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EVALUATION OF ACUTE AND SUB ACUTE TOXICITIES OF LEAF EXTRACT OF *CAESALPINIA BONDUCELLA* (L.) FLEM

KAVITHA SAGAR[*1] *and G M VIDYASAGAR*[2]

[1] Central Institute of Medicinal and Aromatic Plants, Allalasandra, Bangalore-65.
[2] Microbial biotechnology laboratory, Department of P.G. Studies and Research in Botany, Gulbarga University, Gulbarga- 585 106, Karnataka, India.

**Corresponding author*: kavcsa@gmail.com

ABSTRACT

The acute and sub acute toxicity studies of ethyl acetate extract of *Caesalpinia bonducella* leaves in albino mice were investigated. Various doses of ethyl acetate leaf extract from 800 to 2000 mg/kg body weight were administered orally to the test groups of mice, while distilled water was given to the control group to evaluate the toxicity in mice after ingestion of the extract for one day (acute model) and for 15 days (sub acute model). The behavioral response profile of the treated mice was also evaluated along with other parameters such as, body weight, absolute and relative weight of various organs. Biochemical, hematological and histopathological parameters were analyzed in order to study the time-dependent effect and correlation of the extract. The results indicated that LD_{50} of the extract is higher than 2000 mg/kg and no changes were observed in any behavioral parameters in mice. Body weight of mice increased along with the time in sub acute model. The biochemical (total protein, DNA, RNA, alkaline phosphatase, acid phosphatase, cholesterol, and carbohydrate) and hematological parameters (total RBC and WBC) and histopathological studies of liver, heart, lung and kidney indicated either no or less alteration in the treated group. However, a slight change in liver cellular architecture with hydropic changes in the epithelium, congested sinusoids and dilation of central vein was observed in liver of test animal.

KEYWORDS

Caesalpinia bonducella, acute toxicity, biochemical and histopathology

INTRODUCTION

Indigenous knowledge about the use of plants is time tested through trial and error methods, and is passed on from generation to generation by oral tradition. The traditional use of plants in the treatment of different infectious diseases is widely practiced in India and other developing countries. Natural products in general, and medicinal plants in particular,

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are believed to be an important source of chemical substances with potential therapeutic efficacy. In recent years, there has been a gradual revival of interest in the use of medicinal plants for their pharmacological evaluation in Indian traditional system of medicine as some of the herbal drugs are associated with both harmful as well as beneficial effects to the biologic system.

Caesalpinia bonducella (Fam: Caesalpiniaceae), commonly known as Nata karanja (Hindi), is a prickly shrub found throughout the hotter parts of India, Myanmar and Srilanka. The leaves of this plant are traditionally used for the treatment of tumour, inflammation and liver disorder[1,2]. The plant also exhibits many other therapeutic properties such as, antipyretic, antidiuretic, anthelmintic and antibacterial[3], anticonvulsant[4], anti-inflammatory[5], antiasthamatic[6], nematocidal[7], antihyperglycemic[8], and abortifacient[9] activities. Recently, the hepatoprotective, anti-inflammatory and antipyretic activity[10,11] of methanol extract of *C. bonducella* leaves has been reported. However, the toxicity of ethyl acetate extract of *C. bonducella* leaves has not been studied so far and is attempted here.

MATERIALS AND METHODS

(i) *Plant material:*

Fresh leaves of *C. bonducella* were collected from the plants grown in the campus of Gulbarga University, Gulbarga, Karnataka. The plant was identified and the herbarium was prepared. The voucher specimen was deposited in the Department of Botany, Gulbarga University, Gulbarga with the number HGUG-208.

(ii) *Preparation of extract:*

The plant material was dried under shade, pulverized and finely sieved. 100 g of leaf powder was subjected to soxhlet extraction with ethyl acetate as solvent. The extract obtained was evapourated and stored at 4^{o} C for further use.

(iii) *Experimental animals*:

Sexually matured male and female albino mice weighing 16-25 g were obtained from the University of Agricultural Sciences, Veterinary College, Hebbal, Bangalore, Karnataka, India. The animals were kept in polythene cages (5 mice/cage) and allowed to acclimatize for a week prior to experimental use and maintained under standard environmental conditions (27 ± 2^{o}C) for 12 h light and 12 h darkness. All the animals had free access to water and standard diet. The procedures for laboratory animal care were followed.

(iv) *Acute toxicity:*

The bioassay was conducted according to the World Health Organization guideline for the evaluation of the safety and efficiency of herbal medicines[12]. The animals were divided into control and 14 groups of 6 animals in each (male and female). The control group received distilled water per o.s and remaining groups (2-14) received ethyl acetate extract in a dose ranging from 800-2000 mg/kg body weight. The animals were observed continuously for 4h, and then observed each hour for 24 h for behavioral and physiological activities, body weight and mortality rate. The LD_{50} was determined at the end of 24 h based on Lorke[13] method.

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(v) *Sub acute toxicity*:

For the sub acute toxicity studies two groups of 10 mice each (5 male and 5 female) were given daily with the lowest lethal dose of 1/12th (166.7 mg/kg) and 1/8th (250 mg/kg) of LD_{50} for 15 days. The aim of this test was to evaluate the maximum tolerated dose and also to study the nature of toxic reactions and toxic potential of the extract. Control group received distilled water every day. During the study, the animals were weighed, and food and water intake were monitored every day. On 15th day, all the surviving animals were left for fasting overnight and then sacrificed by decapitation. The blood samples were collected directly by puncturing the heart in the heparinized tube for haemotological parameters. The heart, lung, kidney and liver were collected and weighed. After instantaneous washing, all the collected organs were kept in frozen containers (-20^{o}C) for further analysis of histopathological parameters. A part of liver was used for biochemical analysis.

(vi) *Haemotological assay*:

The total red blood cell (RBC's) and white blood cell (WBC's) counts were determined using haemocytometer.

(vii) *Clotting time*:

A drop of blood of control and treated groups (female and male mice) was collected on a clean slide from tail vein of mice and observed for clotting time. The clotting time was determined by moving a clean needle on the blood drop and as soon the thread like fibrin formed, the coagulation time was recorded.

(viii) *Histopathological study*:

Histopathological investigations of liver, kidney, lung and heart were carried out using the methods given by William[14] and Poddar[15]. The tissue were made into small pieces and fixed in bouin's fixative for 24 h and washed in 70% alcohol, 3-4 changes were given till the colour of the fixative was removed; then the tissue was passed through 90% alcohol for 30 min. After 3-4 changes in 90% alcohol, the tissues were transferred to the mixture of xylene : absolute alcohol (1:1) until the tissue became clear and transparent. Then the tissue was rinsed in benzene and xylene (1:1) and later transferred to xylene for 5-10 min. Embedding was done by passing the cleared samples through three cups containing molten paraffin at 50^{o}C and then cubical block of paraffin were made by the 'L' moulds. It was followed by microtome and the slides were stained with diphenyl pthalate plastisizer xylene. The slides were examined.

(ix) *Biochemical estimations*:

50 mg of liver was homogenized with solvent like, 0.9% NaCl for alkaline phosphatase, acid phosphatase, DNA, RNA, 5% TCA 4% TCA and 1:3 petroleum ether: alcohol, 10% TCA separately and this filtrate was used for biochemical analysis of total protein, carbohydrate and cholesterol [16,17,18].

Statistical analysis:

Values were expressed as mean ± SD. Data were analyzed using the SPSS program. Differences between the control and treated groups were analyzed with student's t-tests. A value of $P<0.05$ was considered to represent a statistically significant difference.

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RESULTS

(i) *Acute toxicity studies*:

The acute and sub acute toxicity of ethyl acetate leaf extract of *C. bonducella* were studied. In the acute toxicity, mice treated with doses from 800 to 2000 mg/kg body weight showed some behavioral changes after 130 min of oral administration. These changes included grooming, restlessness, slight excitability, decreased mobility and aggression. However, the changes observed disappeared after 24 h. Thus, the dose did not produce significant change in behaviour and sensory nervous system responses in male and female mice. During 24 h of the experiment, no death occurred in any of the groups (Table 1).

Table -1: Acute toxicity of the ethyl acetate leaf extract of *C. bonducella* in mice (24h).

Number of mice	Doses of extract mg/kg body weight	Number of mice dead	Percentage of mice dead
6	800	0	0
6	1000	0	0
6	1025	0	0
6	1050	0	0
6	1060	0	0
6	1075	0	0
6	1125	0	0
6	1200	0	0
6	1350	0	0
6	1500	0	0
6	1650	0	0
6	1800	0	0
6	2000	0	0

(ii) *Sub acute toxicity*:
Behavioral profile, body and organ weight:

In the sub acute toxicity study, various behavioural parameters like, awareness, mood, motor activity, CNS excitation, motor incoordinations were studied (Table 2). It appeared that the ethyl acetate extract of *C. bonducella* at 166.7 and 250 mg/kg body weight did not produce any marked change in both male and female mice, as evidenced by the parameters examined (Table 3). No mortality was observed during 15 days of drug

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administration. Mice treated with two doses of the extract (166.7 mg/kg and 250 mg/kg) had gradual increase in body weight, which was significantly ($P<0.05$) different from control. The organs were weighed after sacrificing. No statistically significant differences existed in the actual organ weight between treated and control male and female mice (Fig 1). A significant difference in weight of organ in both control and treated groups (female and male mice) was observed ($P<0.05$). But, a significant decrease in liver weight was observed at 250 mg/kg dose in both female and male.

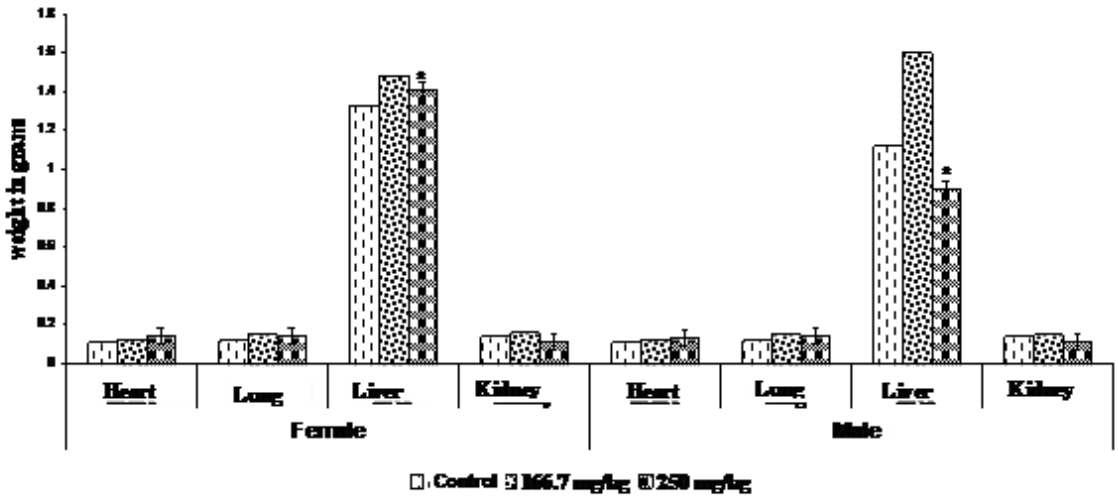
(iii) *Haemotological assay*:

The haematological status after 15 days of oral administration of ethyl acetate extract of *C. bonducella* was assessed (Fig. 2). RBC and WBC counts differed significantly ($P<0.05$) at both the doses, i.e. 166.7 and 250 mg/kg body weight.

(iv) *Clotting time of blood*:

In the sub acute toxicity study, the treatment of extract up to 15 days did not reveal any change in blood clotting time as compared to the control group. Although there was a significant difference in clotting time of both treated and control groups (Table 4).

Fig 1: Effect of Ethyl acetate leaf extract of C. bonducella on organs weight in mice



Data represent mean ± SE of 6 animals. *P<0.05 compared to control.

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Table 2: Behavioural profile of mice in the acute toxicity study of ethyl acetate extract of *C. bonducella* in mice.

Doses mg/ kg	Awareness		Mood					Motor activity			CNS excitation		Motor incoordinations			Autonomic				
	Alertness	Stereotypy	Grooming	Vocalization	Restlessness	Irritability	Fearfulness	Touch response	Pain response	Sedation	Startle response	Convulsions	Body position	Limb position	Pinna reflex	Pupil size	Urination	Salivation	Writhing	Skin color
800	+	-	+	-	-	-	+	+	+	-	+	-	N	N	+	N	-	-	-	N
1000	+	-	+	-	-	-	+	+	+	-	+	-	N	N	+	N	-	-	-	N
1025	+	-	+	-	-	-	+	+	+	-	+	-	N	N	+	N	-	-	-	N
1050	+	-	+	-	-	-	+	+	+	-	+	-	N	N	+	N	-	-	-	N
1060	+	-	+	-	-	-	+	+	+	-	+	-	N	N	+	N	-	-	-	N
1075	+	-	+	-	-	-	+	+	+	-	+	-	N	N	+	N	-	-	-	N
1125	+	-	+	-	-	-	+	+	+	-	+	-	N	N	+	N	-	-	-	N
1200	+	-	+	-	-	-	+	+	+	-	+	-	N	N	+	N	-	-	-	N
1350	+	-	+	-	-	-	+	+	+	-	+	-	N	N	+	N	-	-	-	N
1500	+	-	+	-	-	-	+	+	+	-	+	-	N	N	+	N	-	-	-	N
1650	+	-	+	-	-	-	+	+	+	-	+	-	N	N	+	N	-	-	-	N
1800	+	-	+	-	-	-	+	+	+	-	+	-	N	N	+	N	-	-	-	N
2000	+	-	+	-	-	-	+	+	+	-	+	-	N	N	+	N	-	-	-	N

+ = Present; - = Absent; N = Normal

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Table-3: Body weights of mice in sub acute toxicity after 15 days of oral administration of ethyl acetate leaf extract of *C. bonducella* (Time dependent study).

Body weight (g)			
Female	**Day 0**	**Day 15**	**Weight gained in 15 days**
Control	22.8	31.6	8.8
166.7 mg/kg	21.2	30.6*	9.4
250 mg/kg	20.2	30.8*	10.6
Male			
Control	22	32.2	10.2
166.7 mg/kg	21	31*	10
250 mg/kg	20.4	31*	10.6

*Values are expressed as mean ± SD. n=6. ,*P<0.05 compared with control.*

Fig 2: Effect of Ethyl acetate leaf extract of C.bonducella on haematological parameters

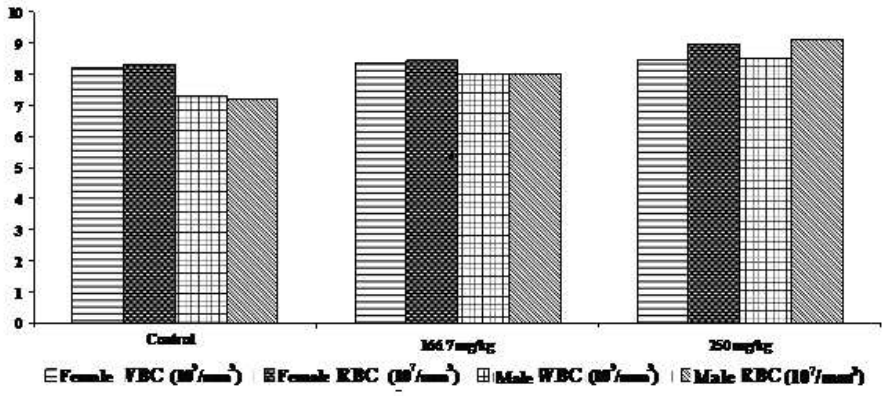

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Table- 4: Clotting time of blood in sub acute toxicity studies of ethyl acetate leaf extract of *C. bonducella* in mice.

Treatment	Female	Male
Control	133.4 ± 4.84	132.9 ± 2.91
166.7 mg/kg	129.9 ± 3.47	132.2 ± 5.49
250 mg/kg	133.6 ± 3.85	134.6 ± 4.72

Values are expressed as mean ±SD. Parenthesis are expressed as range values, n=6

(v) *Histopathological study*:

The macroscopic analysis of the target organ of the treated animal (liver, lung, heart and kidney) did not show significant change in color and texture. Histopathological observations made in kidney, lung and heart revealed no changes, while a slight change in cellular architecture with hydropic changes in the epithelium, congested sinusoids and dilation of central vein was observed in liver (Plate-I).

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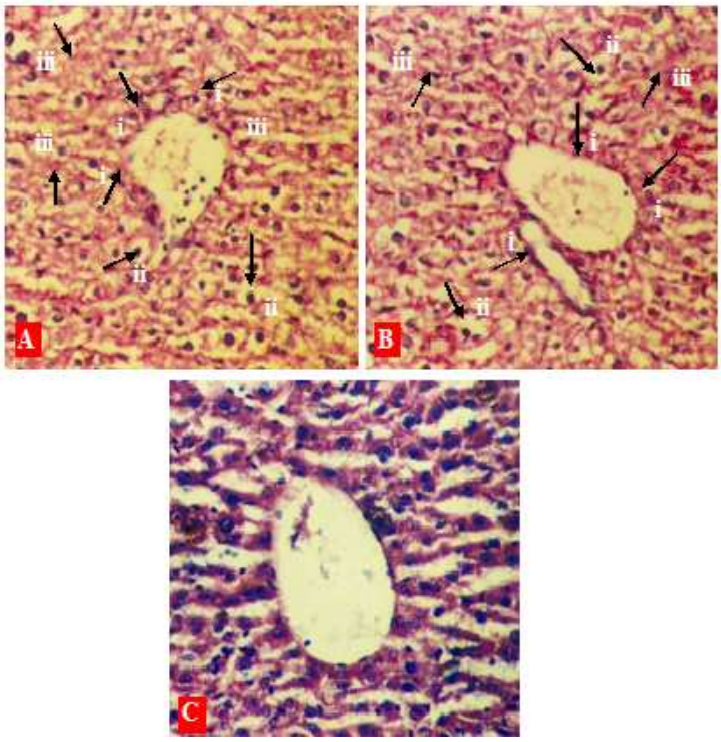

Plate-I: Liver section from ethyl acetate leaf extract of *C. bonducella* intoxicated mice.

A= Liver section of 166.7 mg/kg treated mice
B= Liver section of 250 mg/kg treated mice.
C= Liver section of control mice.
i = Cellular infiltration around the dialated and congested portal vessels and multifocal centrilobullar coagulative necrosis.
ii = Hydrophic changes in epithelium. Vacuolated hepatic cells and size of Von Kupffer cells in dialated blood sinusoids.
iii = Dialated sinusoids.

(vi) *Biochemical estimations*:

The results of the biochemical estimations carried out in liver are presented in figure 3 and 4. On 15th day of treatment, the enzymes estimated in the liver treated with 166.7 and 250 mg/kg body weight concentration of ethyl acetate extract were significantly different ($P<0.05$) in female and male mice. The levels of all the enzymes were found to be gradually increased along with the dose

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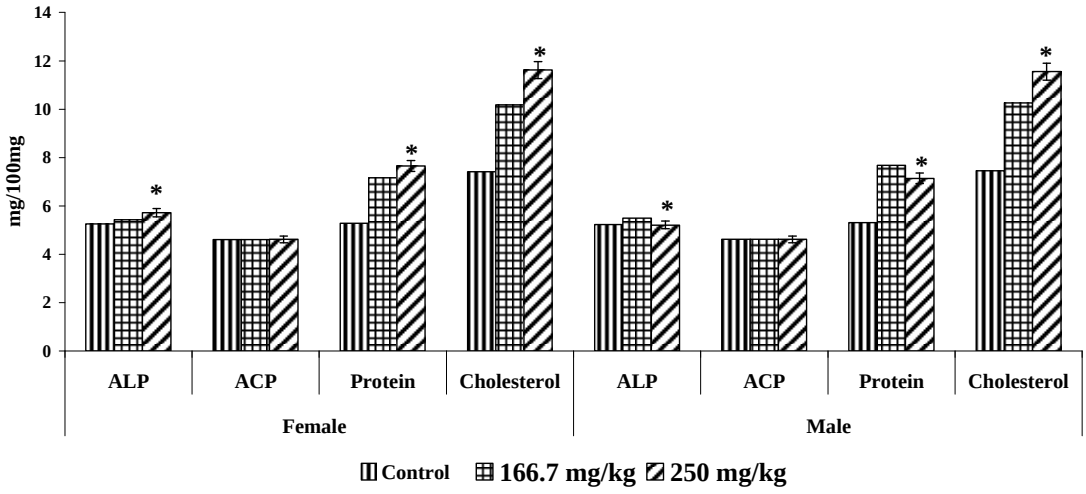
concentration. Only ALP was found decreased at 166.7 mg/kg (female and male mice) when compared to control, but elevated at 250 mg/kg body weight. However, these elevations in all levels of enzymes are not statistically significant enough to protect the liver from hepatotoxicity. Total protein estimated significantly increased at 166.7 and 250 mg/kg body weight in female and male mice as compared to control. However, a significant increase in protein was observed in male mice over female mice at both the doses tested. The cholesterol level also increased at 250mg/kg dose (Fig 3). The DNA and RNA contents decreased at 166.7 mg/kg in both female and male mice when compared to control but, elevated at 250 mg/kg body weight significantly. The amount of DNA increased in female and declined in male at 166.7 mg/kg, while at 250 mg/kg, the DNA increased in male and decreased in female. Similarly, the RNA contents elevated in male over control at both 166.7 mg/kg and 250 mg/kg doses. The carbohydrate content also slightly increased at 250 mg/kg (Fig 4).

Fig 3: Biochemical estimations of liver treated with ethyl acetate leaf extract of *C. bonducella* in mice



*Data represent mean ±SE of 6 animals. *P<0.05 compared to control*

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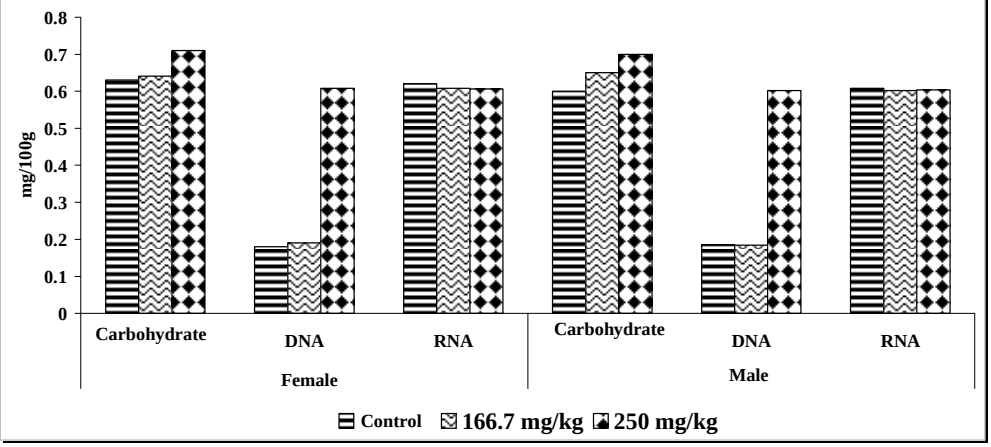
Fig 4: Biochemical estimations in liver treated with ethyl acetate leaf extract of *C. bonducella* in mice



DISCUSSIONS

In the present investigation, the ethyl acetate leaf extract of *C. bonducella* at 166.7 and 250 mg/kg body weight doses was found non-toxic and safe in oral administration during 15 days treatments in mice. The behavioral profile revealed that all the animals were alert and were responding to pain and touch. Stereotypy was not observed, as the animals did not undergo stimulation or depression. Vocalization, restlessness and irritability in animals were also not observed. The animals responded to loud noise, indicating the CNS excitation. The motor incoordination, reflexes, optical signs like, pupil size and secretory signs were normal in all the animals. Feron[19] reported that in sub acute toxicity experiments, the calculation of organ weight to whole body weight ratio serves as useful index of toxicity. The progressive increase in body weight in this study resulted in all the animals of both control and treated groups which may be due to the increased intake of food and water by the animals. The progressive increase in body weight in the animals treated with both the doses (166.7 and 250 mg/kg) for 15 days indicated the improvement of nutritional state in the animals. Kluwe[20] documented that the absolute organ weight has been observed to be a relative sensitive indicator of nephrotoxicity for known nephrotoxicants and thereafter defined nephrotoxicity as increased kidney weight. However, no correlation between the relative weight of the organ and the doses of the extract administered was observed in the present studies. The ethyl acetate extract of *C. bonducella* did not induce any toxic effect on kidney and other organs. These results are in agreement with the results of Sambath Kumar[21] where, they reported no significant changes in animals treated with methanol extract of *C. bonducella* at 100 and 200 mg/kg body weight, but a significant increase in organ weight was observed at 400 mg/kg. Similarly, Gupta *et al.*,[22],

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reported no change in the weight of liver, kidney, brain and spleen at 50, 100, 200 and 300 mg/kg of methanol extract of *C. bonducella* in 14 days of treatment.

The RBC counts at 166.7 mg/kg varied in female and male mice, but there was a marginal increase in RBC count at 250 mg/kg. The WBC counts were found to be decreased at 166.7 mg/kg in male mice as compared to female mice, but RBC and WBC remained unaltered at 166.7 mg/kg in male mice. The small transient of values observed in blood haematology did not show any dose responsiveness. Sambath Kumar *et al*.,[21] reported similar results in the methanol extracts of *C. bonducella* at low and median doses (100 and 200 mg/kg). Similarly, Gupta *et al*.,[23] studied the effect of methanol extract of *C. bonducella* at 50, 100, 200 and 300mg/kg, where RBC counts were unaltered, but there was a marginal increase in WBC count. As there was no significant difference in the clotting time between the treated and control groups at both the doses in male and female mice, the ethyl acetate extract of *C. bonducella* tested in the present study may be non-toxic.

Kramp *et al*.,[24] stated that the functional studies in toxicology should be coupled with the appropriate histological studies, because appropriate morphological studies are useful especially during the anatomical localization of action of toxin. Hence, based on the histological and morphological studies conducted, the ethyl acetate leaf extract of *C. bonducella* seems to be devoid of any toxic effects in mice upto 250 mg/kg body weight. However a slight change observed in cellular architecture of liver may be due to the presence of various chemical constituents present in the extracts. Further work need to be done to find out the exact mechanism of toxicity and loci of fatal action of the extract at more than 250 mg/kg concentration.

The biochemical analysis of liver revealed a few minor changes in the enzymes tested. DNA and RNA contents were found decreased at 166.7 mg/kg (female and male mice) when compared to control, but elevated the amount at 250 mg/kg body weight. The alkaline phosphatase (ALP) and acid phosphatase (ACP) protect the liver from toxicity[26]. The ALP activity decreased at 166.7 mg/kg and elevated at 250 mg/kg in both female and male mice. These results are in agreement with the reports of Sambath Kumar *et al*.,[21] where they reported increased ALP at higher concentration (400 mg/kg body weight). In this study, a slight increase in acid phosphatase activity was noticed in the liver. The total proteins significantly increased at 166.7 and 250 mg/kg body weight in both female and male mice when compared to control. In various liver dysfunctions, generally the protein content increases so as to maintain the protein concentration in liver. The carbohydrate and cholesterol also elevated slightly at both the doses in liver. Elevated level of cholesterol is found in obstructive jaundice or in chronic hepatitis of any type. The amount of DNA increased in female and decreased in male at 166.7 mg/kg, while at 250 mg/kg, the amount of DNA increased in male and decreased in female. These results are well supported by Aiad[25] and Tripathi *et al*.,[26] (2003). They have reported that the hepatotoxins compared to their test compounds produced alterations in DNA and RNA levels.

CONCLUSION

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The present study demonstrated that ethyl acetate extract of *C. bonducella* was non toxic at 250 mg/kg body weight to Swiss albino mice as evidenced by behavioural, haematological, histopathological and biochemical studies. However, a slight change in the cellular architecture was revealed in histology of liver. Further, study on the mode of mechanism of toxicity of the drug should be undertaken in order to identify the site of action.

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EVALUATION OF ACUTE AND SUB ACUTE TOXICITIES OF LEAF EXTRACT OF *CAESALPINIA BONDUCELLA* (L.) FLEM

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