N	N	N	N	N	N	N
Diarrhoea	N	N	N	N	N	N	N	N	N	N	N	N
Morbidity	N	N	N	N	N	N	N	N	N	N	N	N
Mortality	N	N	N	N	N	N	N	N	N	N	N	N

Table 8: Post mortem microscopic observation of the varoius organs from s.d. rats in group a, fedwith 300mg/ kg ETHYL acetate extracts**.**

Serial no.	Experimental animals	Lungs	Liver	Spleen	Kidney	Heart	GIT
1	AEI	N	N	N	N	N	N
2	AE2	N	N	N	N	N	N
3	AE3	N	N	N	N	N	N
4	AC4	N	N	N	N	N	N
5	AC5	N	N	N	N	N	N
6	AC6	N	N	N	N	N	N

N = normal, **A =** group**, E =**extract and **C = control**

RESULTS

The following parameters were observed after dosing the rats with 300mg/kg, 2000 mg/kg and 5000 mg/kg body weight for acute toxicity: Changes in body weight recorded in (Table 1).

Skin and Fur, Eyes, Mucous membrane, Salivation, Lethergy, Sleep, Coma, Convulsion, Tremor, Diarrhoea, Morbidity and Mortality recorded in (Tables, 5, 6 and 7). These parameters were observed during the first 30 min, 4h, 24h, 48h, 1 wk and 2 wks respectively. All observations were systematically recorded with individual records being maintained for each animal.

There was a significant difference in body weights of the rats fed with the extract and the control (table 1). At 300mg/kg (mean weight with the extract 130.95g) and control (mean weight with the extract 130.90g). There was a significant difference in body weight of rats fed with the extract with 2000mg/kg . The mean weight value of rats fed with extract (mean 155.26 g) and with control (155.3g).There was a significant difference in body weight of rats fed with the extract with 5000mg/kg . The mean weight value of rats fed with extract (mean 130.9g) and with control (131.7 g).

There was no difference in the following parameters observed during the following period: first 30 min, 4h, 24h, 48h, 1 wk and 2 wk respectively (Lethergy, Sleep, Coma, Convulsion, Tremor, Diarrhoea, Morbidity and Mortality (Tables 5, 6 and 7). This is an indication that the extract was good for consumption. The differences in body weights among the rats fed with the extract and controls (table 1) may have occurred because the test animals did not have the same initial weight at the beginning of the experiments. During the first 30min and 4h after dosing the rats with the extracts with 300mg/kg and 2000mg/kg (table 5 and 6), they were observed to be sleepy.

Since there were no signs of death of the test and control rats the dosage was increased from 300mg/kg, 2000mg/kg and 5000mg/kg of the body weight of the animal respectively. The addition of the upper dose of 5000 mg/kg body weight was considered because garlic is known not to have any major effects on the consumers (Balch, 2000).OECD (2001) recommends use of this dose when there are facts to indicate that use of the substance is not harmful to animals.

Tables 8, 9 and 10 indicates post mortem microscopic observation of the various organs from S. D. Rats fed with 300mg/kg, 2000mg/kg and 5000mg/kg of the extract and controls. No microscopic lesions were observed on the lungs, liver, spleen, kidney, heart and intestines (git) of both the extract and controls. This is an indication that ethyl acetate extract from garlic is safe for human consumption in food.

Table 9: Post mortem microscopic observation of the varoius organs from s.d. rats in group b, fed with 2000mg/ kg ETHYL acetate extracts**.**

Serial no.	Experimental animals	Lungs	Liver	Spleen	Kidney	Heart	GIT
1	BEI	N	N	N	N	N	N
2	BE2	N	N	N	N	N	N
3	BE3	N	N	N	N	N	N
4	BC4	N	N	N	N	N	N
5	BC5	N	N	N	N	N	N
6	BC6	N	N	N	N	N	N

Table 10: Post mortem microscopic observation of the varoius organs from s.d. rats in group c, fed with 300mg/ kg ETHYL acetate extracts**.**

Serial no.	Experimental animals	Lungs	Liver	Spleen	Kidney	Heart	GIT
1	CEI	N	N	N	N	N	N
2	CE2	N	N	N	N	N	N
3	CE3	N	N	N	N	N	N
4	CC4	N	N	N	N	N	N
5	CC5	N	N	N	N	N	N
6	CC6	N	N	N	N	N	N

DISCUSSION

In accordance to the OECD Guidelines 423, the results revealed that garlic ethyl acetate extract was found to be non toxic at all levels from 300mg/kg, 2000mg/kg and 5000mg/kg body weight of experimental animals as shown in tables 5, 6 and 7. Death of experimental animals did not occur and therefore LD_{50} of garlic ethyl acetate value could not be calculated. The addition of the upper dose of 5000 mg/kg body weight was considered because garlic is known not to have any major effects on the consumers (Balch, 2000).OECD (2001) recommends use of this dose when there are facts to indicate that use of the substance is not harmful to animals. The extract was safe for human consumption. In all the three levels of body weights and wellness parameters used in the evaluation of toxicity, no significant changes were observed. On test rats and their controls, in regards to the skin and fur, eyes, mucus membrane, coma, lethargy, convulsion, tremor, diarrhoea, morbidity and mortality they were found to be normal. However, there was a significant difference in body weights for all test treatments and controls. This could have been contributed by the fact that garlic ethyl acetate extract, being a spice commonly used by man, had nutritive values that resulted to weight increase (Doughari and Obidah, 2008).

Salivation and Sleepiness was observed at levels 300mg/kg and 2000mg/kg body weight of the experimental animals within the first 4h. of oral administration of the extract. At 5000mg/kg body weight of experimental animals 2/3 were found to salivate within the first 30 minute of oral administration of the extract. Due to oral administration of the extracts salivation may have occurred due to the garlic ethyl acetate extract taste described as ‘hot’(Groppo et al., 2007; McGee, (2004). Significant increase in body weight of the experimental animals indicates that the administration of the extracts does not affect the growth of the animal because there was tremendous growth. Microscopic observation of test treatment revealed that all tissues observed were normal, Tables 6, 7 and 8. This is in contrast to (Garty, 2000), who concluded that the side effects of garlic consumption include bleeding, and menstrual irregularities. However gastrointestinal discomfort and dizziness may occur as observed in the sleepy rats. The side effects of garlic extracts are largely unknown and no American, Food and Drug Agency - approved study has been performed. However, garlic has been consumed for several thousand years without any adverse long-term effects, suggesting that modest quantities of garlic pose, at worst, minimal risks to normal individuals (Hellen, (2005).

CONCLUSION

Garlic ethyl acetate extract does not exhibit toxic effects when given orally at a concentration of 5000mg/kg body weight. However the normalcy and insignificant changes in wellness parameters and body weight reveals the safety of garlic ethyl acetate extract at a dose of 5000mg/kg.

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