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Evaluation of the Acute Hepatotoxicity of *Medemia Argun* Seed's Extract by Determining of Ld_{50} Value And Dose Response Curve

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ABSTRACT

Determination of median lethal dose (LD_{50}) is one way to measure the potential short-term toxicity (acute toxicity) of a substance to a biological system that sufficient to kill 50 percent of a population of tested animals. Acute toxicology studies in vivo are essential to drug development experiments that estimate the LD_{50} in rodents. Toxicity tests are usually performed by pharmaceutical manufactures in the investigation of a new drug. *Medemia argun* fruits have been found in the tombs of ancient Egypt including celebrated tomb of Tut Ankh Ammon. Nowadays, little detailed phytochemical and pharmaceutical studies have been reported for *Medemia argun*. Scientists exhibited by in vitro experiments that the proanthocyanidin fraction from *Medemia argun* nuts can be useful as a protecting factor against oxidative/nitrative stress associated with different diseases such as cancer, cardiovascular and neurodegenerative diseases. In this study, we determined the LD50 of the *Medemia argun* seed's crude ethanolic extract in vivo using the experimental mice' model to open the field for further scientific researches. Several doses of *Medemia argun* ethanolic extract was used in LD50 experiment that carried out on male albino mice administered intraperitoneally for 14 days. The results showed that the LD50 of the ethanolic *Medemia argun* crud extract was (200 mg/kg) where half of the tested animals in such group were dead, our data substantiate an evidence that *Medemia argun* seed's extract could be safe up to (150 mg/kg) that there was no mortality was reported and may be effective at dose (100 mg/ kg).

Keywords

Acute toxicity, ethanolic extract, *Medemia argun*, Median lethal dose, Mice.

1. INTRODUCTION

Medemia argun is known as one of the most mysterious plant species celebrated in ancient Egypt, Medemia fruits were discovered by explorers in the Egyptian tombs, including the celebrated tomb of Tut Ankh Ammon [1].

Medemia argun's nuts contained 636.88 mg/g proanthocyanidins (as equivalent of (þ)-catechin) and a small amount of phenolic acids (1.10 mg/g as equivalent of chlorogenic acid) and flavonoids (1.55 mg/g as equivalent of 3-O-rutinoside quercetin) [2]. Proanthocyanidins have antioxidative properties and may protect biomolecules (lipids, DNA, and proteins) exposed to reactive oxygen and nitrogen species, including peroxynitrite (ONOO-) [3]. The effects of proanthocyanidin fraction from *Medemia argun* nuts on oxidative/nitrative protein damages and on the amount of glutathione in human blood platelets and plasma after treatment with peroxynitrite were studied in vitro [2].

The proanthocyanidin fraction from *M. argun* nuts can be useful as a protecting factor against oxidative/nitrative stress associated with different diseases such as cancer, cardiovascular, and neurodegenerative diseases so, proanthocyanidins of M.argun nuts may be a promising antioxidant. [4]

Acute toxicity of a drug can be determined by the calculation of median lethal dose (LD_{50}), i.e., the dose that will kill 50% of animals of a particular species of the tested animals [5].

Dose-response determines the required dose and frequency as well as the therapeutic index for a drug in a population. The therapeutic index (ratio of the minimum toxic concentration to the median effective concentration) helps in determining the efficacy and safety of a drug. Increasing the dose of a drug with a small therapeutic index increases the probability of toxicity or ineffectiveness of the drug [6].

In the present work, we have reported median lethal dose (LD_{50} value) of *M.argun* and dose response curve of several doses of the extract against carbon tetrachloride (CCL4) treated animals to evaluate its hepatotoxicity.

2. MATERIALS AND METHODS

2.1 Preparation of *Medemia argun* seed's extract

in collaboration with researchers in the botanical garden, Aswan University, we kindly got *Medemia argun* (MA) fruits. Extract was prepared by using the method of Azwanida (2015) [7] who described that, The seeds of the plant were separated from their fruits and put in a dry and clean place for 5 days, then crushed into small particles by a grinder, the particles of the seeds were weighted and kept for 2 days in a conical flask containing 70% ethanol, The mouth of the conical flask was covered with aluminum foil and kept in a shaker for 48 hours for continuous agitation at 150 rev/min for full mixing of active materials to dissolve in the respective solvent. The solvent was filtered by using filter paper, then extract was removed by using rotary vacuum evaporator at temperature of 45°C. Finally, the crude extract were collected in petri dishes and kept in a dry and clean place for 2 days to be ready for use in any biological experiment.

2.2 Median lethal dose (LD_{50}) experiment

Median lethal dose (LD_{50}) experiment was carried out on 40 male healthy male albino mice (20-25 g) using method of Vahdati-Mashhadian et al, (2005) [8] with brief modifications as shown in (Table 1).

2.2.1Animals and experimental design of the experiment

Adult male albino mice were used for the experimental investigation in this study. Animals were supplied by animal house of Biochemistry Department, Faculty of Science, Cairo University, the animals were housed in polyethylene cages in a moderately humid room under a controlled 12 hours light and 12 hours dark cycle, they were kept under constant environmental and nutritional conditions with free access of food and water *ad libitum*. Animals were kept 2 weeks for acclimatization before starting the experiment.

Table (1): Design of LD_{50} experiment of M.argun ethanolic extract on male albino mice,

the extract was administered intraperitoneally in graduated doses from 25 mg/kg to 300 mg/kg. The vehicle of extract was saline.

Groups	Doses (mg/ kg)	Total no. of mice	no of injection
1.	25	4	Daily for 2 weeks
2.	50	4	Daily for 2 weeks
3.	75	4	Daily for 2 weeks
4.	100	4	Daily for 2 weeks
5.	125	4	Daily for 2 weeks
6.	150	4	Daily for 2 weeks
7.	175	4	Daily for 2 weeks
8.	200	4	Daily for 2 weeks
9.	250	4	Daily for 2 weeks
10.	300	4	Daily for 2 weeks

2.3 Histopathological study

In the present work, liver tissues were checked by histological examination. The liver specimens were rinsed in saline solution to remove excess of blood, then fixed in 70% ethyl alcohol. Fixed tissues were routinely processed for obtaining paraffin blocks which were cut by using microtome to 5 µm sections [9]. Those sections stained with hematoxylin and eosin (H.E) stains for histological examination [10], and the second group were stained with Masson's Trichrome for immunohistochemical examination to access collagen accumulation [11].

2.4 Dose response curve

This experiment was carried out on 36 healthy male albino rats with a body weight (100-150 g). Animals were randomly divided into 9 groups, 4 animals in each group, animals were injected intraperitoneally by CCL4 (3ml/kg), 50% v/v solution of CCL4: olive oil (Twice/week for 6 weeks) to induce fibrosis [12], After that, *M.argun* extract with different doses was injected to the groups, after 6 weeks of treatment, blood samples were collected and centrifuged to obtain serum. Alanine transaminase (ALT) was measured in the serum of all animals to obtain dose response curve.

Table (2): Design of Dose response curve experiment, animals were injected intraperitoneally by CCL4 for six weeks, after that treated with *M.argun* extract at different doses for 3 weeks.

Groups	Saline	CCL4 (3ml/kg) twice / week for 6 weeks	MA (mg/ kg) Twice/ week for 3 weeks
1	(3 ml/kg) Twice/ week for 9 weeks	____	____
2		(3ml/kg) of 50% v/v solution of CCL4 in olive oil,	25
3			50
4			75
5			100
6			150
7			175
8			200
9			300

3. RESULTS

3.1 Median lethal dose of *Medemia argun* seeds' crude extract

The results showed that the LD_{50} of the ethanolic extract was (200 mg/kg) that half of animals in the group were dead (Table 2). The result showed the safe dose was up to (150 mg/kg) where there was no mortality was reported (Figure 1), so half of median lethal dose (100 mg /kg) was more effective according to histological examinations (Figure 2).

3.2 Gross and histological examination of liver in the different groups.

Gross examination of liver showed that the treatment with half of median lethal dose of *M.argun* (100 mg/kg) showed normal pattern with reddish colored appearance of liver. Microscopic examination of liver sections stained with Hematoxylin/eosin stain showed normal hepatic appearance with normal hepatic cords, normal architecture and uniform nuclei. Examinaion of liver sections stained with Masson's Trichrome showed normal distribution of the collagen fibers. (Figure 2).

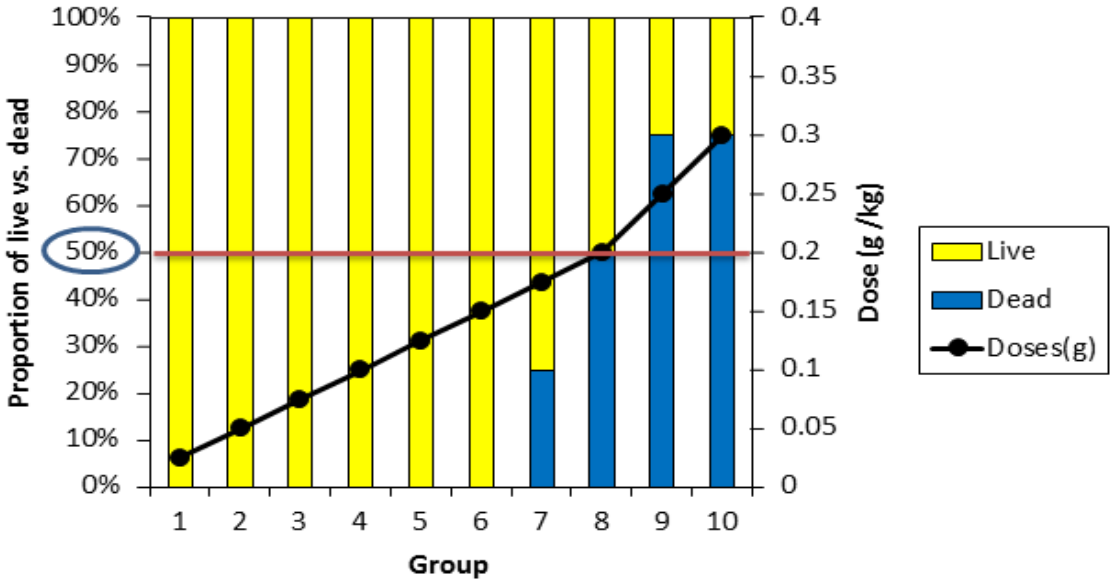

Figure 1. Calculation curve of LD_{50} experiment, the figure showed that the LD_{50} of the *M.argun* ethanolic extract was (200 mg/kg) "group8" , as the result show the safe dose" group 6" was up to(150 mg/kg) that there was no dead animals.

Table (3): Determination of median lethal dose (LD_{50}) of *Medemia argun* seeds' crude extract, the results showed that the LD_{50} of the ethanolic extract was (200 mg/kg), and show no mortality up to dose (150 mg/kg).

Groups	Doses (mg)	no. of mice	Dead mice	Live mice	% Dead	% Live
1	25	4	0	4	0	100
2	50	4	0	4	0	100
3	75	4	0	4	0	100
4	100	4	0	4	0	100
5	125	4	0	4	0	100
6	150	4	0	4	0	100
7	175	4	1	3	25	75
8	200	4	2	2	50	50
9	250	4	3	1	75	25
10	300	4	3	1	75	25

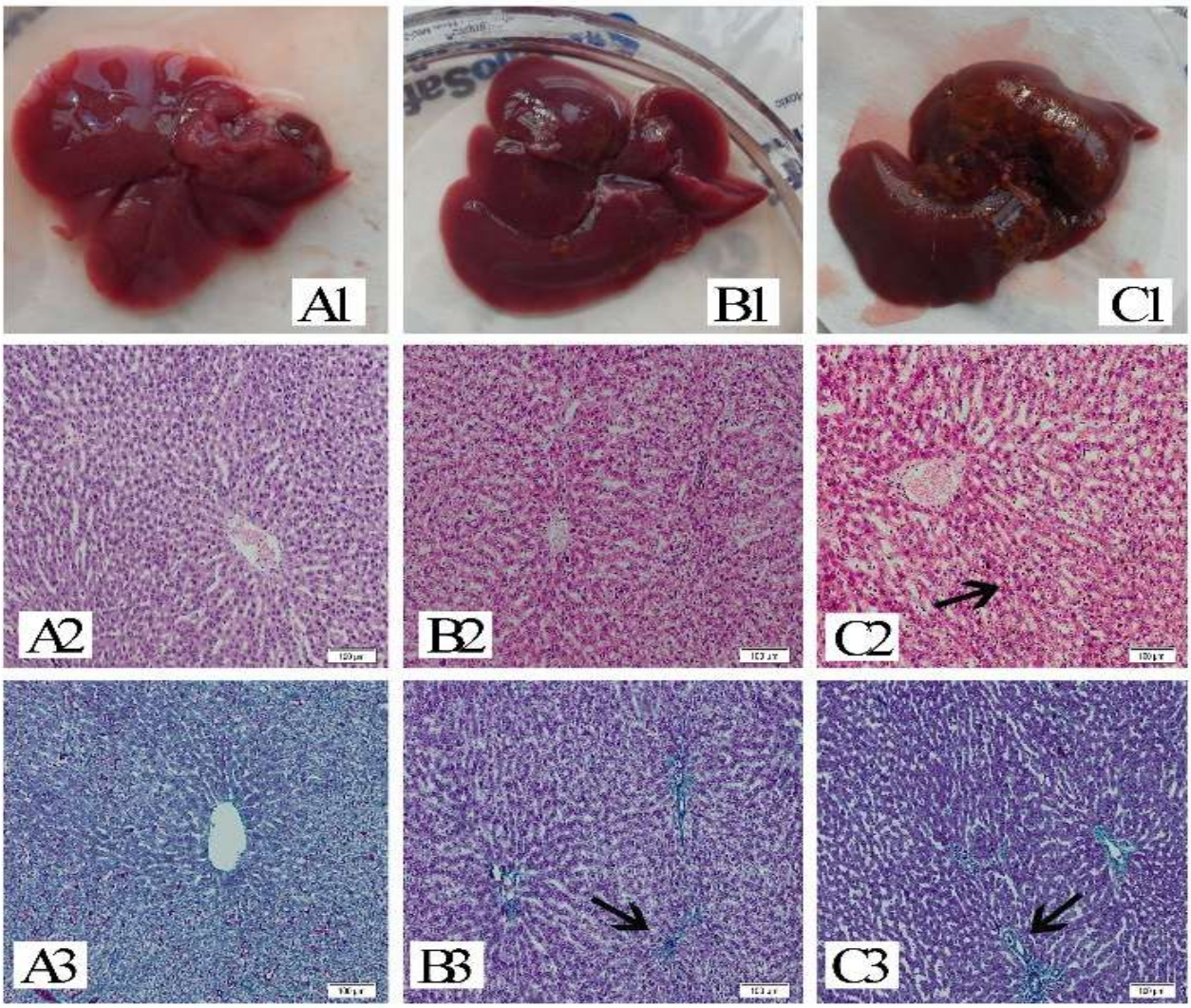

Figure 2. Gross morphology and T.S sections of experimental animal's liver tissue

(A) Normal liver tissue of mice injected with saline only (0.9 NaCl), **(B)** Liver tissue of mice injected by *M.argun* extract with a dose (100mg/kg), **(c)** Liver tissue of mice injected by *M.argun* extract with dose (300mg/kg), power of magnification was 10 X.

(A1) Liver morphology showed normal pattern with reddish color appearance, (B1) Normal hepatic appearance, and (C1) Normal appearance with darker color than normal. (A2, B2) Photomicrographs of liver sections stained with Haematoxylin and eosin stain showed normal hepatic appearance with normal hepatic cords (C2) shows normal hepatic cords with patches of inflammatory cells (arrow). (A3, B3) Examination of liver sections stained by Masson's trichrome of these groups revealed normal distribution of the collagen fibres with fine few collagen fibres (stained blue) around the portal tracts as well as around the central veins (arrow). (C3) showing few periportal proliferation of collagen fibers (arrow).

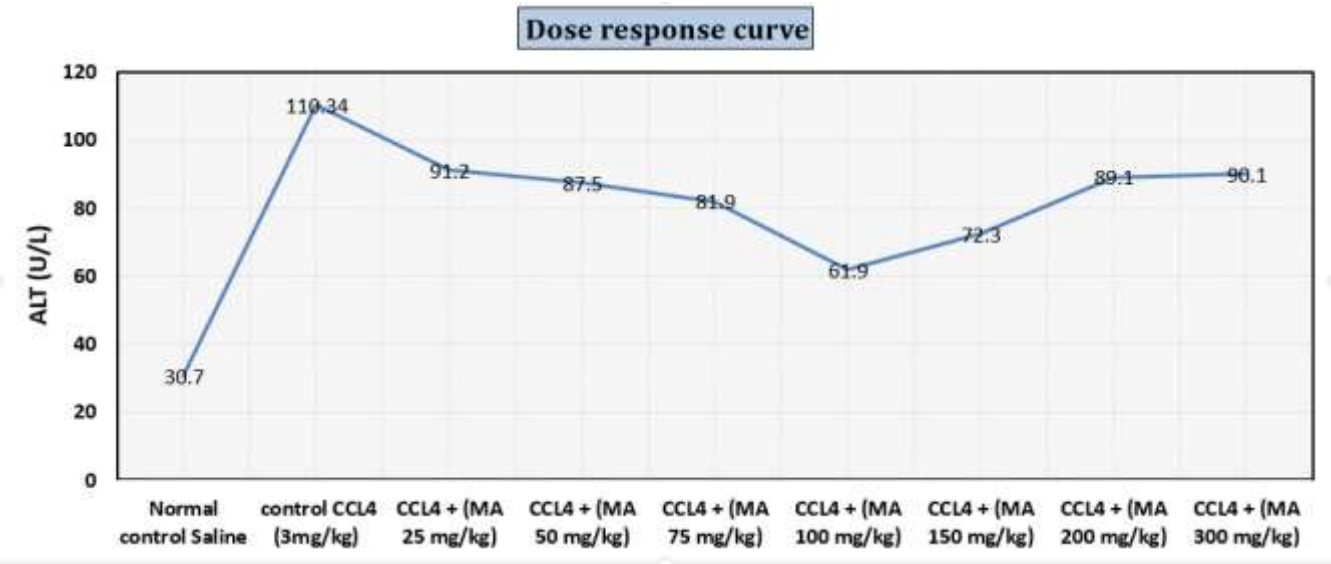

Figure 3. Dose response curve, showed ALT level after treatment with CCL4 and *M.argun* at different doses. ALT was highly increased in CCL4 treated group and decreased after *M.argun* treatment. The dose (100 mg/kg) was more effective than other doses.

4. DISCUSSION

Medemia argun is an extremely rare species of fan palm from the Nubian Desert in southern Egypt and northern Sudan. It is ecologically important as one of the few, large trees in the Nubian Desert, providing shelter and food for desert wildlife. In ancient Egypt, the fruits of this palm were widely distributed and were highly valued, as confirmed by their frequent occurrence in offerings in the tombs [1].

Results of acute toxicity test with ethanolic extracts of *Medemia argun* seeds showed a wide margin of effectiveness. These results are in agreement with other studies that showed a high degree of safety in the acute systemic administration of seed extracts because proanthocyanidins of *medema argun* as well as *Nigella sativa* seed is a major component of their ingredients [19]. Proanthocyanidins have antioxidative properties and may protect biomolecules exposed to reactive oxygen and nitrogen species [2].

Gross anatomy of normal liver tissue showed normal pattern with reddish colored appearance gradually become darker than normal in the dose (300 mg/kg) of *M.argun* extract. The current results are in agreement with Kambara et al, (1970) [13] who found that most of the hepatic cells showed a higher electron density than those of the control animals. Such cells corresponded to cells staining darkly with toluidine blue, observed with light microscopy. These cells are similar to the so-called dark cell described in rat liver following administration of ethionine, thioacetamide, after circulatory arrest of the liver or ligation of the common bile duct, or in partial starvation.

Histopathological examination of mice liver tissues of normal saline treated group and dose (100 mg/kg) of *M.argun* showed normal hepatic appearance with normal architecture and uniform nuclei. On the other hand, treatment with *M.argun* extract at the dose of (300 mg/kg) showed normal hepatic cords with patches of inflammatory cells which indicated that the large doses of *M.argun* caused hepatic injury to the healthy liver that may lead to fibrosis. Our results are in agreement with Husain et al, (2018) [14] who found that growth factors such as cytokines and other chemical messengers are secreted by inflammatory immune cells which in turn activate hepatic stellate cells that lead to the synthesis of collagen.

Examination of liver sections stained by Masson's trichrome to access collagen accumulation in the liver, the dose of (300 mg/kg) of *M.argun* showed few periportal proliferation of collagen fibers as a result of healing response due to inflammation as fibrosis is one of the wound healing response of inflammatory tissues that collagen, proteoglycan, and glycoprotein deposition in the liver tissues leading to fibrosis. [15].

Serum transaminase (ALT) is one of the known indicators for liver function and its elevated levels is a sensitive marker of hepatic injury [16]. Alaline transaminase is a good indicator for liver damage

because elevated activity of serum ALT due to CCL4 induced hepatic injury and subsequent leakage of these enzyme from the neoplastic cell into circulation [17]. Dose response curve showed that ALT enzyme was highly increased in CCL4 treated group due to fibrosis and showed significant decrease after *M.argun* treatment, and the dose (100 mg/kg) was more effective than other doses where the ALT enzyme was decreased but still more than normal control. [18].

5. CONCLUSION

The intraperitoneal acute toxicity in the present study reported that the LD_{50} of *M.argun* was (200 mg/kg). On the other hand, the safety doses were up to (150 mg/kg). The promising results of this study emphasize a great margin of effectiveness and safety for *Medemia argun* seeds in many scientific fields could be approach of further research in the future.

List of abbreviations

MA: *Medemia argun*;
LD_{50}: median lethal dose;
H&E: Hematoxylin/Eosin;
IP: Intraperitoneal;
PACs: Proanthocyanidins;
CCL4: carbon tetrachloride.

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