Acute Toxicity Studies of Ethanolic Extract of Eucalyptus Camaldulensis Dehnh Leaves

AZZA DAWOUD H. DAWOUD
PHARMACY PROGRAM, AL-YARMOUK COLLAGE KARTOUM, SUDAN

MOHAMED El HASSAN SHAYOUB
PROFESSOR, PHARMACY PROGRAM, AL-YARMOUK COLLAGE, KARTOUM, SUDAN

SARA MOHAMED El HASSAN SHAYOUB
PHARMACY PROGRAM, AL-YARMOUK COLLAGE, KARTOUM, SUDAN

Abstract – The acute toxicity of the ethanolic leaf extract of *Eucalyptus camaldulensis* leaves was investigated. Acute toxicity was assessed by median lethal does (LD50) using fixed dose procedure (FDP), the LD50 of the extract is ≥5000mg/kg b. wt. The ethanolic extract of *E.camaldulensis* did not show any obvious symptom or signs of toxicity were found in any rat throughout the experimental period, there was no alteration on the liver, renal function and the hematological profile in the rats. (Was in significant difference).

Index Terms – Eucalyptus camaldulensis, Letal dose, fixed dose procedure, Acute toxicity.

1. INTRODUCTION

Medicinal plants have been identified and used throughout human history. Ethno botany (the study of traditional human uses of plants) is recognized as an effective way to discover future medicines. In 2001, researchers identified 122 compounds used in modern medicine which were derived from "ethno medical" plant sources, 80% of these have had an ethno medical use identical or related to the current use of the active elements of the plant. Many of the pharmaceuticals currently available to physicians have a long history of use as herbal remedies including aspirin, digitalis, quinine, and opium. Chemical compounds in plants mediate their effects on the human body through processes identical to those already well understood for the chemical compounds in conventional drugs, thus herbal medicines do not differ greatly from conventional drugs in terms of how they work.

This enables herbal medicines to be as effective as conventional medicines, but also gives them the same potential to cause harmful side effects [13].

Eucalyptus camaldulensis (River Red Gum) is a tree of the genus Eucalyptus, it is one of around 800 in the genus and it is a plantation species in many parts of the world, but it is native to Australia, where it is widespread, especially beside inland water courses.

The tree produces welcome shade in the extreme temperatures of central Australia and plays an important role in stabilizing river banks [10].

2. RELATED WORKS

Many methods have now been developing for measuring the potential harmful effects chemicals irritancy, mutagenic effect, reproductive effect and active toxicity.

In general, the smaller the LD50 value, the more toxic the chemical is. Also, the larger the LD50 value, the lower the toxicity [16].LD50 value can be compared to other values using a toxicity scale. Confusion sometimes occurs because several different toxicity scales are in use.

The two most common scales used are the “Hodge and Sterner scale” and the “Gosselin, Smith and Hodge scale [16].”

Toxicity Rating	Commonly used term	LD50 (Rat , Oral)
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 mg/Kg

Table 1. Hodge and Sterner toxicity scale

These tables/scales differ in both the numerical rating given to each class and the terms used to describe each class. It is important to know that the actual LD50 value may be different for a given chemical depending on the route of exposure (Oral, dermal, inhalation [16].

3. PROPOSED SYSTEM

3.1. Plant material collection and Authentication

The leaves of *Euclayptus camaldulensis* plant were collected during August 2013, from Forest Research Center, they were authenticated by Prof. Mohammed El-mokhtar and prof. Dawoud H. Dawoud, Agricultural Research Corporation (ARC), Federal Ministry of Agricultural and Irrigation, Khartoum, Sudan.

The freshly collected leaves were cleared from any foreign materials and dried in a shade then powdered in a suitable powder form.

3.2. Animals used in screening of hypoglycemic activity

Albino rats (200- 230g) of both sexes were used. Animals were supplied by Medicinal and Aromatic Research Institute (MAPRI), National Center of Research (NCR), Ministry Of Science and Technology (MOST), Sudan.

They were housed under standard environmental conditions at temperature (25±2º C) and light and dark cycles (12/12 h). Rats were fed standard balance diet and water *ad libitum.*

3.3. Preparation of Ethanolic Extracts

60gm of *Eucalyptus camaldulensis* powdered leaves were taken and extracted with soxhlet apparatus ethanol 70%. The solvent was removed under reduced pressure in a rotary evaporator until they become completely dry. The residue was stored at 4°C for further use. Each residue was weighed and the yield percentage was 9.34% [2].

3.4. Toxicological Screening

3.4.1. Determination of Median Lethal Dose (LD50) of Ethanolic extract of *E.camaldulensis* in Albino Rats

The LD50 test was carried out using the fixed dose procedure (FDP). A single dose of 3000mg/kg b.wt. of the Ethanolic extracts of *E.camaldulensis* was administered to 4 healthy albino rats. If the mortality is more than 2, the dose is reduced, but if there is no mortality observed, the dose is increased to 5000mg/kg but thereafter there would be no need to increase the dose [9].

3.4.2. Determination of Acute Toxicity of Ethanolic extract of *E.camaldulensis* in Albino Rats

A modification of [17] was used. 20 healthy Wister albino rats were randomized into 2 groups of 10 animals each. Group1 which served as the control was administered with distilled Water, the control vehicle, Groups 2, was orally administered daily with 5000mg/kg b. wt. of the Ethanolic extracts of *E.camaldulensis* for 30 days. The test animals were observed for lethargy, restlessness, weight loss, appetite and deaths.

After 30 days, the control and surviving rats were sacrificed and organs (the heart, liver, kidney, intestine, lung and spleen) were collected in sterile saline. Freshly dissected organs from each animal were isolated rapidly and fixed in buffered neutral formalin (10%) for at least 24 hours and then were used for histopthological study.

The blood was used for two purpose hematological screening such as: Hb, RBC and WBC, other part was put in test tube and centerifuge at 3000 r.p.m for 5 minutes to seperate serum which then was used to estimate the GOT, GPT, Total protein, albumin, urea, creatinine Na and K.

Groups	Drug administered (mg/kg b. wt.)	Rats number
Group 1	Distilled Water	10
Group 2	5000 mg of ethanolic extract of *E. Camaldulensis*	10

Table 2. Experimental design for Toxicity Test

3.4.3. Histopathological Study

After sacrificing the rats, parts of the liver, kidney, heart, lung, spleen and intestine tissues were collected for histological studies. The tissues were washed in normal saline and fixed immediately in for a period of at least 24 h, dehydrated with alcohol, embedded in paraffin Wax, cut into 4-5µm thick sections, and stained. The sections were examined and photographed by electronic microscope [15]. The microscopic features of the organs of rats were compared with the control group.

3.5. Statistical analysis

The data obtained was statistically analyzed by one way ANOVA and expressed as Mean ± S.E.M.

4. RESULTS AND DISCUSSIONS

4.1. Clinical signs

The rats which received daily 5000mg/kg of *E.camaldulensis* ethanolic extract for 30 days did not show any obvious symptom of toxicity, no obvious clinical signs were found in any rat throughout the experimental period. Physical observation of the treated rats throughout the study indicated that none of them showed signs of toxicity in their skin, fur, eyes, mucus membrane, or behavioral changes, diarrhea, tremors, salivation, sleep, and coma.

4.2. Effect on body weight

Groups	Dose	Mean weight (gm) at zero day	Mean weight (gm) at 30th day	% of weight change
Group 1	5ml of Distilled Water	210.27 ±2.20	216.19 ±3.20	2.81
Group 2	5000 mg/kg b. wt.	221.30 ±1.45	228.4±1.22	3.20

(Data are expressed in mean ± SEM)

(Number of Rats = 10 for each group)

Table 3. Percentages of weight change in Wistar albino rats after oral administration of ethanolic extracts of *E. camaldulensis* for 30 days

Body weights of control and treated rats were presented in table no.3. All the animals of both groups gained weight over the test period and this is considered positive sign. Animals that treated with extracts of *E. camaldulensis* had a higher percentage weight gain than those received distilled Water.

The percentage of increase in weight for treated group was (3.20%), while for control group was (2.81%).

The increase in food and water intake is considered as being responsible for the increment in body weight gain. As mentioned earlier the loss of appetite is often synonymous with weight loss due to disturbances in the metabolism of carbohydrate, protein, or fat. Therefore, the normal food and water intake without loss of appetite are suggested as being responsible for the observed increase in body weight in this study. In addition, the observed increase in body weight could be attributed to insulin mimics effect in the *E. camaldulensis* extract.

4.3. The Mortality rate and LD50

Within the whole test period, there was no any rat death neither from the control group nor treated group with 5000 mg/kg of ethanolic extract of *E.camaldulensis*.

From this result can concider the *E.camaldulensis i*s practically Nontoxic according to [3];(Table No.1) because the LD50 value of extract of *E. camaldulensis* was found to be >5000mg/kg b.wt.

This finding agres with [9], who have also reported that the extract of *E. camaldulensis* is safe.

If during the evaluation of the toxic characteristics of medicinal plants a high dose (e.g., 5,000 mg/kg) is found to be survivable, no further acute testing will be conducted.

In this study, *E.camaldulensis* ethanolic extract at a dose of 5000 mg/kg had no adverse effect on the tested rats up to 30 days of observation, which saddest that the extract is practically nontoxic [16].

4.4. Hematology and *Clinical Biochemistry Analysis*

Table no.4 shows, the results of serum hematology and clinical biochemistry analyses which were done to evaluate the possible alterations in hepatic and renal functions influenced by the extracts. Liver and kidney function analysis is very important in the toxicity evaluation of drugs and plant extracts as they are both necessary for the survival of an organism [12]. High levels of GOT, GPT, and alkaline phosphatase are reported in liver diseases or hepatotoxicity [8].The non-significant changes ($p>0.05$) in GOT, GPT, and alkaline phosphatase in rats of treated group suggest that administration of *E.camaldulensis* extract did not affect the hepatocyte function in the rats.

Parameters	Control group	Group treated with 5000mg/kg exctract of *E.camaldulensis*
Haemoglobin (g/dl)	14.53± 0.04	14.64± 0.24[a]
red blood cells(10^6 /mm^3)	7.56± 0.41	7.66± 0.22[a]
Total white blood cells(10^3/mm^3)	4.93± 0.79	4.70± 0.88[a]
GOT (U/L)	27.17 ± 0.66	26.93 ± 1.47[a]
GPT (U/L)	20.18 ± 13.23	23± 13.63[a]
ALP (U/L)	72.45± 1.17	72.56 ± 1.67[a]
Total protein(g/dL)	6.58± 1.10	6.53± 1.16[a]
Albumin (g/dL)	4.2± 1.68	3.6± 1.2[a]
Creatinine (mg/dL)	0.91± 0.63	0.73± 0.51[a]
Urea (mg/dL)	6.90± 2.23	5.20±3.23 [a]
Na (mg/dL)	137.3± 11.47	141.24± 7.42[a]
K (mg/dL)	3.4± 2.72	4. 1± 2.28[a]

Data are expressed in mean ± SEM) ; [a] = $P > 0.05$

(Number of Rats = 10 for each group)

Table 4. Hematology and clinical biochemistry analysis of wistar albino rats after oral administration of ethanolic extracts of *E. camaldulensis* for 30 days

A decrease in total protein and albumin is a sign of the reduced synthetic function of the liver or might be due to impaired hepatocellular function. Low serum albumin content may suggest infection or continuous loss of albumin [11].Thus, the in-significant change ($p>0.05$) in serum concentration of total protein and albumin in the *E.camaldulensis* extract treated and control group, confirms that the extract does not damage the hepatocellular or secretory functions of the liver at any of the doses tested.

Renal dysfunction can be assessed by concurrent measurements of urea, creatinine and uric acid and their normal levels reflect at reduced likelihood of renal problems [7].In this study, changes in plasma urea and creatinine levels in *E.camaldulensis* extract treated groups showed non-significant differences ($p>0.05$) indicating a normal renal function. As illustrated in table no. 4.

Evaluation of hematological parameters can be used to determine the extent of the harmful effect of *E.camaldulensis* extract on the blood of an animal.

It can also be used to explain blood relating functions of a plant extract or its products [5].Furthermore, such analysis is relevant to risk evaluation as changes in the hematological system have higher predictive value for human toxicity when the data are translated from animal studies [1].The results show no significant effects ($p>0.05$). The non-significant effect of the extract on total red blood cells, total white blood cells and Hb indicates that the *E.camaldulensis* extract does not affect the erythropoiesis, or morphology of the red blood cells [13]. A normal hematological profile of *E.camaldulensis* extract treated groups also further justified the non-toxic nature of *E.camaldulensis* extract.

4.5. Macropathology a*nd Histopathology finding*

The macroscopic examinations of the organs of rats treated with 500mg doses of *E.camaldulensis* extract did not show any changes in colour compared with control group rats' organs. Hypertrophy of organs is first hand indication of toxicity of chemical or biological substance. In addition, the microscopic examination revealed that none of the organs from the extract treated rats showed any alteration in cell structure or any unfavorable effects when viewed under the light microscope using multiple magnification powers.

No pathologies were recorded in the histological sections of the vital organs (heart, liver, intestine, spleen, kidney, and lung) of the control group. Equally, there was also no significant increase in urea and creatinine after administration of *E.camaldulensis* extract when compared to the control group. Any rise in urea and creatinine levels is only observed if there is marked damage to functional nephrons [4].This finding was further confirmed by histopathological observations of the kidney tissue in this study. Therefore, the results recorded in this study demonstrate that the *E.camaldulensis* extract did not alter the liver or renal function and further support the non-toxic nature of *E.camaldulensis* extract.

5. CONCLUSIONS

These results demonstrate that ethanolic extract of *E.camaldulensis* leaves is practically Nontoxic when administered orally safety and nontoxic effect of the.

REFERENCES

[1] Guyton and J. E. Hall, (2000), Textbook of Medical Physiology, W.B Saunder, Philadelphia, Pa, USA.

[2] Harborne,(1999). Phytochemical dictionary: Handbook of bioactive compounds from plants 2nd (Edn.). Health Perspect. 109 Suppl 1 (Suppl 1): 69–75. PMC 1240543.

[3] Hodgson E.,(2004), A text book of modern toxicology; Wily-intersecting, 3rd Edition .

[4] Lameire, W. Van Biesen, and R. Vanholder, 2005 "Acute renal failure," The Lancet, vol. 365, no. 9457, pp. 417–430,.

[5] Olson, G. Betton, D. Robinson et al., 2000 "Concordance of the toxicity of pharmaceuticals in humans and in animals," Regulatory Toxicology and Pharmacology, vol. 32, no. 1, pp. 56–67,.

[6] Slichter 2004, "Relationship between platelet count and bleeding risk in thrombocytopenic patients,"Transfusion Medicine Reviews, vol. 18, no. 3, pp. 153–167.

[7] Yakubu, L. S. Bilbis, M. Lawal, and M. A. Akanji, 2003 "Effect of repeated administration of sildenafil citrate on selected enzyme activities of liver and kidney of male albino rate," Nigerian Journal of Pure and Applied Sciences,vol.18, pp.1395–400

[8] Brautbar and J. Williams II,(2002). "Industrial solvents and liver toxicity: risk assessment, risk factors and mechanisms," International Journal of Hygiene and Environmental Health, vol. 205, no. 6, pp. 479–491

[9] Dickson Achimugu Musa, Fred Okwesili Chiletugo Nwod and Eleojo Ojogbane ,(2011), Phytochemical, antibacterial and toxicity studies of the aqueous extract of *Euclayptus camaldulensis* Dehnh, *Asian* Asian Journal of Plant Science and Research1 (3): 1-10

[10] Fransworth, N.R. (1993). Ethnopharmacology and future drug development: the North American experience. J. Ethnopharma, 38, 145-152.

[11] Klink, B. (1997). Alternative medicines: is natural really better. J. Drug Top. 141, 99-100.

[12] Olorunnisola, G. Bradley, and A. J. Afolayan,(2012)., "Acute and sub-chronic toxicity studies of methanolic extract of Tulbaghia violacea rhizomes in Wistar rats," African Journal of Biotechnology, vol. 11, pp. 14934–14940

[13] Taylor, J.L.S., Rabe, T., McGraw, L.J., Jäger, A.K., Staden, J. (2001). Towards the scientific validation of traditional medicinal plants. J. Plant growth Regulation, 34, 23-37.
[14] Roy Mahoney, (1973), Laboratory techniques in zoology
[15] Senin, R. (2006). Acute toxicity study .Retrieved from (http://www.ccohs.ca/oshanswers/chemicals/ Id50.html) on 27/3/2010.
[16] Sharma, S.C. Mehta, G. Dubey, B. Lakshmayya, S.,(2011),Kaushik, *Der Pharmacia Sinica*, , 2, 4, 99
[17] Gulla, A.J. Singer, R. Gaspari ,(2001), Academy of Emerging Medicine., 8, 450.

Authors

AZZA DAWOUD H. DAWOUD is currently a head of department of pharmacognocy –pharmacy programme- Yarmouk collage Khartoum,Sudan.

Prof. MOHAMED El HASSAN SHAYOUB is currently a dean of pharmacy programme- Yarmouk collage Khartoum,Sudan.

SARA MOHAMED El HASSAN SHAYOUB is currently a teaching assistant at pharmacy programme- Yarmouk collage Khartoum,Sudan