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ORIGINAL ARTICLE

Effect of *Caesalpinia bonducella* seed extract on histoarchitecture of some vital organs and clinical chemistry in female albino rats

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Abstract The effect of an ethanolic seed extract of *Caesalpinia bonducella* F., (Caesalpiniaceae) on the histoarchitecture of the some vital organs and clinical chemistry was evaluated in Wistar female albino rats. The study was divided into four groups. Group I received distilled water and served as vehicle treated control. Groups II, III and IV were orally administered 100, 200 and 300 mg/kg b.wt dose of seed extract respectively for 10 consecutive days and subsequently euthanized twenty four hours after the last dose. Histoarchitecture of the vital tissues in treated groups appeared normal. Hematological analysis showed a significant increase in RBC count, various types of WBCs, platelet count, hemoglobin levels and packed cell volume levels in all the seed extract treated groups. Serum biochemistry revealed significantly decreased cholesterol, triglycerides and creatinine levels whereas HDL level was found to be significantly increased in all the treated groups. Our results suggest that the ethanolic seed extract of *C. bonducella* probably possesses chemical constituents of cytoprotective potential and should be further explored as a source of natural medicine.

1. Introduction

Herbs have attracted attention as a potential natural health care approach that focuses on protecting and restoring the health (Badakhshan et al., 2011). Recently herbal medicines are being increasingly utilized to treat a wide variety of clinical diseases, with relatively little knowledge regarding their modes of action. India is sitting on a gold mine of well-recorded and traditionally well-practiced knowledge of herbal medicines; therefore, any scientific data on such plant derivatives in order to determine the toxic or beneficiary effects when used for a short or long period of time as food or medicine to various tissues and organs could be of clinical importance (Desai, 2005).

Caesalpinia bonducella F., (Caesalpiniaceae) commonly known as Nata Karanja, is a prickly shrub found throughout

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the hotter parts of India, Myanmar and Sri Lanka. Seeds consist of a thick, brittle shell with a yellowish white bitter fatty kernel (Nadkarni, 1954). It is reported to have multiple therapeutic properties like adaptogenic and antidiabetic (Kannur et al., 2006a,b), antitumor (Gupta et al., 2004) antifilarial (Gaur et al., 2008), immunomodulatory antioxidant (Shukla et al., 2009a,b), anti-inflammatory, antipyretic, analgesic (Shukla et al., 2010), hypoglycemic, antihyperglycemic, hypolipidemic (Sharma et al., 1997; Chakrabarti et al., 2003), muscle contractile activity (Datte et al., 2004), antimicrobial (Arif et al., 2009), antifertility (Sukhdev et al., 2011) and diuretic agent (Khedkar et al., 2011). Its leaf powder is used to treat gynecological disorders like menorrhagia and leucorrhoea (Vidyasagar and Prashantkumar, 2007).

The seeds of *C. bonducella* are found to contain various chemical constituents such as furanoditerpenes, phytosterinin, β-sitosterol, flavonoids, bonducellin, aspartic acid, arginine, citrulline and β-carotene (Williamson, 2002).

Despite its many uses for medicinal purposes, the seed of this plant has not yet been scientifically evaluated for its effect on vital organs by adopting histological procedure in parallel with clinical chemistry. Therefore, the present study was conducted to assess its effect on the histoarchitecture of some vital organs and clinical chemistry in Wistar female albino rats.

2. Material and methods

2.1. Plant material

The seeds of *C. bonducella* F., (Caesalpiniaceae) were procured from the local market of Dharwad district, Karnataka, India. Further taxonomic identification was conducted by Dr. G.S. Mulgund and a voucher specimen number Bot/H/484 was deposited in the Department of Studies and Research in Botany, Karnatak University, Dharwad, India.

2.2. Preparation of the extract

The seed extract was prepared by following the method of Shukla et al. (2009b). Briefly, 50 g powder of *C. bonducella* was extracted with 500 ml of 95% ethanol by using a Soxhlet apparatus for 16 h. The crude extract obtained was filtered through Whatman paper and the filtrate was evaporated to dryness on rotary flash evaporator. The yield of the extract was 4% (w/w). *C. bonducella* seed extracts (henceforth referred to as CBSE) obtained were preserved in a sterile glass container at 4 °C until further use.

2.3. Animal model

Colony bred virgin Wistar female albino rats (*Rattus norvegicus*), approximately twelve weeks old and weighing between 190 and 200 gm exhibiting regular estrous cycle were housed in polypropylene cages (35 cm long × 23 cm wide × 15 cm high) under standard animal house conditions and controlled environmental conditions (24 ± 2 °C) for 12 h light and 12 h darkness. They were fed pelleted standard rat feed (Sai Durga Feeds & Foods, Bangalore, India) and allowed free access to water.

2.4. Treatment protocol

Animals were equally distributed into four treatment groups containing eight animals each. The group I animals were given distilled water (2 ml/kg b.wt) alone for 10 days and served as a control group. Groups II, III and IV animals were treated with CBSE at the dose of 100, 200, 300 mg/kg b.wt /day for 10 consecutive days, respectively. The selected tested doses and duration were based upon the work of Chakrabarti et al. (2003) and Salunke et al. (2011). The required drug was dissolved in distilled water and administered orally with a straight ball-tipped needle.

2.5. Behavioral profile, body and organ weight

The animals were observed daily for behavioral activities according to the methods of Whishaw et al. (2006). Body weight was recorded every day during the study period. Twenty-four hours after the last dose, the rats were weighed and sacrificed under sodium pentobarbital anesthesia (40 mg/kg). The liver, heart, kidney, adrenal, spleen and pancreas were carefully dissected out, cleaned off any fat and weighed (absolute weight). The relative organ weight (ROW) of each organ was then calculated according to the following equation:

$$\text{ROW} = \text{Absolute organs weight (mg)}/\text{Body weight of rat on sacrifice day (g)} \times 100$$

2.6. Clinical chemistry

Blood samples were collected by cardiac puncture technique under sodium pentobarbital anesthesia (40 mg/kg) in sodium heparin coated tubes. The clinical chemistry parameters viz. hemoglobin level, RBC count, various types of WBCs count, platelet count and packed cell volume were analyzed by Swelab Alfa Mindray 5640 Cell Counter. For serum separation, the blood was allowed to stand for an hour at room temperature to clot and then centrifuged at 3000 rpm for 10 min. The supernatant (serum) was then tipped off into a separate vial and subsequently subjected to an assessment of cholesterol, triglycerides, high-density lipoprotein, low-density lipoprotein, very low-density lipoprotein and creatinine levels by Stat Fax 2000: Semi Auto Analyser.

2.7. Histological examination

The tissues were first examined for gross pathology and then fixed in 10% neutral buffered formalin solution. After proper fixation, the tissues were dehydrated in graded series of alcohol, cleared in benzene and embedded in paraffin wax. The tissue sections were cut at 5 μm thicknesses by Leica RM 2255 microtome and stained with hematoxylin and eosin. The stained slides were photographed under Axio Imager M.2 microscope for histological examination.

2.8. Ethical aspects

The study was approved by the Institutional Animal Ethics Committee, Department of Zoology, Karnatak University,

Dharwad, India. CPCSEA (Committee for the Purpose of Control and Supervision on Experiments on Animals) (Animal House Registration No. 639/02/a/ CPCSEA) guidelines were followed for maintenance, use and disposal of the experimental animals.

2.9. Statistical analysis

One way-ANOVA followed by Tukey's HSD post hoc multiple comparison tests was employed to analyze data. *P* values less than 0.05 were considered significant.

3. Results

3.1. Behavioral profile, body and organ weight

There were no treatment related changes in the behavioral profile at any tested dose level. All animals both in control and extract treated groups appeared healthy, alert and were responding to pain and touch. No significant difference was observed in the body and relative organ weight in seed extract treated groups when compared with vehicle treated group (Table 1).

3.2. Hematolgy

The results of hematological analysis are presented in Table 2. The value of hemoglobin level showed a significant increase ($p \leqslant 0.05$) at 300 mg/kg dose level when compared with vehicle treated control group. RBC and WBC counts showed a significant increase ($p \leqslant 0.05$) at 200 and 300 mg/kg dose when compared with their respective vehicle treated control group. Platelet count and PCV levels increased significantly ($p \leqslant 0.05$) in a dose dependant manner in all the extract treated groups. Neutrophil, lymphocyte and eosinophils percent were also significantly increased ($p \leqslant 0.05$) in a dose dependant

Table 1 Effect of *Caesalpinia bonducella* seed extract on body (g) and relative organ weight per 100 g b.wt (mg).

Parameters	Vehicle treated control (Group I)	100 mg (Group II)	200 mg (Group III)	300 mg (Group IV)
Body weight	201.62 ± 2.68	200.37 ± 3.67	202.36 ± 2.79	201.66 ± 2.47
Liver	3413.81 ± 222.95	3519.46 ± 259.44	3242.59 ± 259.63	3214.76 ± 221.45
Adrenal	11.11 ± 0.47	10.93 ± 0.57	11.3 ± 1.61	10.32 ± 0.72
Kidney	315.56 ± 18.33	313.98 ± 20.78	321.33 ± 12.66	310.62 ± 9.02
Spleen	246.15 ± 12.02	243.24 ± 18.81	240.13 ± 25.32	247.16 ± 11.27
Heart	250.47 ± 7.73	250.79 ± 7.15	253.26 ± 8.23	247.39 ± 8.93
Pancreas	299.50 ± 5.58	302.09 ± 12.02	302.49 ± 7.46	298.17 ± 3.69

$n = 8$, data represent mean ± SD. There is no significant difference ($p \leqslant 0.05$) between the groups.

Table 2 Effect of *Caesalpinia bonducella* seed extract on hematological parameters.

Test	Vehicle treated control (Group I)	100 mg (Group II)	200 mg (Group III)	300 mg (Group IV)
Hemoglobin (gms%)	13.68 ± 0.23	13.62 ± 0.30	13.81 ± 0.24	14.55 ± 0.15*
RBC(million/cumm)	6.87 ± 0.31	6.68 ± 0.37	7.38 ± 0.25*	7.65 ± 0.12*
WBC(cells/cumm)	4672.87 ± 894.12	5017.50 ± 415.47	5586.25 ± 208.45*	7915.62 ± 69.52*
Platelet(lakhs/cumm)	2.85 ± 0.29	4.20 ± 0.37*	4.26 ± 0.14*	5.54 ± 0.52*
PCV (%)	35.12 ± 0.88	36.62 ± 1.02*	38.86 ± 1.21*	40.60 ± 0.96*
Neutrophil (%)	6.37 ± 0.74	23.50 ± 0.75*	36.25 ± 0.88 *	48.62 ± 5.04*
Lymphocytes (%)	49.87 ± 4.76	54.50 ± 0.92*	67.25 ± 1.28*	86.50 ± 1.51*
Eosinophils (%)	3.50 ± 0.53	4.62 ± 0.51*	4.87 ± 0.64*	4.87 ± 0.83*
Monocytes (%)	0.37 ± 0.51	0.50 ± 0.53	0.62 ± 0.51	2.75 ± 0.70*

$n = 8$, data represent mean ± SD.
* Significant difference ($p \leqslant 0.05$) as compared to the control.

Table 3 Effect of *Caesalpinia bonducella* seed extract on serum biochemistry.

Test	Vehicle treated control (Group I)	100 mg (Group II)	200 mg (Group III)	300 mg (Group IV)
Cholesterol (mg/dl)	82.35 ± 1.11	78.11 ± 1.06*	70.37 ± 1.40*	53.12 ± 1.55*
Triglycerides (mg/dl)	113.17 ± 0.97	108.25 ± 1.90*	99.73 ± 1.53*	98.37 ± 0.51*
HDL (mg/dl)	39.08 ± 0.79	40.18 ± 0.71	40.50 ± 2.07	42.21 ± 0.67*
LDL (mg/dl)	20.62 ± 1.02	16.28 ± 1.39*	8.21 ± 1.03*	7.05 ± 0.62*
VLDL(mg/dl)	22.63 ± 0.19	21.65 ± 0.38	19.94 ± 0.30*	19.67 ± 0.10*
Creatinine(mg/dl)	1.57 ± 0.04	1.40 ± 0.09*	0.95 ± 0.13*	0.56 ± 0.05*

$n = 8$, data represent mean ± SD.
* Significant difference ($p \leqslant 0.05$) as compared to the control.

manner in seed extract treated when compared with vehicle treated group. Monocyte percent elevated significantly ($p \leqslant 0.05$) at a dose level of 300 mg/kg b.wt.

3.3. Serum biochemistry

The results of serum biochemical analysis are presented in Table 3. There was a significant decrease ($p \leqslant 0.05$) in the cholesterol and triglycerides levels in a dose-dependent manner in all the seed extract treated groups when compared to that in their respective vehicle treated groups. The HDL level showed a significant increase ($p \leqslant 0.05$) at 200 mg/kg b.wt. dose level. No significant changes were noticed in LDL and VLDL levels. The value of creatinine showed a significant decrease ($p \leqslant 0.05$) in a dose-dependent manner in the seed extract group when compared to the vehicle treated group.

3.4. Histological observations

The light microscopy examinations of vital organs are presented in Fig. 1. The liver of normal control animals showed complete hepatic lobules with well formed hepatocytes with

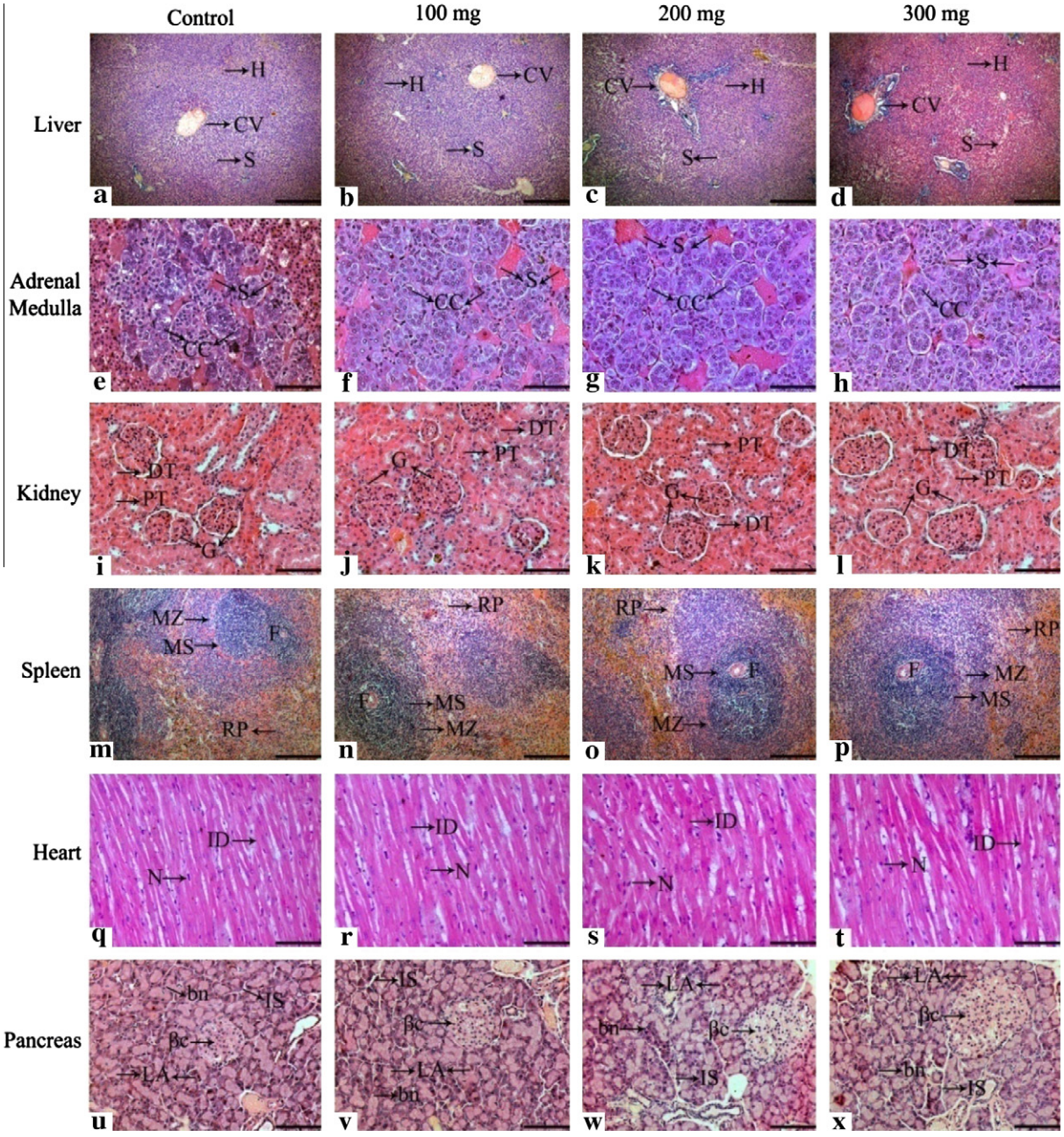

Figure 1 Histoarchitecture of the vital organs of female rats of vehicle treated control and following treatment with *Caesalpinia bonducella* seed extract at the doses 100, 200, 300 mg/kg b.wt for 10 days. Abbreviation-CV-central vein, H-hepatocyte, S-sinusoids, CC-chromaffin cell, G-glomerulus, DT-distal tubule, PT-proximal tubule, RP-red Pulp, F-follicle, MZ-marginal zone, MS-marginal sinus, ID-intercalated disk, N-nucleus, βc-beta cells, bn-basal nucleus, IS-interlobular septa, LA-lobular acini.

distinct portal triads. Hepatic cells arranged in cord like fashion, separated by sinusoids and central vein were seen clearly (Fig. 1a). These structures were also found intact in all extract treated animals (Fig. 1b–d). Chromaffin cells and sinusoid areas of the medulla region of adrenal gland were devoid of any treatment related alteration (Fig. 1e-h). Kidney (Fig. 1i-l), spleen (Fig. 1m-p) and heart (Fig. 1q-t) showed no evidence of vacuolations or distortion in all tested doses when compared with vehicle treated control. The beta cells of endocrine pancreas seemed to proliferate in treated group over the vehicle treated group. Exocrine pancreas showed no evidence of deformation (Fig. 1u-x).

4. Discussion

Considering the complexity of herbals in general and their inherent biological variation, it is now necessary to evaluate their safety, efficacy and quality (Castro et al., 2009). In the present study, we investigated the effect of oral administration of CBSE for 10 consecutive days. There were no noticeable changes in the behavioral profile, body, organ weights and gross pathology of vital organs at all tested dose levels. These records can be considered as a part of pharmacological effects of herbs but not as toxicological signs. The research finding of Sagar and Vidyasagar (2010) is in favor of the present investigation where administration of ethyl acetate extract of *C. bonducella* leaves at a dose level of 166.7 and 250 mg/kg b.wt in albino mice for fifteen days did not produce marked changes in various clinical parameters and organ weight except liver at 250 mg/kg b.wt. Moreover, Gupta et al. (2004) have also supported the present study where the treatment of methanolic leaf extract of *C. bonducella* against Ehrlich ascites carcinoma in Swiss albino mice did not produce any significant change in the weight of liver, kidney, brain and spleen at 50, 100, 200 and 300 mg/kg b.wt in fourteen days of treatment.

Histological studies play an important role in anatomical localization of toxic/beneficial action of various phytochemicals. In the present study, liver showed intact hepatocytes, central vein and sinusoids in all the seed treated animals. A number of scientific reports indicated certain flavonoids, triterpenoids and steroids have a protective effect on the liver due to their antioxidant properties (DeFeudis et al., 2003; Takeoka and Dao, 2003). Therefore, the presence of these phytochemicals in ethanolic seed extract of *C. bonducella* rules out any possibility of degenerative changes in liver histoarchitecture, in the present study. Moreover, Sambath et al. (2010) have also reported hepatoprotective and antioxidant effects of *C. bonducella* on carbon tetrachloride-induced liver injury in rats at 50, 100 and 200 mg/kg b.wt for 10 days.

Histoarchitecture of medulla of adrenal gland also indicated that the seed extract does not have an adverse effect in treated animals. This is probably due to adaptogenic potential (Kannur et al., 2006a) of *C. bonducella* seed extract.

The significant decrease in the creatinine level and well organized glomerulus, proximal and distal tubule in seed extract animals clearly indicate that the kidneys were working efficiently in extract treated animal. Khedkar et al. (2011) have also reported diuretic effects of *C. bonducella* seed extracts with a dose-dependent increase in urine excretion and specific conductivity at dose levels of 150 and 300 mg/kg b.wt in rats and support the present findings.

The significant increase in the RBC,WBC, Platelet counts, hemoglobin, PCV levels in extract treated animals may be attributed to the immunomodulatory potential of *C. bonducella* seed extract which could have boosted up the immune system and site of hematopoiesis. To strengthen this possibility, histological analysis of spleen, a site of hematopoiesis in rodents (Cesta, 2006) was carried out. It was found that spleen exhibited well organized marginal sinus, marginal zone, follicle with germ center and red pulp region in extract treated animals. Moreover, Shukla et al. (2009a) have also reported immunomodulatory potential of ethanolic seed extract of *C. bonducella* (200–500 mg/kg b.wt) by oral administration which evoked a significant increase in percent neutrophil adhesion to nylon fibers as well as a dose-dependent increase in antibody titer values and potentiated the delayed type hypersensitivity reaction induced by sheep red blood cells. Also it prevented myelosuppression in cyclophosphamide drug treated rats and good responses toward phagocytosis in carbon clearance assay.

It is a well established fact that the hypertriglyceridemia, hypercholesterolemia and high LDL levels are major pathological conditions that contribute to the cardiac cell death. In the present study, significantly decreased levels of cholesterol, triglycerides, LDL and an increased level of HDL in the seed extract treated animals indicate normal heart function. Moreover, histoarchitecture of cardiac cells have also exhibited normal intact structure in treated rats and supports the above fact. Thus, seeds of *C. bonducella* may be used as a cardioprotective agent.

The beta cells in Islet of Langerhans seemed to proliferate in the seed extract treated groups when compared to vehicle treated control group. This could be due to the high contents of flavonoids in seeds of *C. bonducella* which exhibits high ability of pancreas β-cell regeneration (Szkudelski, 2001). Moreover, Sharma et al. (1997) Chakrabarti et al. (2005) and Kannur et al. (2006b) have also reported the antidiabetic potential of *C. bonducella* seed extract in diabetic animal models at different doses and support the present finding.

5. Conclusion

The ethanolic seed extract of *C. bonducella* probably possesses phytochemicals of cytoprotective nature. Further, extensive experimental studies should be conducted to establish the exact mechanism of action and for the isolation and characterization of the active principle.

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