IJPSR (2012), Vol. 3, Issue 04 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 30 December, 2011; received in revised form 28 January, 2012; accepted 27 March, 2012

EVALUATION OF ACUTE AND SUB-ACUTE TOXICITY OF ETHANOLIC EXTRACT OF SEED KERNELS OF *CAESALPINIA CRISTA* (LINN.) IN ALBINO MICE

S. N. Kshirsagar*[1], D.M. Sakarkar [1] and S.S. Deshpande [2]

Department of Pharmacology, Sudhakarrao Naik Institute of Pharmacy [1], Pusad- 445 204, Maharashtra India
Department of Pharmacology, Dr. P. D. Medical College [2], Amaravati, Maharashtra, India

Keywords:
Caesalpinia crista,
Ethanolic extract,
Acute oral toxicity,
Sub acute toxicity,
Gross abnormality,
Hematological analysis

Correspondence to Author:

S. N. Kshirsagar

Assistant Professor, Department of Pharmacology, Sudhakarrao Naik Institute of Pharmacy, Pusad- 445 204, Maharashtra, India

ABSTRACT

Acute and sub-acute toxicity of ethanolic extract of *Caesalpinia crista* (Linn.) was evaluated in Albino mice. The acute toxicity studies were conducted as per the OECD guidelines 420, here the limit test dose of 2000mg/kg used. Observations were made and recorded after treatment at 2 hrs, 4 hrs, 8 hrs and then for seven days regularly for respiration rate, heart rate, and behavioural signs like apathy, reduced locomotor activity as well as licking. Three groups of 6 mice were made for the sub-acute toxicity study. Group I (control) received distilled water, group II 200 mg/kg and group III 400 mg/kg received ethanolic extracts every 24 hr orally for 28 days. No toxic effects of the Ethanolic extract were observed on body and organ weights between the control and the treated group after 28 days of treatment. No significant variation was found in the Hematological analysis and clinical blood chemistry. No mortality was found during study.

INTRODUCTION: Plants used in traditional medicine contain wide range of ingredients that can be used to treat chronic as well as infectious diseases. A vast knowledge of how to use the plants against different illness may be expected to have accumulated in areas where the use of plants is still of great importance [1]. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The important constitutent of these plants are alkaloids, Flavonoids,tannins and phenolic compounds [2].

Caesalpinia crista (Linn.) synonym: *Caesalpinia bonduc* (L.) Roxb.), Caesalpinia bonducella belongs to family Caesalpiniaceae and is commonly known as kat-takaranja in Hindi and sagargota in Marathi. It is prickly shrub found throughout the hotter regions of India and srilanka [3]. The seeds of plant are almost globular in shape, grey, hard with a smooth shiny surface [4]. The seeds of the plant contain bonducin, proteins, saponin, starch, sucrose, an enzyme, two phytosterols namely sitosterol and hepatsane, fattyacids such as palmitic acid, stearic acid, lognoceric, oleic, linolenic acid. The seed kernals of the plant contain furanoditerpenes- ά-caesalpin, ß-caesalpin, γ-caesalpin, δ-caesalpin, ε-caesalpin,and F-caesalpin [5]. The leaves of *Caesalpinia crista* (Lin) are tradionally used for tumors, inflammation and liver disorders.

In India, various parts of this plant has been used in various therapeutic uses like adaptogenic [6], antimicrobial [7], antiproliferatve [8], antidiabetic [9], anti-filarial [10], contractility on uterus [11], hepatprotective [12], antitumor and antioxidant activities [13]. Because of their various pharmacological activities, animal toxicity studies are required to identifyl adverse effects of *Caesalpinia crista* (Linn.). The present work was undertaken to study the acute and sub- acute toxicity of ethanolic extract of seed kernels of *Caesalpinia crista* (Linn.)

MATERIALS AND METHODS:

Plant Material: The *Caesalpinia crista* (Linn.) seeds were collected from the local market Yogesh Pharmacy Nanded Maharastra. It was authenticated by Dr. Mr. Krishna G. Kadaskar, Department of Botany P. N. College, Pusad (Maharastra).

Preparation of Extract: The seeds kernels of *Caesalpinia Crista* (Linn.) were shade dried at room temperature. Then the shade dried seeds kernels were powdered to get a coarse powder. 100g of coarse powder was defatted with petroleum ether and extracted exhaustively with 95% ethanol at Temperature 60oC, in a soxhlet extractor. The extract was concentrated in a rotary flash evaporator residue was dried in desiccator over sodium sulfite. This procedure was repeated for 5-6 times to receive sufficient quantity of ethanolic extract [14].

Phytochemical Investigation: Ethanolic extract of *Caesalpinia crista* (Linn.) seeds kernels were subjected to further preliminary qualitative phytochemical investigation [15].

Toxicology Study:

Experimental Animals: Healthy adult Albino mice of either sex weighing 24-32 gm acclimatized for 14 days. The animals were housed under standard conditions and room temperature (25±2^{o}C). During the acclimatization period of 14 days, animals were observed for general condition every day and weighed on the next day of arrival and on the last day of acclimatization. The animals were fed with balanced pellet and water *adlibitum*. The experimental protocol of toxicological studies was reviewed and approved by the Institutional Animal Ethical Committee of Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Acute Toxicity Study: The acute toxicity studies were conducted as per the OECD guidelines 420 (OECD 2001) where the limit test dose of 2000 mg/kg used [16]. Observations were made at 2, 4, 8 hrs for seven days for body weight, treatment related changes like respiration rate and heart rate and behavioral signs like apathy, reduced locomotor behavior, licking activity.

Sub Acute-Toxicity Study: Healthy adult Albino mice of either sex weighing 28-32 gm were divided in to 3 groups of 6 animals each and were housed under standard conditions and room temperature (25±2^{o}C). The control animals (Group-I) received 0.5ml of vehicle alone and the other two groups (Group-II &III) received *Caesalpinia crista* (Linn.) seeds kernels ethanolic extract for 28 days at doses of 200, 400 mg/Kg body weight respectively. Toxic manifestations such as body weight, mortality, food and water intake was monitored. After 28 days, all surviving animals were fasted overnight and anesthetized with ether. The blood samples were collected in heparinized tubes for determining hematological parameters and the serum was used for clinical blood chemistry. Animals were sacrificed after blood collection and the internal organs were removed and preserved in 10 % formalin solution for histological examination.

Biochemical Estimations: Blood collected in heparinized tubes were then centrifuged at 3000 rpm for 10 minute. The serum separated was analyzed for various parameters such as serum bilirubin, Serum glutamic oxaloacetic Transaminase, Serum Glutamic pyruvic Transaminase, Serum alkaline phosphatase, Serum total proteins, serum total albumin, serum total globulin, serum cholesterol, electrolytes like Na, K, Cl, Ca, P, Blood Urea Nitrogen and creatinine by autoanalyzer.

Haematological Assay: Blood sample collected in the heparinized tubes were used to estimate hemoglobin, W.B.C, R.B.C, Platelet count, Reticulocyte count, Mean corpuscular volumn, mean corpuscular hemoglobin concentration, Percent of Neutrophils, Eosinophils, Lymphocytes and Monocytes, Packed cell volumn and mean corpuscular hemoglobin [17, 18].

Histopathological Study: Histopathological investigation of the organs was done. The organs were fixed in 10% formalin for 24 hrs and washed in running water for 24 hrs. Samples were processed for weighing [19].

Statistical Analysis: The values are expressed as mean ± Standard deviation (S.D.). Results were analyzed statistically using one way Anova. The significant difference between the groups are considered at $P<0.05$ level.

RESULTS:

Acute Toxicity Study: The acute toxicity study was conducted as per the OECD guidelines 420, where the limit test dose of 2000mg/Kg used. No test substance related mortality was observed at 2000mg/Kg and throughout the observation period there were no significant changes in behavior (i.e., apathy, hyperactivity, hypoactivity) in any of the mice. The results of acute toxicity were illustrated in **Table 1 and 2**. No changes were found in body weight and changes in respiration rate and heart rate.

TABLE 1: EFFECT OF ORAL ADMINISTRATION OF ETHANOLIC EXTRACT OF *CAESALPINIA CRISTA* (LINN.) SEEDS KERNELS ON BODY WEIGHT (G) AND ORGANS WEIGHT (G) OF MICE

Organs	Group I	Group II	Group III
Liver	1.30 ± 0.01	1.36±0.11	1.38±0.06
Heart	0.09 ± 0.01	0.10±0.01	0.11±.03
Lungs	0.35 ± 0.02	0.36±0.03	0.36±0.03
Spleen	0.12 ± 0.03	0.14±0.02	0.13±0.03
Kidney	0.30±0.03	0.31±0.05	0.32±0.02
Body weight	29.78±0.46	30.43±1.20	30.74±1.20

Values are expressed as mean ± S.D of 6 mice in each group.

TABLE 2: OBSERVATIONS OF ACUTE TOXICITY STUDY (TREATMENT RELATED CHANGES)

Animal no	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	C1	C2	C3	C4	C5	C6	C8	C9	C10
Dose mg/ Kg	2000	2000	2000	2000	2000	2000	2000	2000	2000	2000									
Body weight(gm)	31	29	32	31	28	32	31	30	32	30	28	32	31	31	32	30	31	30	32
Apathy	-	-	-	-	-	-	-	-	-	-	N	N	N	N	N	N	N	N	N
Ataxia	-	-	-	-	-	-	-	-	-	-	N	N	N	N	N	N	N	N	N
Circling	-	-	-	-	-	-	-	-	-	-	N	N	N	N	N	N	N	N	N
Compulsive behaviour	-	-	-	-	-	-	-	-	-	-	N	N	N	N	N	N	N	N	N
Excitability	+	+	+	+	+	+	+	+	+	+	N	N	N	N	N	N	N	N	N
Locomotor behaviour	+	+	+	+	+	+	+	+	+	+	N	N	N	N	N	N	N	N	N
Moribund	-	-	-	-	-	-	-	-	-	-	N	N	N	N	N	N	N	N	N
Drinking	-	-	-	-	-	-	-	-	-	-	N	N	N	N	N	N	N	N	N
Edema	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Paralysis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Reflexes	-	-	-	-	-	-	-	-	-	-	N	N	N	N	N	N	N	N	N
Heart rate	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Respiratory rate	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Pruritis	-	-	-	-	-	-	-	-	-	-	N	N	N	N	N	N	N	N	N
Eyelid closure	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Diarrhea	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Depression	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Body weight change	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Hunched/stiff posture	-	-	-	-	-	-	-	-	-	-	N	N	N	N	N	N	N	N	N

+ Significant changes, - no change noticed, C- Control, N- normal

Sub Acute Toxicity Study: Ethanol extract of *Caesalpinia crista* (Linn.) seeds kernels at dose of 200,400 mg/kg orally for every 24 h for 28 days did not produce any mortality in tested animals. No sign of toxicity was observed during the experimental period. All haematological parameters such as hemoglobin, W.B.C, R.B.C, Platelet count, Reticulocyte count, Mean corpuscular volume, mean corpuscular hemoglobin concentration, percent of neutrophils, eosinophils, lymphocytes and monocytes, packed cell volume and mean corpuscular hemoglobin and biochemical parameters such as serum bilirubin, serum glutamic xaloacetic transaminase, serum glutamic pyruvic transaminase, serum alkaline phosphatase, serum total proteins, serum total albumin, serum total globulin and serum cholesterol and serum triglyceride were found within the normal range.

The results of hematological and biochemical parameters are showed in **table 3, 4, 5**.

TABLE 3: HEMATOLOGICAL PARAMETERS AFTER 28 DAYS ORAL TREATMENT WITH ETHANOLIC EXTRACT OF *CAESALPINIA CRISTA* (LINN.) SEEDS KERNELS

Parameters	Group-I Control	Group-II (200 mg/Kg b.wt)	Group-III (400mg/Kg b.wt.)
Hemoglobin G%	14.70±0.54	14.69±0.57	15.29±0.39
RBC X 106/cmm	8.03±0.40	8.98±0.41*	8.65±0.28*
WBC X 103/ cmm	3.86±0.51	4.84±0.8*	5.08±0.70*
PLT lakhs/cmm	5.43±0.71	5.22±1.22	5.80±1.21
Reticulocyte%	0.92±0.14	0.96±0.23	0.83±0.21
Neutrophil %	19.47±3.95	27.71±2.85	26.91±8.34
Eosinophil %	0.10	0.28±0.47	0.31±0.47
Lymphocyte %	74.26±4.1	65.88±2.98	66.66±8.48
Monocyte %	1.26±0.47	1.11±0.37	1.15±0.37
PCV%	43.52±1.13	43.30±2.27	44.8.4±0.89
MCVFI	51.52±1.66	51.49±1.86	49.64±0.90
MCH pg	17.38±0.50	17.43±0.24*	17.52±0.58*
MCHC gm/dl	32.07±0.37	32.22±0.98	32.37±0.45

Values are expressed as mean ± S.D of 6 mice in each group. Comparisons were made between Group-I with Group-II and Group-III. P <0.05 was considered significant. The * symbol also represent the statistical significance at P <0.05

TABLE 4: EFFECT OF TREATMENT WITH ETHANOL EXTRACT OF *CAESALPINIA CRISTA* (LINN.) SEEDS KERNELS ON BIOCHEMICAL PARAMETERS

Parameters	Group-I (Control)	Group-II (200 mg/Kg b.wt)	Group-III (400mg/Kg b.wt.)
SGOT IU/L	117.01±22.62	136.96±32.96	122.71±24.53
SGPT IU/L	82.96±6.72	91.67±12.67	94.52±5.1
ALP IU/L	470.25±26.83	469.93±11.86	469.13±13.45
BILI mg/dl	0.40±0.094	0.45±0.11	0.47±0.12
PRO g/dl	4.84±0.49	5.33±0.76	5.68±0.76
ALB g/dl	2.21±0.12	1.97±0.46*	1.54±0.43*
GLB g/dl	2.61±0.22	3.35±0.40*	4.13±0.37*
Cholesterol mg/dl	87.4±4.16	87.87±29.35	88.18±10.07
TG mg/dl	86.13±3.60	85.66±5.18	86.13±8.98

TABLE 5: EFFECT OF TREATMENT WITH ETHANOL EXTRACT OF *CAESALPINIA CRISTA* (LINN.) SEEDS KERNELS ON BIOCHEMICAL PARAMETERS

Parameters	Group-I (Control)	Group-II (200 mg/Kg b.wt)	Group-III (400mg/Kg b.wt.)
Na mEQ/L	142.95±7.10	141.67±3.03	143.19±3.30
K mEQ/L	6.46±1.01	5.51±0.8	5.53±0.36
Cl mEQ/L	110.97±6.96	107.16±3.17	107.99±1.61
Ca mg/dl	8.07±0.33	9.00±0.91	8.75±0.56
P mg/dl	6.67±0.471405	6.20±0.32	6.43±0.32
BUN mg/dl	17.43±8.50	12.27±1.98	10.22±.78

Values are expressed as mean ± S.D of 6 mice in each group. Comparisons were made between Group-I with Group-II and Group-III. P <0.05 was considered significant. The * symbol also represent the statistical significance at P <0.05

DISCUSSION: In acute toxicity study, there was no any mortality observed up to the maximum dose level of 2000mg/kg body weight of the extract administered orally, which the single high dose is recommended by OECD guidelines 423 for testing acute toxicity. No changes were found in body weight, respiration rate, heart rate and behavioural changes. Thus, our test suggested that ethanolic extract of *Caesalpinia crista* (Linn.) seeds kernels does not cause any acute toxicity. The changes in body weight have been used as an indicator of adverse effects of *Caesalpinia crista* (Linn.) and chemicals.

In subacute toxicity study mice treated with 200,400 mg/kg doses of ethanolic extract of *Caesalpinia crista* (Linn.) seeds kernels had a progressive increase in body weight. The increase in weight was not significantly different from that of the control. The progressive increase in body weight at dose of 200,400 mg/kg of mice during 28 days of administration of ethanolic extract of *Caesalpinia crista* (Linn.) seeds kernels may indicate the improvement in the nutritional state of the animal. The growth response effect could be as a result of increased food and water intake.

The haematological status after 28 days of oral administration of ethanolic extract of *Caesalpinia crista* (Lin) seeds kernels was also assessed. Table 3 shows the effect of *Caesalpinia crista* (Linn.) seeds kernels on hematological parameters. The red blood cell was found to be significantly increased (P<0.05) in Group – III and white blood cell count were found to be significantly increased in Group-II and III *Caesalpinia crista* (Linn.) seeds kernels treated animals and slight changes in mean corpuscular hemoglobin also. With the exception of a transient increase in RBC and WBC count there were no significant alterations in the hematological parameters. Increase in WBC may indicate the impact of *Caesalpinia crista* (Linn.) seeds kernels in boosting the immune system of treated groups.

However, slight changes in RBC and WBC did not show any dose responsiveness. All the other parameters in all treated group remained normal without any significant difference. Transaminases (GOT and GPT) and ALPs are good indices of liver and kidney damage respectively. Results of serum biochemistry were showed in Table 4. There were no deleterious changes found in the level of transaminases and ALPs in serum of treated groups with control animals. Serum albumin and globulin level of treated groups showed significant changes when compared to control group. All other biochemical parameters were remained normal without any significant difference.

Furthermore, gross examination of internal organs like Liver, Lung, Heart, spleen and kidney were also found to be normal. No abnormalities were detected in treated animals when compared to the control. In conclusion this study presents strong evidence of nontoxic effect of ethanolic extract of *Caesalpinia crista* (Linn.) seeds kernels. These results showed that the use of extract of *Caesalpinia crista* (Linn.) seeds kernels is safe and explained the extensive utilization of the plant in traditional medicine.

REFERENCES:

1. Ahsan Rajib MD., Islam Monirul Km., Haque E. Md., Mossaddik. A. MD: *In-vitro* antibacterial screenings and toxicity study of Some Different Medicinal Plants, world Journal of Agriculture Science 2009. 5(5):617-621.
2. Edeoga H.O., Okwu D. E. and Mbaebie B.O.: Phytochemical constituents of some Nigerian medicinal plants. African Journal of Biotechnology 2005, (4):685-688
3. Kirtikar K.R., Basu B.D.: Text book of Indian medicinal plants, International Book Distributors, Dehradun, 2nd edition. Vol.II 2005: 842-844.
4. Rastogi P. Ram, Mehrotra B.N.: Compendium of Indian medicinal plants, CDRI and NISC. Cataloguing-in-publication Data,Vol.I 1990:67
5. Gurunath H.M., Sidramayya M.S., Patil K.S.,Wadkar G.H.: Effect of *Caesalpinia bonducella* seed extracts on human neutrophil; Journal of Pharmacy 2010; 3(3):467-469
6. Kannur D.M., Hukkeri V.I, Akki K.: Adaptogenic activity of *Caesalpinia bonducella* seed extracts in rats, Journal of Ethno Pharmacology 2006 ;(108):327-331
7. Tasleem A., Mandal T.K., Kumar N., Bhosale J.D., Hole Archana.,Sharma G.L., Padhi M.M,Lavekar G.S, Dabur R.: *In-vitro* and *in- vivo* anti microbial activities of seeds of *Caesalpinia bonducella* (L) Roxb. Journal of Ethno Pharmacology 2009; (123):177-180
8. Yadav P., Arora A.,BID H.K., Know A.R., Khanojiya S.: New cassane butenolide hemiketal diterpenes from the marine creeper *Caesalpinia bonducella* and their anti proliferative activity, Tetrahedron Letters 2007; (48):7194-7198
9. Aswar P.B., Khadabadi S.S., Deokate U.A., Jain.S.p, Biyani K.R.: Evaluation of antidiabetic activity of *Caesalpinia bonducella* (L) Roxb leaves in Alloxan Induced Diabetic Mice, Indian Journal of Pharmaceutical Education Research. 2009;43(3):280-283
10. Gaur R.L., Sahoo M.K., Fatma N., Rastogi S., Murthy P.K.: Antifilarial activity of *Caesalpinia bonducella* against experimental filarial infections, Indian Journal of Medical Research. 2008;28:65-70
11. Datte J.Y., Offoumou A.M, Ziegler. A.: Effects of leaf extract of *Caesalpinia bonducella* on the contractile activity of uterine smooth muscle of pregnant rats, Phytomedicine1998; (60):149-155.
12. Ahsan Rajib MD., IslamMonirul Km., Haque Md., Mossaddik.A.M.D.: Hepatoprotective activity of Methanol Extract of some Medicinal Plants against Carbon Tetra chloride Induced Hepatoprotective in rats, Europian Journal of Science Research 2009 ;Vol.37(2) :302-310
13. Malaya Gupta, Mazumder U.K., Sambath R., Sivakumar T.: Antitumor activity and antioxidant status of *Caesalpinia bonducella* against Ehrlich Ascites Carcinoma in swiss albino Mice, Journal of Pharmacology Science. 2004; (94):177-184
14. Kokate C. K.: Practical Pharmacognosy. Nirali Prakashan NewDelhi, 1999, 14-19
15. Khandelwal K.R.: Practical Pharmacognosy Techniques and experiments, Nirali Prakashan, 17th edition, Pune2007, 149-156.
16. Mounnissamy V.M; Kavimani S.,Sankri G.: Evaluation of acute and sub-acute toxicity of ethanol extracts of *Cansjera rheedii* J. Gmelin (Opiliaceae). Journal of Brewing and Distilling2010,1(1),011-014,
17. 17. Sim K.S., Nurestri A.M., Kim K.H.: Acute oral toxicity of *Pereskia bleo* and Pereskia grandifolia in mice, Phcognosy Magazine2010. 6(21), 67-70.
18. 18. Teo S., Stirling D.,Thomas S., Hoberman A., Khetani V.: A 90 day oral gavage toxicity study of D-methyl phenidate and D, L-methyl phenidate in Sprague-Dawley rats.Toxicology, 2002., 179, 183-196.
19. Godkar P. B., Godkar D.P.: Text book of Medical Laboratory Technology, Bhalani Publishing House, Mumbai 2nd edition, 2003. 1017-1024.