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STUDIES ON ACUTE TOXICITY OF MANGROVE DERIVED PHYTOCOMPOUND BERBERINE IN ALBINO RATS

Thirunavukkarasu Palaniyadi[1], Asha Sivaji[2]

[1]Department of Biotechnology, Dr.M.G.R. Educational and Research institute (Deemed to be University), Maduraivoyal, Chennai-95

[2]Department of Biochemistry, D.K.M College for Women, Vellore, Tamil Nadu India.

***Corresponding author E-mail:** drpthiunavukkarasu@gmail.com

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ABSTRACT

Ceriops decandra is used for traditional folklore medicine in fisherman community. It have diverse pharmacology activities in varies communicable and non communicable diseases. In the previous study we have isolated characterization of berberine bioactive compound from *C.decandra* through analytical techniques.In present study was designed for the acute toxicity of *C.decandra* isolated berberine compound the studies was conducted albino rat. The berberine at dose of 25, 50 and 100mg/kg /bw to three test groups and with normal saline at a dose of 100mg/kg to a negative control group of albino rats were given.The berberine was found to be safe up to the dose of 100mg/kg .The results indicate that the studies plants presents potential for many pharmacological studies with high safety profile.

INTRODUCTION

Medicinal plants (crude extract, pure compound or derivative) are an unlimited source for the discovery of new medicines. Most of the natural products used in traditional medicine have solid scientific evidence regarding their biological activities [1-3].However, little information or evidence is available on the possible toxicity of medicinalplants to the consumers [3].Regarding the discovery and development industry and patients must be taken into consideration [2].The prompt access to safe and efficient medicines, as well as animal welfare, are of primary interest to the general public, patients and consumers. Plant used in traditional medicine could be expected to have Low side effects due to their long term use by local population. Nonetheless, the surveys have indicated that many medicinal plants applied in traditional medicine showed adverse effects [1]. Therefore, the traditional used of any plant for medicinal proposes does not guarantee the safety of this plant. Thus, concerns remain about the potential toxic effect of the short-term and long term use of the medicinal plants. The date from toxicity studies on medicinal plants or other derivatives must be obtained in order to establish their safety for humans essential for the development of pharmaceutical products. Toxicity is the fundamental science of poisons. The organization for Economic and Development (OECD) mentioned acute toxicity as the advance effect occurring within a short time of

oral administration of a simpledose of a substance or a multipledoses given within 24 hours. Phyto chemical interactions of poisons lead to injury or death of living tissues Toxicology is like science and an art like medicine. It includes observational data gathering & data utilization to predict outcome of exposure in human and animals. The ancient humans categorized some plants as harmful and some as safe[4-7]. In the recent years, attention has been focused at the traditional (Herbal) way of therapy. It is presumed that Ayurveda Medicines (drugs), which is popular in ourcountry, have lesser side effects as compared to allopathic drugs. Therefore, considerable attention has been directed towards identification of plants with no toxicity that may be used for human consumption.Coastal vegetation has been traditionally used in fisher -folk medicine [8-9] and an under-explored source of anticancer drugs [10]. The mangrove plants *Ceriops decandra* has been used traditionally and scientifically for its biological activities such as antiviral, antibacterial, antioxidant and chemo-preventative potential [11]. Mangrove like *C.decandra* has been proved to be a potential source of black tea to have the aflavins and the arubigins which resemble constituents of the commercial tea plant. The present investigation is undertaken to determine the acute toxicity present in the extracts by analysis in the extracts of ethanol and n-butanol from mangrove plant, *C. decandra.*

MATERIALS METHODS

1.1. Collection of plant material

Leaves of the mangrove plant, *Ceriopsdecandra* was collected from the Pichavaram mangrove forest (Lat.11^{o} 27'N; Long.79^{o} 47' E), Southeast coast of Tamil Nadu, India. After that the dried specimen was identified (AUOCAS0072)and its halotype has been deposited to herbarium at C.A.S. in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai. The fresh leaves washed in distilled water and air dried at room temperature. The dried leaves were made powder form using electrical blender and stored at 4^{o}C for further extraction.

1.2. Preparation of extracts

One kg of powdered material of *C.decandra* was soaked in 4 L of Ethanol and n butanol for 24 hrs. at 25^{o}C. The extraction was repeated thrice to obtain a sizable quantity of extract, after that the extract were pooled, filtered using Whatmann No. 1 paper and concentrated by using rotary evaporator (Buchi Rotavapor R-124). Finally, the resultant residues of crude extracts were kept at 4^{o}C for further investigation.

1.3. Acute toxicity study and determination of LD50

Prior to administration, animals were weighed and allowed to keep fasting overnight with regular water intake. In order to determine LD50 five groups of rats with similar weight were used. Each group consist of five male wistar rats. Berberine doses that included 20, 40, 60, 80,100 mg/kg were selected, prepared with distilled water and gavaged to rats. Rats were monitored individually during the first 30 min, then for first 24 hr and then thereafter 14 days. Mortality and behavioral symptoms such as mortality, respiratory pattern, changes in general behavior, skin, eyes, fur, and somatomotor activity were noted.

1.4. Haematology and biochemical analysis

For haematological parameters, blood was collected in EDTA tubes and analysed for Total white blood cells, total red blood cell count (RBC), haemoglobin content (Hb), Neutrophils(NP),lymphocytes (LC), monocytes (MC), eosinophils (EP), hemoglobin (Hb), platelet count (PL) and packed cell volume(PCV) were evaluated by automated analyzer (KX-21-Hematology-analyzer, Sysmex Corporation, USA). A portion of the blood was collected in non-heparinized tubes, and the serum was separated after centrifugation at 5000 rpm for 10 min, which was used for the biochemical analysis. Serum glutamic oxaloacetate transaminase (SGOT)[12], Alkaline phosphatase (ALP) [13], urea [14], creatinine [15] was performed.

Table 1: Haematological and biochemical analysis of Acute toxicity study of Berberine in rats

S.No	Parameters	units	Control	100mg/Kg. B. Wt
1.	Total white blood cells	10^9/L	7.42±0.11	7.41±0.09
2.	Total red blood cell count	10^{12}/L	7.59±0.22	7.45±0.13
3.	Neutrophils	%	11.11±1.13	11.23±1.26
4.	Lymphocytes	%	84.49±1.51	84.77±1.23
5.	Monocytes	%	2.25±0.00	2.08±0.04
6.	Eosinophils	%	1.00±0.09	1.07±0.10
7.	hemoglobin	g/L	138.33±3.55	141.02 ± 0.71
8.	Platelet count	10^9/L	868±10.33	870±0.55
9.	Packed cell volume	L/L	0.47±0.05	0.48±0.02
10.	Serum glutamic oxaloacetate transaminase (SGOT)	U/L	73.35±3.21	74.02±3.01
11.	Alkaline phosphatase (ALP)	U/L	136±8.41	135±8.37
12.	Urea	mmol/L	6.19±0.04	6.76±0.48
13.	Creatinine	µmol/L	44.19±0.46	43.78±0.65

1.5. Weight

On completion of the treatment, necropsy was performed on animals. Organs such as heart, liver, spleen and kidney were isolated to determine their weights.

2. RESULTS

2.1. Acute toxicity study and determination of LD50

To achieve acute toxicity, rats were treated with single doses of berberine, orally at various concentrations (20, 40, 60, 80, 100mg/Kg) and observed. There were no significant changes in the physical and behavioural parameters (changes in skin, eye color, diarrhea and sedation) of all groups of rats on 24 h of monitoring. It was also observed that administration of BR at different doses did not cause any mortality. Our experimental results showed that BR in high values, up to 100mg/kg, has no lethal toxicity, therefore, LD50 values were considered to be beyond the concentration for this compound. Clinical records were represented in Table

2.2. Haematological parameters

Data of hematological analysis of 14 day acute toxicity were given in Table 1. No significant differences were observed in haematological parameters (Total white blood cells, Total red blood red cells, Neutrophils, Lymphocytes, Monocytes, Eosinophils, Hemoglobin, Platelet count, Packed cell volume) between the treated groups and the control groups.

2.3. Biochemical parameters

The effects of Berberine on biochemical parameters of test and control rats are presented in Table 12. On oral administration of berberine at the different doses did not cause any significant differences on creatinine, ALP, SGOT and urea levels when compared to control rats(Table1)

3. DISCUSSION

In our study acute oral toxicity of berberine was conducted to evaluate the safety efficacy of berberine at different doses. The study was conducted upto 100mg/kg dose. Administration of berberine for 14 days showed no significant changes in clinical signs in treated groups when compared to control groups. Since there was no mortality it could also be concluded that oral administration of berberine has no effect on rats evaluating the safety of this compound at the concentrations studied. The haematopoietic system is the best profound parameter for evaluating the toxicity of drugs in humans and animals [16]. Present study indicated that berberine has no effect on blood cells production or on their circulation. If the compound has any toxicity, it directly affects red blood cells, white blood cells, platelets, and hemoglobin components, significantly either by increase or decrease in

ranges compared to normal. Since hematological system is an important target for toxic compounds, especially acts on bone marrow where the red blood cells production occurs[17]. This indicates that toxicity of the compound can affect the immune system of the body as well as the organ functions. [18]. Increase in platelet count represents the formation of thrombosis in blood vessel while its decrease indicates the risk of hemorrhage [19,20,21].Liver and kidney are the two vital organs required to assess the proper function of body. Liver is used for metabolism and kidney is used for excretion of waste products [22]. To assess the toxicity of any new compound it is very essential to check the functions of the above said two vital organs through biochemical estimations. To assess liver function, SGOT, SGPT and ALP parameters [23,24]were used and for kidney function assessment the serum urea and creatinine were used. If any changes in those biomarkers from the normal range after the intake of any compound indicates the toxic nature of the compounds in animals [25,26]. Changes in both ALT and AST in serum indicates the liver damage [27,28]. ALT, a cytoplasmic enzyme, present abundantly in liver, released into the blood stream during hepatocellular damage[29]. While AST is present extracellularly in tissues like the heart, skeletal muscles, liver, kidneys, pancreas, and erythrocytes [30]. In addition to hepatic damage any change in membrane permeability of these tissue cells AST gets released into the blood stream. Hence, ALT is more associated with hepatic damage than AST. Moreover, liver is the major site cholesterol breakdown, synthesis of free glucose from glycogen [31]. Relatively to enzymes, elevated level of cholesterol, bilirubin and glucose indicates hepatic function failure [32,33]. During hepatic damage fat gets accumulated in hepatocytes and obstruction in intrahepatic bile duct resulted in elevation of cholesterol level which develops as hypercholesterolemia as persists for prolonged period [34,35]. Renal functions as concern, creatinine and urea level in blood used as a good indicator to evaluate renal damage. High level indicates the renal failure which may be due to damage in nephrons [27,36]. From the study results it was suggested that the levels or activities of examined biochemical parameters in animals after 14 days of treatment showed no significant variations in SGOT, ALP, urea and creatinine levels in tested doses when compared with that of the control animals. These results suggest that acute administration of berberine did not alter liver and renal function.

5. CONCLUSION:

This study provides information on the toxicological profile of *C.decandra* derived berberine. The results obtained suggest that berberine is relatively non-toxic in daily oral administration for a period of 14 days. However, it becomes toxic for 50 days at the doses of more than 100mg/kg bw. These uphold indigenous knowledge on its safe folkloric use in fisher man community and provide justification for specifically designed studies to investigate other beneficial pharmacological effects and clinical studies.

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Abbreviations:

ALT-AlanineAminotransferase, ALP-Alkaline phosphatase, SGOT-Serum glutamic oxaloacetate transaminase, AST-Aspartate Aminotransferase, LD 50-Lethal dose 50, EDTA- Ethylenediaminetetraacetic acid, OECD-organization for Economic and Development.

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