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Acute and Sub-Acute Toxicological Evaluation of Ethanolic Leaves Extract of *Prosopis juliflora* (Fabaceae).

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Abstract
Prosopis juliflora (Sw.) DC. (Fabaceae), an exotic tree introduced in Kenya, is one of the World's top 100 least wanted species due to its invasive nature and tendency to form impenetrable thickets. Goats that constantly feed on the plant's sugary pods are known to lose their teeth. Pastoralists have previously taken the Kenya government to court due to possible death arising from starving animals which hardly feed. However, *P. juliflora* is known to contain tannins, alkaloids, saponins and other phytochemicals that can be exploited in the development of anti-helmintic herbal drugs. Alkaloids and saponins have been associated with numerous other pharmacological activities such as anti-bacterial, anti-cancer, anti-inflammatory and anti-viral. This study investigated the toxicity and safety levels of *P. juliflora* with a prospect of exploiting its bioactive compounds in the development of herbal remedies. Acute toxicity of *P. juliflora* ethanolic leaves extract was evaluated using *Swiss albino* rats. Oral dosages used were 175 mg/kg, 550 mg/kg, 1750 mg/kg, 2000 mg/kg and 5000 mg/kg body weight respectively. All clinical signs and symptoms were recorded within 24 hours. Toxicity symptoms were moderately observed and post mortem did not show any major gross effects on the internal organs.
Key words: *Prosopis juliflora,* acute toxicity, clinical signs, doses, symptoms.

INTRODUCTION

1.0 General Introduction

For many years, man has used natural herbs and potions as medicines, but it is only since the mid-nineteenth century that serious efforts were made to isolate and purify the active principles of these remedies. Since then, a large variety of biologically active compounds have been obtained and their structures determined e.g. morphine from *opium*, cocaine from coca leaves, and quinine from the bark of the *cinchona* tree (Graham *et al.,* 1995). Owing to growing demand for herbals, the current need is to intensify research in the field of medicinal herbs and to get authentic information on the subject. Herbal products are often questioned for quality control and assurance. Preclinical trials are essentially toxicity and other biological tests carried out on tissue samples, animals and sometimes organ cultures to determine whether it is safe to test the drug on humans. The animal tests investigate the effect of the drug on various body systems such as the respiratory, nervous and cardiovascular systems. They are carried out under both in vivo (in the living organism) and in vitro (in an artificial environment) conditions. These preliminary tests provide information concerning the drug's pharmacokinetic properties and its interaction with other drugs and over-the-counter medicines.

Prosopis juliflora is an evergreen tree native to northern South America, Central America and the Caribbean (Pasiecznik et al., 2004). It is fast growing, nitrogen-fixing and tolerant to arid conditions and saline soils (Anonymous 2003, Pasiecznik et al., 2004). In Kenya *P. juliflora* was first planted in the beginning of the 1970s to rehabilitate a quarry in Bamburi near Mombasa (Ebenshade & Grainger 1980, Maghembe *et al*., 1983). In the early 1980s it was introduced in the Lake Baringo area through the Fuelwood Afforestation Extension Project (FAEP) (Kariuki 1993, Lenachuru 2003). The major objectives of the project was to involve the local people in tree planting to overcome problems such as lack of firewood and overgrazing (Kariuki 1993, Lenachuru 2003).

In the initial introductory stages, the tree was appreciated due to its ability to grow where nothing else seemed to be able to grow. It was easy to plant, prevented soil erosion and sandstorms, provided shade and its pods served as a source of food for livestock (Lenachuru 2003). After about ten years, problems with *P. juliflora* started to occur. It started to spread rapidly and its ability to survive cutting by coppicing made it uncontrollable. However, the

problems associated with the *Prosopis* invasion vary considerably between regions. In Ngambo area of Marigat district, where the invasion has been most severe, the community noted the most severe problems are the reduction of pastures for livestock grazing, reduced farm lands and associated opportunities for cultivation, disfiguration of livestock gums (especially goats) and tooth decay, both of which result in deterioration of livestock health and sometimes death. In Loboi area, where the invasion is far less severe, the incidence of malaria associated with the expansion of Prosopis thickets was the most frequently mentioned problem (Mwangi and Swallow, 2005).

The underlying motivation to study toxicological profiles of *Prosopis juliflora* was guided by the magnitude of this species invasion in arid areas of Kenya, the level of public and government outcry, environmentalists concern about the invasion and its adverse effects in both human and animals.

2.0 Materials and Methods

2.1.0 Sample collection

The *Prosopis juliflora* pods, root barks, stem and leaves were obtained from mature trees in Marigat district, Kenya. The samples were authenticated and Voucher specimens stored at Chemistry Department, JKUAT.

2.1.1 Preparation and extraction of samples

The collected *Prosopis juliflora* leaves were washed in water, shade dried for 3weeks at room temperature (30±3°C), and made into fine powder using an electrical mill in readiness for extraction. The extraction procedures were carried out using cold sequential extraction on plant materials with analytical grade organic solvents of increasing polarity, which include n-hexane, dichloromethane, ethyl acetate and ethanol. (Harborne, 1998).

2.2 Phytochemical screening

This was performed using standard procedures (Sofawara, 1993; Trease *et al.,* 1989; Harborne, 1998; Siddiqui and Ali, 1997). The leaves extracts were tested for tannins, alkaloids, saponins, flavonoids, steroids, terpenoids, anthraquinones, reducing sugars and glycosides. The results obtained were recorded in tabular form (Table 1).

2.3 Acute Oral Toxicity

Acute oral toxicity refers to adverse effects occurring following oral administration of a single dose of a substance, or multiple doses given within 24 hours. In this test, rats of either sex were randomly selected, marked to permit individual identification, and kept in their cages for 7 days prior to the start of dosing to allow for acclimatization to the laboratory conditions. The animals were kept fasting overnight providing only water, after which the extracts were administered orally at the selected dose levels by intragastric tube (OECD, 1998) and observed for 14 days according to OECD (2001) recommendations.

2.3.1 Experimental animals

30 Wistar *Albino* rats were obtained from random breeding in a closed colony from the animal house at Jomo Kenyatta University of Agriculture and Technology (JKUAT). They were kept in a temperature-controlled environment (23 ± 2ºC) with a 12 h light-dark cycle. Food and water were provided *ad libitum*. They were then marked to permit individual identification, and kept in their cages to acclimatize for 7 days before the dose administration.

2.3.2 Preparation of doses

The ethanolic leaves extract was suspended in distilled water and a stock solution was prepared. The doses selected were 175mg/kg, 550mg/kg, 1750mg/kg, 2000mg/kg and 5000mg/kg. These doses selected were based on the Organization of Economic Cooperation Development (OECD/OCDE) regulations which fix the upper limit at 2000mg/kg and exceptional dose at 5000mg/kg body weight.

2.3.3 Administration of doses

The ethanolic leaves extract was administered orally in a single dose using an intubation cannula in graduated doses to 5 groups of experimental animals, one dose being used per group. The animals were fasted for 16 hours prior to dosing. Their weight were taken and recorded before administration. After the administration, food was withheld for a further 3-4 hours. All the clinical signs were recorded within the 24 hours test period. Clinical signs to be observed included increased motor activity, anaesthesia, tremors, arching and rolling, clonic convulsions, ptosis, tonic extension, lacrimation, Straub reaction, exophthalmos, pilo-erection, salivation, muscle spasm, opisthotonus, writhing, hyperesthesia, loss of righting reflex, depression, ataxia, stimulation, sedation, blanching, hypnosis, cyanosis and analgesia (OECD, 2000). The control group received water.

2.4 Sub-acute Toxicity

30 Wistar Albino rats were kept in a temperature-controlled environment (23±2ºC) with a 12 h light-dark cycle. Food and water were freely available and were recorded each 3 days. The animals were divided into one control group and three treated groups (250,500 and 1000 mg/kg), each group consisting of 6 animals. The control group received

saline and each treated group received the ethanolic leaves extract by gavage for 14 days (once a day). At the end of the experiment, blood was collected from the orbital sinus under ether anesthesia for biochemical analysis. After the blood collection, the animals were sacrificed by cervical displacement and selected organs (liver, heart, spleen, kidneys and lungs) were removed for macroscopic analysis. The biochemical parameters evaluated included plasma; creatinine, alanine aminotransferase (ALT), bilirubin and serum alkaline phosphatase and were assessed using an auto analyser.

3.0 RESULTS AND DISCUSSION

3.1 Phytochemical Screening Results

Many plants phytochemicals exhibit various pharmacological activities, such as, antimicrobial (Moleyar and Narasimham, 1992; Kareru *et al*., 2007 a), anthelmintic (Wasswa and Olila 2006), cytotoxicity (Alluri *et al.,* 2005), antiprotozoal (Burapadaja and Bunchoo 1995; Camacho *et al*., 2003), antibacterial (Sathiya *et al*., 2008) and many others. These bioactive components have potential roles in health care and can be used to synthesize chemicals for new drug development. Drugs are derived from substances, such as alkaloids (e.g., caffeine, from the coffee shrub—Coffea arabica—used as a stimulan), glycosides (e.g., digoxin and other digitalis glycosides, from foxglove—Digitalis spp.—used to treat heart failure), alcohols, esters, aldehydes, or other constituents or mixtures of constituents isolated from the plant or animal (.Monika *et al.,* 2008*).* The quantities in percentage ratio of extractives in stem barks, roots, pods and leaves of *P. juliflora* are reported below in figure 1. Extraction using ethanol gave the highest yield in all the different plant parts while hexane gave the lowest yields. This could be attributed to the polar nature of the secondary metabolites present in this plant. Root ethanolic extraction gave the highest yield of 16.789% while dichloromethane extraction of the stem barks gave the lowest yield of 0.200%. Analyses of some phytochemicals present in *P. juliflora* extracts are summarized in table 1 below.

Different secondary metabolites were found to be present in all the solvents extractives of *P.juliflora*. Sterols and triterpenes were present in all the extracts while alkaloids were absent in the DCM extract. In general, the polar metabolites of alkaloids, saponins, tannins and flavonoids were predominantly present in ethyl acetate, ethanolic and aqueous extracts. Ethanolic and aqueous extracts had the highest concentration of reducing sugar as compared to the other extracts of the different plant parts.

3.2 Acute toxicity studies

In this study, male and female rats were dosed in separated groups via oral route and a total of 22 rats were used. Some of the clinical symptoms of toxicity noted included: hypo-activity, pilo-erection, loss of appetite, salivation and hyperventilation (i.e. abnormal rapid breathing which could be attributed to stress). These signs also varied depending on the dosage used (i.e. they were more pronounced at the doses of 2000mg/kg and 5000mg/kg) as compared with the other lower doses. However, all the signs were reversed within 72 hours duration. The no-observed-adverse-effect level *(NOAEL)* for ethanolic leaves extract of *P. juliflora* administered to both the female and male rats via the oral route was 1750 mg/kg while the minimum lethal dose and the estimated LD_{50}, according to OECD425 method, was > 5000 mg/kg. The results are summarised in the Table 2 below.

In both sexes two rats died at the dose of 1750mg/kg. Gross post mortem observations were characterized by bloated stomach, mild lung inflammation and dehydration. However, the rats that survived had no significant change compared to the control group.

According to Clarke and Clarke (1977), any substance with LD_{50} values of 1000mg/kg and above are regarded as safe or with low toxicity. In our studies we used the fixed dosage procedure as provided by the OECD test guidelines 423 and 425 (OECD, 1998; 2001). The moderate toxicity observed could result from any of the organic secondary metabolites like saponins, alkaloids and tannins as indicated by the results of the phytochemical analysis in table 1.

3.3 Sub acute toxicity studies

3.3.1 Clinical signs and mortality

Sub acute toxicity data have been used for several decades to predict the hazard of long-term, low-dose exposure to a particular compound. The present investigation was conducted on *Wistar albino* rats of approximately 10-12 weeks old. Three groups of rats were administered orally with doses of 250mg/kg, 500mg/kg and 1000mg/kg of ethanolic leaves extracts of *Prosopis juliflora* daily for 14 days. Another one group received distilled water as vehicle control. Live body weight was recorded after every two days. All the rats were observed keenly for the first 3 hours and at least twice daily with the purpose of recording any symptoms of ill-health or behavioral changes during the study period. The clinical symptoms observed during the study period were dose dependent and included: hypo-activity, pilo-erection, loss of appetite, salivation and hyper-ventilation (i.e. abnormal rapid breathing which could be attributed to stress). These signs were more pronounced at the dosage of 1000mg/kg as compared with the other

lower doses. These signs were more pronounced between the 3rd to the 11th days during which 5 rats died. In the lower dosage level of 500mg/kg, clinical signs were evident to a lesser extent and only two rats died. However, the rats in the lowest dosage of 250mg/kg had only minor signs of pilo-erection, loss of appetite and hyper-ventilation in which case none of the rats died. No clinical signs were recorded among the animals pertaining to the Control group.

3.3.2 Gross Pathology

There were no major differences in gross pathology for any organ is the sub-acute test. Figure 3 below shows the relative organ weights in treated and control groups. There was a slight decrease in the relative liver weights for treatment group at 500mg/kg in comparison to the rest of the groups. This could be attributed to the adverse effect observed from the 11th day of administration. The slight differences in relative heart, lungs and spleen weights could not be connected with any pathological processes. The macroscopic analysis of the target organs of the treated animals (liver, lung, heart, spleen and kidney) did not show significant changes in color and texture when compared with the control group.

3.3.3 Biochemical Analysis

The bilirubin value in the control group was (16.0125±7.6230µmol/l) while at the dosage level of 1000mg/kg the value was recorded at (22.233±3.9716 µmol/l). At this dosage the value was above the normal range which is between (3.4-17.1µmol/l) and could be attributed to mild liver malfunction, obstruction of the common bile or hepatic duct or hemolytic anemia. When the liver is not filtering normally, bilirubin builds up in the blood, which in turn results in to a damaged liver (cirrhosis). The createnine clearance values obtained from all the three groups were similar at (<44.2 µmol/l) which was within the normal range of between (15-61 µmol/l). This implies that none of the rats suffered from impaired renal functioning. The alkaline phosphate values obtained were all below the normal range which lies between (20-70 u/l). The value of (<20µ/l) could be due to malnutrition induced by fasting the rats for a period of 16 hours prior to the test. These observations could indicate that liver functions were to a large extent preserved by oral administration of *P. juliflora* ethanolic leaves extract.

Conclusion

In general, our study demonstrated that *P. Juliflora* ethanolic leaves extract seems to be destitute of major toxic effects, which could compromise the medicinal use of this plant in folk medicine. However, further studies are necessary, such as histological and morphological experiments, to confirm this evidence.The presence of bioactive metabolites in this plant can be used in development of new pharmaceuticals that address largely unmet therapeutic needs in our society. However, further research should be conducted to investigate on the long term effects.

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Table 1: Phytochemicals determined from *P.juliflora* sequential leaves extracts.

Extracts	Alkaloids	Saponins	Tannins	Flavonoids	Reducing sugars	Sterols/ Triterpenes
n-Hexane	-	-	-	-	-	+
DCM	+	+	+	-	+	+
Ethyl acetate	+	+	+	+	++	+
Ethanolic	++	+	++	+	+++	+
Aqueous	++	+	++	+	+++	+

Key: - absent, +present, ++ present in high concentration

Table 2: Acute toxicity study of *P. juliflora* LEE after oral administration to both female and male rats.

Dose (mg/kg) body weight	Sex				Total number of rats used
	Male		Female		
	O (survived)	X (died)	O(survived)	X (died)	
175	1	0	1	0	2
550	1	0	1	0	2
1750	2	1	2	1	6
2000	3	0	3	0	6
5000	3	0	3	0	6

Table 3: Effect of treatment with *P.juliflora* leaves ethanolic extract on biochemical parameters

Dose (mg/kg)	Control (n=5)	250 (mg/kg) (n=5)	500 (mg/kg) (n=5)	1000 (mg/kg) (n=5)
Createnine (µmol/l)	<44.2	<44.2	<44.2	<44.2
Bilirubin (µ mol/l)	16.0125±7.6230	14.29±3.9010	14.77±6.7722	22.233±3.9716
ALT (µ/l)	161.46±60.1686	240.22±292.2966	66.94±23.1954	108.667±10.0167
Alkaline phosphatase (µ/l)	<20	<20	<20	<20

Values are mean ± S.D. ALT – Alanine Aminotransferase

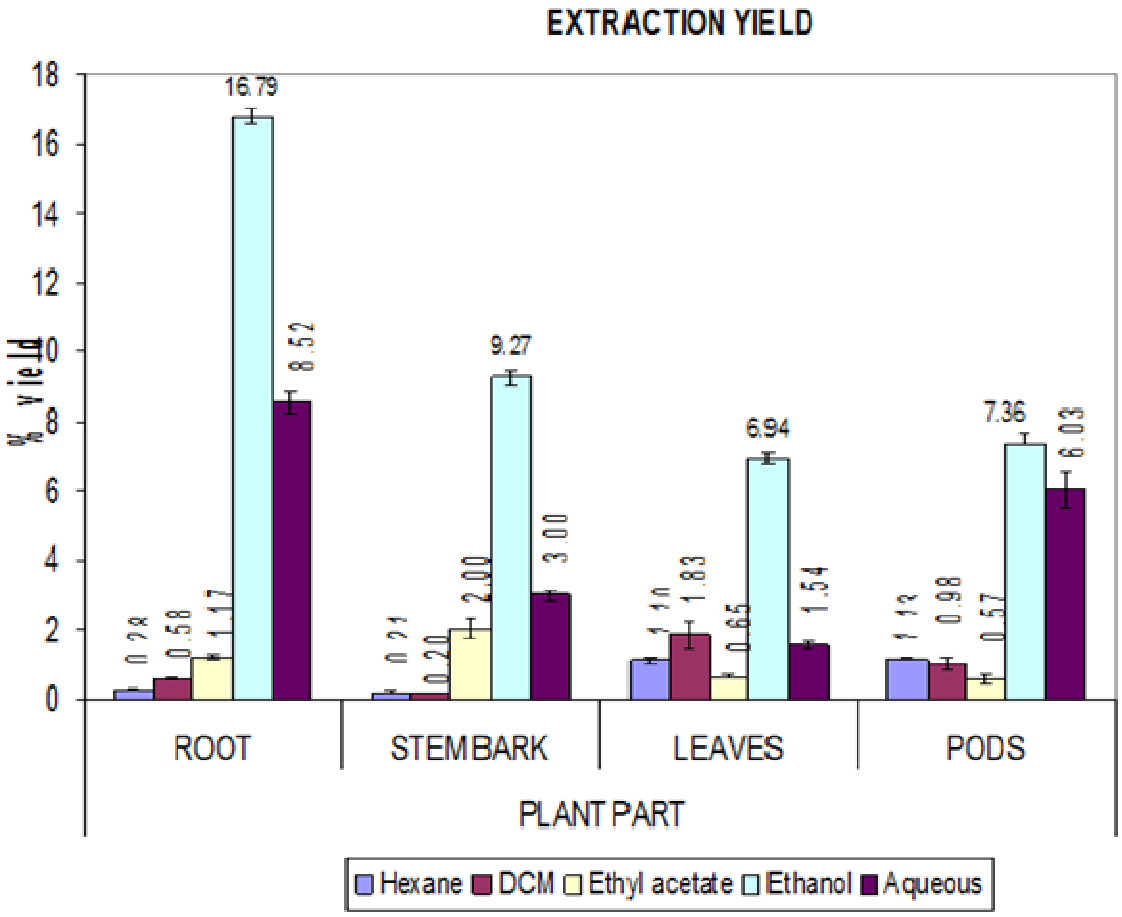

Figure 1: Solvent extractive values for *P. juliflora*

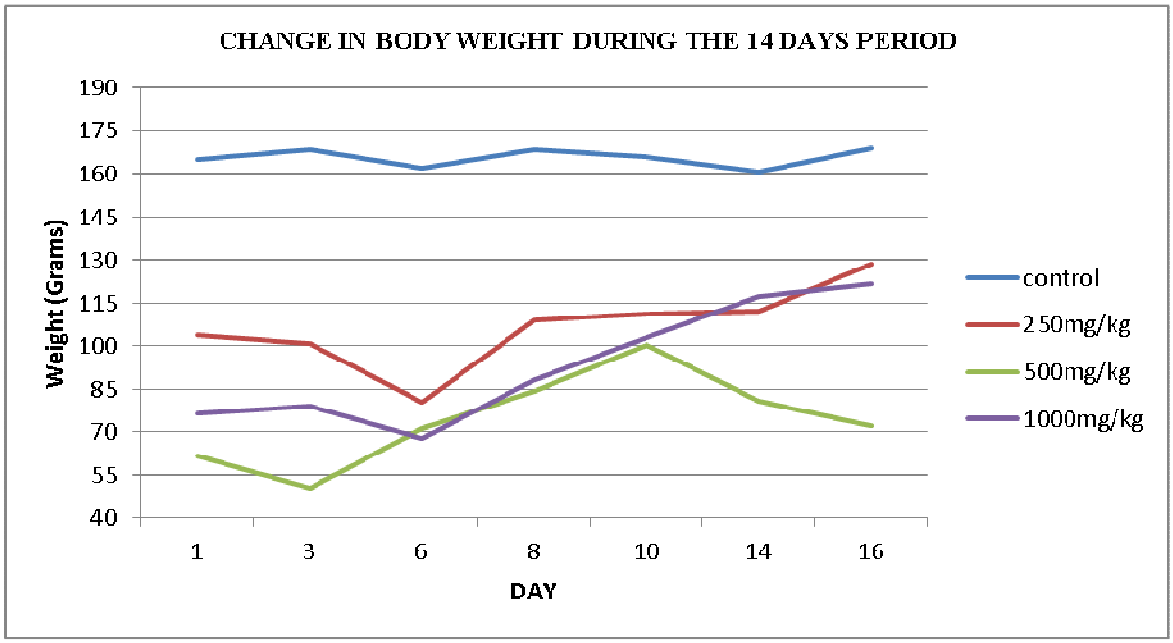

Figure2: Effect of oral administration of *P. juliflora* leaves ethanolic extract on the body weight.

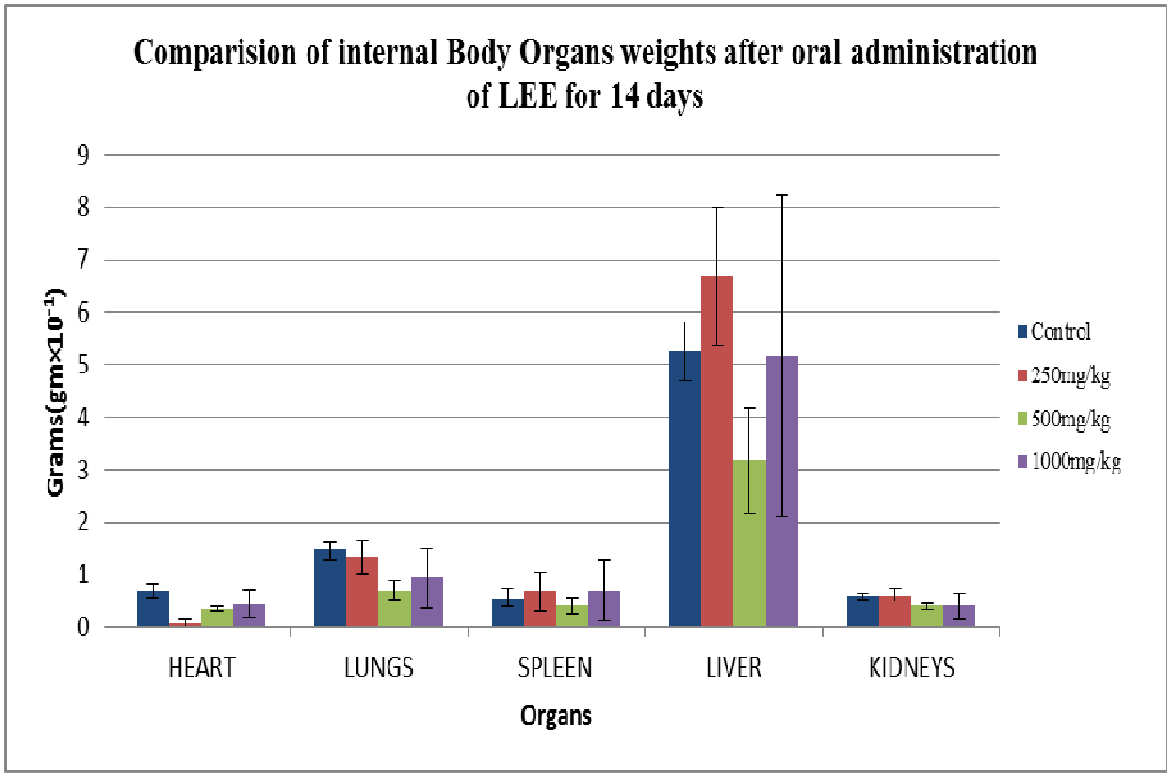

Figure 1: Internal organs weight after oral administration of LEE.

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