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Studies on Acute Toxicity (*LD50*) and Histopathological Effects of Methanolic and Aqueous *Conocarpus lancifolius* Extracts in Mice

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ABSTRACT

The aim of present study was to determine the acute toxicological and histopathology effects of crude extracts (aqueous and methanolic) of *Conocarpus lancifolius* leaves on mice, using arithmetic method of Karber for the calculation of LD50. Test extracts were administered orally to groups of male mice at dose levels of (1000, 2000, 3000, 4000, 5000 and 10000) mg/kg (body wt.) Signs of toxicity and possible death of animals were monitored for (24) hr. to ascertain the median lethal dose LD50 of the extracts. At the end of study, all the animals in all dose groups were sacrificed and the internal organ-body were compared with values from the control group. The result shows that LD50 is higher than (10000) mg/Kg (body wt.) for both types of extracts according to Hodge and Sterner method, the extracts were practically non-toxic while the histopathological results reveal serious pathological changes were found in experimental organs like liver and kidney, hepatoxicity and nephrotoxicity chiefly present at dose (5000) mg/ Kg, which regarded as conclusive evidence to the active ingredients in extracts.

INTRODUCTION

Conocarpus lancifolius, family Combretaceae, which is commonly called buttonwood or button mangrove, is a tropical and subtropical evergreen tree [1]. It is widely distributed as an accessories tree in many countries around the world. The tree is about 6m tall with spreading crown or brown bark with green leaves and greenish flowers in dense cone-like heads in terminal panicles [2]. In folk medicine *C. lancifolius*, has many uses like anemia, catarrh, conjunctivitis, diabetes, diarrhea, fever, hemorrhage, orchitis, skin ulcers and syphilis [3-5]. At the present time, there is the tendency for studying the plants adverse and toxic effects because the safety and efficacy data are available for only a few plants. Acute toxicity LD50 is test used single dose of the material in each animal only for one time to determine the gross behavior changes and death occur to 50 % of the tested animals; which is usually considered as the first step in the assessment and evaluation of the toxic characteristics of a substance to accede the lethality of herbal extract and used histopathology changes of vital organs like liver and kidney to exist the lethality of doses [6].

The aim of present study was to determine the acute toxicological and histopathology effects of crude extracts (aqueous and methanolic) of *Conocarpus lancifolius* leaves on mice.

Further information

Conocarpus lancifolius leaves collection:

The herb plant used in present study was purchased from local garden around the status of research center in Baghdad, Iraq as green leaves and authenticated by botany specialist.

Preparation of aqueous extract:

100 grams powder of *Conocarpus lancifolius* leaves macerated with 1000 ml of water using shaker device (SI-600R) for (2) hr. at room temperature. The extract was filtrated then dried using (BUCHI Mini Spray Dryer B-290). 11.5 grams of aqueous extract was obtained.

Preparation of methanolic extract:

200 grams powder of *Conocarpus lancifolius* leaves macerated with 1000 ml of methanol using shaker device (SI-600R) for 1 week at room temperature. The extract was filtrated then dried using (BUCHI Mini Spray Dryer B-290). 9.5 grams of methanolic extract was obtained.

Animals:

Seventy male mice with a body wt. ranged (25-30) grams, were maintained in an air-conditioned room (25±1)^{0}C with (12) hr. light: (12) hr. dark cycle. Standard pellet diet and water were provided daily. All animals fasted overnight before dosing and terminal necropsy.

Extract dosage preparation:

Each mouse was administered at a dose of 0.1/10 ml/ gm body wt., which was calculated carefully to obtain the LD50 of aqueous and methanolic extract of *Conocarpus lancifolius* used in this study.

Acute Toxicity measurement:

The study was performed to evaluate the acute toxicity of aqueous and methanolic extract of *Conocarpus lancifolius*. Seventy male Balb-C mice were divided equally into two groups and each group treats with one type of extract with doses subsequently (1000, 2000, 3000, 4000, 5000 and 10000) mg/Kg (body wt.) while a control animal received distilled water only, all group treated with extract orally by gastric gavage needle. After 24 hr., the signs of acute toxicity and mortality initially were determined and recorded in each group.

Calculation of Median Lethal dose (LD50):

For each mouse, the observations were made for 24 hr. and symptoms of toxicity and rate of mortality in each group were noted. At the end of study period, expired animals were counted for the calculation of LD50. The arithmetic method of Karber (1931) was used for the determination of LD 50.

$$LD50=LD100-\sum(a \times b)/n:$$

n= total number of animal in group.

a= the difference between two successive doses of administered extract/substance.

b= the average number of dead animals in two successive doses.

LD100= Lethal dose causing the 100% death of all test animals.

Hodge and Sterner scale (Table 2) was used for the evaluation of toxicity with the help of LD50 [7].

Histopathology study:

Immediately after death of animals, the organs (kidney and liver) were fixed in 10 % formalin. After dehydration, clearing and infiltration the tissues were embedded in paraffin wax and sectioned 7 µm by using Leica RM 2145-rotation microtome

RESULTS AND DISCUSSION

LD50 Value:

The study of acute toxicity of *Conocarpus lancifolius* extracts shows different signs. The experimental mice treated with different oral doses of extract shows appreciable changes in physical activity and signs of toxicity in table (1), while there are no signs of mortality up to (72) hr. post-treatment, indicating that the LD50 of the crude extracts in rats is significantly less than (1000) mg/kg. LD50 values were calculated by Karber analysis within 95% confidence limits.

Table 1: LD50 dose determination and mortality rate of aqueous and methanol extract of *Conocarpus lancifolius* after oral administration

Solvent	Dose mg/kg	Log dose	Mortality rate	Mortality ratio (x/N)	Symptom after 24hr.
Aqueous	1000	3	0	0/5	Nil
	2000	3.3	0	0/5	Nil
	3000	3.4	0	0/5	Twitching, increase heartbeat
	4000	3.6	0	0/5	Twitching, increase heartbeat, titan in the skin hair, corner sitting.
	5000	3.7	0	0/5	Accelerated heartbeat, titan in the skin hair, corner sitting with sign of sedation
	10000	4	0	0/5	Accelerated heartbeat, titan in the skin hair, corner sitting with sign of sedation

70% methanol	1000	3	0	0/5	Nil
	2000	3.3	0	0/5	Nil
	3000	3.4	0	0/5	Twitching, increase heart beat
	4000	3.6	0	0/5	Twitching, increase heartbeat, titan in the skin hair, corner sitting.
	5000	3.7	0	0/5	Accelerated heartbeat, titan in the skin hair, corner sitting , sedation
	10000	4	0	0/5	The animal after 2hr. appear sign of accelerated heartbeat, titan in the skin hair, corner sitting with sign of sedation
Control	10 ml/kg of D.W	---	----	----	Nil

Table 2: Hodge and Sterner Toxicity Scale

No	Term	LD50
1	Extremely Toxic	Less than (1) mg/ kg
2	Highly Toxic	(1 – 50) mg/ kg
3	Moderately Toxic	(50 – 500) mg/ kg
4	Slightly Toxic	(500 – 5000) mg/ kg
5	Practically Non-Toxic	(5000 – 15000) mg/ kg

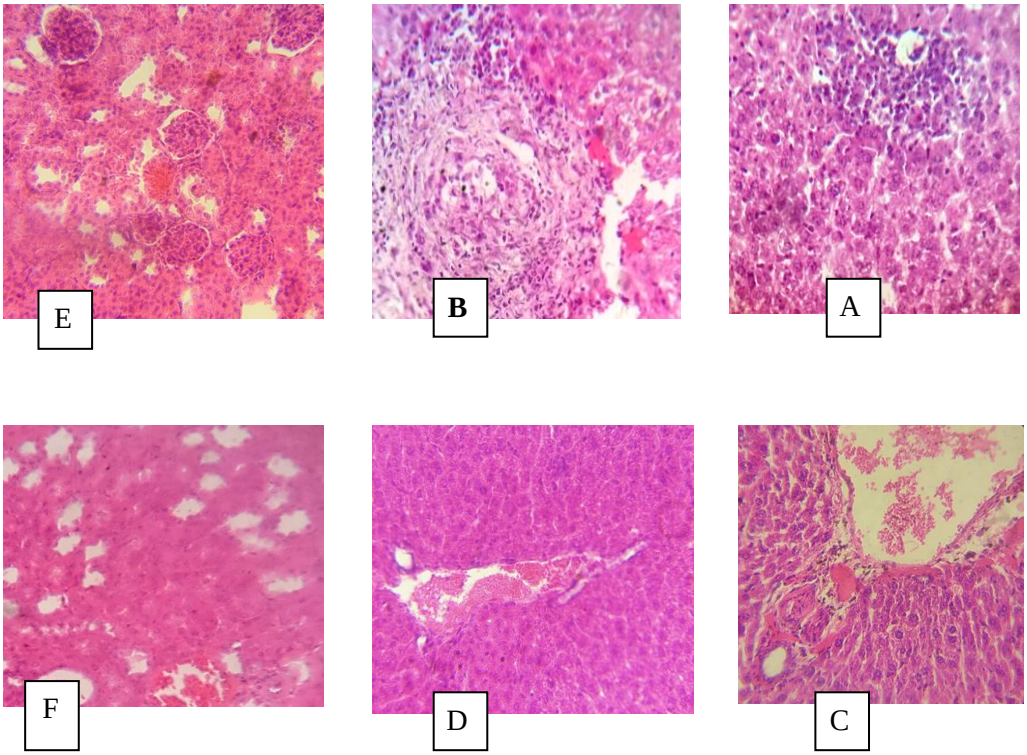

Figure (1): show the histopathological changes in liver and kidney of mice with different solvent and doses: section(A) liver of mice treated with (4000) mg/kg of alcoholic extract, (B) liver of mice treated with (4000) mg/kg of aqueous extract, (C) liver of mice treated with (5000)mg/kg of alcoholic extract , (D) liver of mice treated with (5000) mg/kg of aqueous extract, (E) kidney of mice treated with (5000) mg/kg of alcoholic extract, (F) kidney of mice treated with 5000mg/kg of aqueous extract

Gross pathologic observations:

Gross appearance of internal organs like (liver and kidney) of treated mice show abnormal texture, shape, size or color. The changes like paleness of color and looseness of texture of liver which mainly appear in large doses of both aqueous and methanol extract group compared to the control group.

Histopathological examination:

The results of histopathological changes in kidneys and liver of dead mouse are shown in figures 1. The results revealed that in the kidneys there was congestion of blood vessel, swelling epithelial cells lining of renal tubules that cause obstruction of some renal tubules lumen, hypertrophy and congestion of glomeruli, infiltration of inflammatory cells,

perivascular fibrosis, cystic dilation of some renal tubules, degenerative changes in some renal tubules. On the other hand, in the liver cell show pleomorphism, congestion of some central veins, loss of hepatic tissue architecture, degenerative changes of hepatic tissue single cell necrosis (apoptotic bodies), centrilobular necrosis, perivascular cuffing with inflammatory cells.

DISCUSSION

This study was designed for examining toxicity or lethal dose LD50 of aqueous and methanolic extracts of *Conocarpus lancifolius* herb. Due to lack of resources found, we decided to study acute toxicity LD50 by giving single dose of both extracts and observe behavioral and physiological changes that appear on the laboratory animal during the administration. Finally, to confirm the study, we conducted histological assessment of the tissue changes through a histopathological examination of liver and kidney to evaluate the changes caused by various extracts. In this study, the lethal dose LD50 of *Conocarpus lancifolius* administered to mice orally was up to 5000 mg/kg (body wt.), while there was no evident of death occurred among animals when given different doses of extract but instead it observed signs of change in behavior probably due to affected the function of many organ systems of the mice which that mainly appear in histological section [8].

As a result of high doses that were given, the laboratory animal shown abnormal responses such as increased heart rate, corner sitting, hair erection and sedation may be due to damage to many parts of the body cells may be that lead to great lack of blood and food durations to the cells and thus got shortfalls in the amount of oxygen needed by the body and it was seen clearly through the animal stillness or sedation with increased respiratory rate, offsetting for the shortfall in that. The mice that received 1000-2000 mg/kg dose of the extract survive and doesn't show any signs compare with the other doses from 3000 mg/kg, the signs where slight then increases in (5000) mg/kg (body wt.), the extract could be classified as practically non-toxic, since LD50 was found to lie between 5 gram and 15 gram/kg [9, 10].

From ancient time, folk herbs and botanical drugs have been widely adapted as primary therapeutic agents for treating various illnesses according to that there is a great need to look into their acute toxicity effects. Toxicity tests are not designed to study the safety of these plants, but to point the toxic effects that can produce [11], but to determine the safety margin of the extract. Accordingly to this study, both extracts of *Conocarpus erectus* did not induce

lethality in mice when administered orally at doses of began from 1000 till reach to 10000 mg/kg. This result suggests that LD50 of the extract would be greater than 10000 mg/kg [12]. Hence, the plant extract can be assumed practically non-toxic [13]. In other way, the liver of dead animal seen grossly pale in color and shrinkage in size that given information about the state of the animal and that changes in liver architecture mainly seen at high doses of extract between 4000-5000 mg/kg (body wt.)., that could be due to great damage of liver cells and clear indication of the toxicity and adverse effects of *Conocarpus lancifolius* toxic content present in herb but in small quantity which appear as defect in hepatic cells but did not show any sign of death in lab animal [14,15].

The activity of herbal extract significantly depend on the total phytochemical content and that parameter was mainly dependent on solvent polarity, thus the higher solvent polarity with better solubility given higher activity because that lead to higher solubility of compounds [16,17] while water is universal solvent and both solvent widely used in herbal extraction. According to that fact perhaps revealed the increase in histopathological changes mainly found in high doses due to more phytoconstituents dissolve and the emergence of symptoms [18]. Changes in obvious organ is an important index of physiological and pathological status of animals and diagnose sign to injury of organ [19], shrinking of the liver it may confirm the reality that liver play a central role in metabolizing and excretion of toxic chemicals and that make it more susceptible to the toxicity from another organ [20]. For this reason, we note that most of the side effects of toxic substances in the plant showing its impact on liver. While in kidney, there was hypertrophy and congestion of glomeruli, cystic dilation of some renal tubules, degenerative changes in some renal tubules and reduction in the glomerular filtration was that perhaps good signs to metabolic disturbances and toxic effects of herbal extract were corroborated by the histological deteriorations [21]. On the other side, the damage in cell tissues of selected organ may be due to rupture of cell because the fluid becomes outside the cells in a little amount like sodium and other electrolytes in comparison to that inside then to balance its concentration the fluid shift through into the cells which accompanied mainly with hemorrhage of blood vessels [22]. Also there was hepatotoxicity seen as great damage in interstitial component of cell like mitochondria caused by herb itself or reactive metabolites which can trigger oxidative stress lead to severe ATP depletion and finally cells necrosis and there are many signs pointed to the amount of Alkaloids which have major bioactive compounds in *Conocarpus lancifolius* herb may have slightly accountable for the defect present. In this study, the mainly defect or tissue changes have emerged in high doses, there

is clear sign there was a relationship between a number of toxic substances found in the plant and the extent of the existing poisoning in main organ (kidney and liver). Both extract cause defect in glomerular filtration and decrease in excretion of substances in the urine lead to increased sodium ion concentration and that increase could be an indication of dehydration. So the increase in sodium ion which mainly conjugated with decrease in potassium ion and that may suggest to an adverse effect on the kidney [23]. Also, there was glomeruli atrophy present in mice treated with higher dose of both extracts may be due to slow circulation or tissue hypoxia with signs of congestion and hemorrhage due to impaired outflow of venous blood from the tissue and severe vascular injury or depletion of coagulation factors respectively at high doses. In other way, there were cytoplasmic vacuolations (Hydropic degenerations) in the hepatocytes located towards the periphery of the hepatic lobules around the central veins and observed in the interstitial cells of the kidney mainly in high doses (5000) mg/kg (body wt.) of both extract due to disturbance in lipid inclusions and fat metabolism as result to collect of injured substances in the cell [24] or to the effect of secondary metabolites in the extracts like : alkaloids, flavonoids and phenols [25] which contain free radical scavenging molecules that can cause damage in major tissues [26]. There are increased inflammatory reactions observed in the present study may be associated with the cellular and tissue damage caused by both extracts in both liver and kidney, suggesting that these two organs are capable of being damaged by the extracts.

CONCLUSION

In conclusion, the results of the present study conclude that the confirm lethal doses of both extracts showed clearly that the plant extracts are non-toxic, it is safely less than (1000) mg/ kg while chronic toxicity studies are needed to further support and to increase the safety measurement if decide to use the plant in future.

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