EFFECTS OF METHANOL EXTRACTS OF CAESALPINIA BONDUCELLA AND BAUHINIA RACEMOSA ON HEMATOLOGY AND HEPATORENAL FUNCTION IN MICE

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ABSTRACT — The aim of the present investigation deals with the hematology and hepatorenal function of Caesalpinia bonducella Flem. and Bauhinia racemosa Lam. belonging to the Family: Caesalpiniaceae, and used in the traditional system of medicine. The tribal people of Kolli Hills, Tamil Nadu, India, use the leaves of Caesalpinia bonducella and the stem bark of Bauhinia racemosa in combination with some other herbs for the treatment of various tumors, liver disorders, inflammation and some other diseases. In ancient Ayurveda medicine these plants were mentioned to possess antitumor agents. Since there are no scientific reports regarding the toxicological aspects of these plants, the present investigation deals with the sub-chronic toxicity studies of a methanol extract of Caesalpinia bonducella (MECB) leaves and Bauhinia racemosa (MEBR) stem bark in Swiss albino mice. The MECB and MEBR were administered intraperitoneally (i.p) to Swiss albino mice twice a week for thirteen weeks. No significant alterations in hematological, biochemical and histopathological parameters were observed in the MECB- and MEBR-treated groups at the doses of 100 and 200 mg/kg body weight. Administration of MECB and MEBR at the dose of 400 mg/kg body weight elevated the levels of serum enzymes and altered the hematological parameters. Our results suggested that MECB and MEBR at doses 100 and 200 mg/kg body weight did not induce any toxic effects in the mice. Adverse effect was noted at the dose of 400 mg/kg body weight.

KEY WORDS: Caesalpinia bonducella, Bauhinia racemosa, Hematological profile, Biochemical parameter

INTRODUCTION

Medicinal plants are natural resources yielding valuable herbal products, which are often used in the treatment of various ailments. Crude materials of these herbs are used in Ayurvedic preparations. Medicinal plants have been used in the treatment of various degenerative diseases such as cancer, inflammation, and liver disease. Large numbers of plants have been reported to possess antitumor activity in laboratory animals.

Caesalpinia bonducella F., (Family: Caesalpiniaceae), commonly known as Nata Karanja (Hindi), is a prickly shrub found throughout the hotter parts of India, Myanmar and Sri Lanka. The leaves of this plant are traditionally used for the treatment of tumor, inflammation and liver disorders (Kirtikar and Basu, 1975; Wealth of India, 1950, 1952). It has also been recognized for such multiple therapeutic properties as antipyretic, antiuretic, anthelmintic and antibacterial (Neogi and Nayak, 1958), anticonvulsant (Adesina, 1982) anti-anaphylactic, anti-diarrheal and antiviral (Dhar et al., 1968), antiasthmatic (Gayaraja et al., 1978) anti-inflammatory (Agrawal and Kapadia, 1982), antiamoebic and, anti-estrogenic (Raghunathan et al., 1982). Nematocidal (Kjuchi et al., 1989), antihyperglycemic (Rad et al., 1994) and abortifacient (Datte et al., 1998) activities.

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Bauhinia racemosa L. (Caesalpiniaceae), a small crooked tree with dark scabrous bark commonly known as Ashta (Hindi), is widely distributed throughout India, Ceylon, China and Timor. The bark and leaves of this plant are extensively used for the treatment of inflammation, headache, fever, tumors, skin infection, blood disease, dysentery and diarrhea (Kirtikar and Basu, 1975; Wealth of India, 1950, 1952). The ethanol extract of leaves of this plant was evaluated for analgesic, anti-inflammatory, anti-pyretic and antispasmodic activity (El-Khatib and Khaleel, 1995). The fresh flower buds of this plant were screened for anti-ulcer activity (Akhtar and Ahmad 1995). A dried entire plant showed antimicrobial activity (Ali et al., 1995). The cytotoxic, hypotensive and hypothermic activities of seeds of B. racemosa have also been reported (Dhar et al., 1968).

From our laboratory the hepatoprotective and antioxidant roles of Caesalpinia bonducetla and Bauhinia racemosa (Gupta et al., 2003a, 2004c), anti-inflammatory, analgesic and anti-pyretic activity (Gupta et al., 2003b), antitumor activity and antioxidant status of Caesalpinia bonducetla and Bauhinia racemosa against Ehrlich ascites carcinoma in Swiss albino mice were also reported (Gupta et al., 2004a, 2004b). The present study deals with the effect of methanol extracts of Caesalpinia bonducetla (MECB) leaves and Bauhinia racemosa (MEBR) stem bark on hematological profile and hepatorenal function and metabolism in mice.

MATERIALS AND METHODS

Plant material

The plants Caesalpinia bonducetla and Bauhinia racemosa were collected from Kolli Hills of Tamilnadu, India. The plant material was taxonomically identified by the Botanical Survey of India, Kolkata, India. Voucher specimens (No.GMS-2 and GMS-1) have been preserved in our laboratory. The leaves and stem barks were dried under shade and then powdered with a mechanical grinder and stored in an airtight container.

Extraction

The dried powder material of the leaves (500 g) of Caesalpinia bonducetla was defatted with petroleum ether (60-80°C) in a soxhlet apparatus. The defatted powder material thus obtained was further extracted with methanol for 72 hr in the soxhlet. The solvent was removed by distillation under suction and the resulting semisolids mass was vacuum-dried using a rotary flash evaporator to yield (8.78%) a solid residue (methanol extract). Phytochemical screening of the extract revealed the presence of alkaloids, saponins, flavonoids, triterpenoids, tannins, and steroids.

The dried powder material of the stem bark of Bauhinia racemosa was extracted with methanol (Yield 9.25%) in a soxhlet apparatus. The methanol extract was then distilled, evaporated and dried in vacuum. The chemical constituents of the extract were identified by qualitative analysis followed by their confirmation by thin layer chromatography, which indicate the presence of flavonoids, triterpenoids, steroids and tannins.

Experimental animals

Male Swiss albino mice weighing 23-25 g were used. They were obtained from the animal house, Indian Institute of Chemical Biology (IICB), Kolkata, India. The animals were grouped and housed in polyacrylic cages (38 × 23 × 10 cm) and maintained under standard laboratory conditions (temperature 25 ± 2°C) with dark and light cycle (14/10 hr). They were allowed free access to standard dry pellet diet (Hindustan Lever, Kolkata, India) and water ad libitum. The mice were acclimatized to laboratory condition for 10 days before commencement of the experiment. All procedures described were reviewed and approved by the Animal Ethical Committee of the University.

Chronic toxicity study

The Swiss albino mice were divided into eight groups containing 12 animals. Group 1 (Normal) received normal saline (0.9% NaCl w/v, 5 ml/kg body weight, intraperitoneally i/p) twice a week. Group 2 (Vehicle control) received propylene glycol 5 ml/kg body weight twice in a week. MECB at the doses of 100, 200 and 400 mg/kg, body weight were administered to Groups 3, 4 and 5 i.p. twice a week, respectively. Groups 6, 7 and 8 were administered MEBR i.p. at doses of 100, 200 and 400 mg/kg body weight, twice a week, respectively. At the end of the 13th week (Muto et al., 2003), mice were fasted for 18 hr, then anesthetized with ether. Blood samples were collected from the inferior vena cava and used for the estimation of different biochemical parameters.

Body and organ weight

Body weights and food intake were measured weekly and the animals were observed for signs of
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abnormalities throughout the study. The positions, shapes, sizes and colors of internal organs, namely brain, heart, kidneys and lungs, stomach, liver, and spleen, were visually observed for any signs of gross lesions. These organs were collected and weighed.

Hematological parameters
Hematological analysis was performed using an automatic hematological analyzer (Cell dyne 3500, Abbott). Hematological parameters including hemoglobin content, total count of red blood cells (RBC) and white blood cells (WBC), differential count of leukocytes such as neutrophil (%), lymphocyte (%), monocyte (%), hematocrit (Hct), mean cell volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and platelet count were measured.

Biochemical parameters
Biochemical analysis of serum samples was performed using an automatic chemistry analyzer (Hitachi model 912, Roche). Aspartate aminotransferase (GPT), alanine aminotransferase (GOT), alkaline phosphatase (ALP), bilirubin, total protein, cholesterol, glucose, urea, uric acid, creatinine and nonprotein nitrogen (NPN) were measured.

Histopathology
The organs were then preserved in 10% buffered formalin solution. The organs were processed for histopathological studies (Hawk, 1965; Varley, 1980a). Tissue slides were stained with hemotoxylin and eosin and were examined by a pathologist.

Statistical analysis
The experimental results were expressed as the mean ± S.E.M. Data were assessed by the method of analysis of ANOVA followed by Student’s t-test (Ostle, 1966, Woolson, 1987). p value of < 0.05 was considered as statistically significant.

RESULTS
Effects of MECB and MEBR on food intake and body weight
Water intake and food consumption of the MECB- and MEBR-treated animals at the dose of 100 and 200 mg/kg body weight were more or less normal. The water and food intake of MECB and MEBR at the dose of 400 mg/kg treated groups was significantly lower than that of the control group. The body weights of all animals were measured weekly throughout the study. The body weights of the animals were reported in Fig. 1.

Effects of MECB and MEBR on organ weight
The organs were weighed after being sacrificed. No significant changes in the actual organ weights were seen in all animals treated with MECB and MEBR at the doses of 100 and 200 mg/kg body weight. The organ weights of the animals treated with MECB and MEBR at the dose of 400 mg/kg body weight significantly increased when compared with the control group (Fig. 2).

Effects of MECB and MEBR on hematological parameters
There was no significant change in the number of white blood cells, neutrophils (%), lymphocytes (%), monocytes (%), the number of red blood cells, hemoglobin, Hct, MCV, MCH, MCHC, and platelet in MECB- and MEBR-treated mice at doses 100 and 200 mg/kg body weight tested group. The above hematological parameters altered in MECB and MEBR at the dose of 400 mg/kg body weight treated mice (Fig. 3, 4 and 5).

Effects of MECB and MEBR on biochemical parameter
It was demonstrated that there was no significant alteration in all biochemical parameters tested in the mice given 100 and 200 mg/kg body weight of the MECB- and MEBR (Fig. 6-9)-treated animals, but the levels of serum enzymes (GPT, GOT and ALP) and bilirubin were significantly increased in the mice treated with MECB and MEBR at the dose of 400 mg/kg body weight. The level of urea, uric acid, creatinine, cholesterol and glucose were slightly altered in the MECB- and MEBR-treated groups receiving 400 mg/kg body weight (Fig. 6-9).

Effects of MECB and MEBR on histopathology of internal organs
No remarkable changes in the internal organs of mice receiving the extract at the doses of 100 and 200 mg/kg body weight were noticed by the gross examinations. The animals receiving MECB and MEBR at the dose of 400 mg/kg body weight exhibited fatty liver.

DISCUSSION
Liver being the detoxification organ of mammals

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Fig. 1. Effect of methanol extracts of Caesalpinia bonducella (MECB) and Bauhinia racemosa (MEBR) on body weight of mice. Values are mean ± SEM. p < 0.05, Experimental groups compared with control group.

Fig. 2. Effect of methanol extract of Caesalpinia bonducella (MECB) and Bauhinia racemosa (MEBR) on organ weight in mice. Values are mean ± SEM. Number of animals = 12. *p < 0.05, Experimental groups compared with control group.
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Fig. 3. Effect of methanol extracts of Caesalpinia bonducella (MECB) and Bauhinia racemosa (MEBR) on hematological parameters.
Values are mean ± SEM. number of mice = 12.
* p < 0.05, ** p < 0.01, Experimental groups compared with control group.

Fig. 4. Effect of methanol extracts of Caesalpinia bonducella (MECB) and Bauhinia racemosa (MEBR) on differential count and hematocrit value in mice.
Values are mean ± SEM. number of mice = 12.
* p < 0.05, ** p < 0.01, Experimental groups compared with control group.
Fig. 5. Effect of methanol extracts of *Caesalpinia bonducella* (MECB) and *Bauhinia racemosa* (MEBR) on mean cell volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). Values are mean ± SEM. number of mice = 12.

* p < 0.05, ** p < 0.01, Experimental groups compared with control group.

Fig. 6. Effect of methanol extracts of *Caesalpinia bonducella* (MECB) and *Bauhinia racemosa* (MEBR) on serum enzymes GOT, GPT and ALP.

Values are mean ± SEM. number of mice = 12.

* p < 0.05, ** p < 0.01, Experimental groups compared with control group.
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Fig. 7. Effect of methanol extracts of *Caesalpinia bonducella* (MECB) and *Bauhinia racemosa* (MEBR) on bilirubin, creatinine and uric acid levels in mice. Values are mean ± SEM. number of mice = 12.

* p < 0.05, ** p < 0.01, Experimental groups compared with control group.

Fig. 8. Effect of methanol extracts of *Caesalpinia bonducella* (MECB) and *Bauhinia racemosa* (MEBR) on Non-protein nitrogen (NPN) and Total protein levels in mice. Values are mean ± SEM. number of mice = 12.

* p < 0.05, ** p < 0.01, Experimental groups compared with control group.
and kidney the most important excretory organ is very susceptible to cancer drugs. For example, hyperuricemia (purine catabolism) induced by alkyl sulphonates and antimetabolites. Hepatotoxicity and jaundice were induced by 6-MP and 6-TG, renal toxicity induced by Mitomycin and hepatic dysfunction by Methotrexate and antimetabolites etc. Apart from nephrotoxic and hepatotoxic, anticancer drugs also produce delayed haemopoietic depression (Leonard, 1996; Goodmann and Gilman, 1991).

The results indicate that SGOT and SGPT remain unchanged in MECB- and MEBR-treated group at low and moderate doses. The SGOT and SGPT were significantly increased at the dose of 400 mg/kg body weight. The present study indicates that the bilirubin level was not altered significantly, but the higher doses of the MECB and MEBR alters the level of bilirubin. Alkaline phosphatase level remains unaltered at the dose of 100 and 200 mg/kg body weight, whereas the alkaline phosphatase levels increased by both extracts treated at the dose of 400 mg/kg body weight. Synthesis of serum proteins (albumin) in liver is affected both qualitatively and quantitatively in liver damage. In many liver disorders, serum globulin may rise to such a level so as to maintain normal or increased total protein concentration, even when there is severe albumin depletion. Decreased albumin and elevated globulins in serum indicate hepatocellular origin or jaundice or liver diseases. In the present study, the MECB and MEBR at the low and median doses did not alter the protein content, but in the case of high-dose (400 mg/kg) treated groups it was slightly increased. This may be due to some lipophilic substance such as triterpenoids and steroids present in the extracts.

Similarly, a high level of cholesterol is found in obstructive jaundice or in chronic hepatitis of any type. The present study indicates that the MECB and MEBR did not alter the level of cholesterol at the dose of 100 mg/kg and 200 mg/kg body weight. On the other hand, the cholesterol content was slightly increased with the higher doses (400 mg/kg body weight) of MECB and MEBR.

Renal parameters were also observed in the present investigation. Kidney eliminates waste products of metabolism from the body. In renal failure, in the same way, other waste products accumulate, particularly nitrogenous substances like non-protein nitrogen, urea and uric acid. It has been reported that the

![Fig. 9. Effect of methanol extracts of Caesalpinia bonducella (MECB) and Bauhinia racemosa (MEBR) on blood levels in mice.](image)

**Fig. 9.** Effect of methanol extracts of *Caesalpinia bonducella* (MECB) and *Bauhinia racemosa* (MEBR) on blood levels in mice. Values are mean ± SEM. number of mice = 12.

* p < 0.05, ** p < 0.01, Experimental groups compared with control group.
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non-protein nitrogen value is elevated in those conditions when blood urea is also raised (Varley et al., 1980). In the present study, both the extracts at the dose of 100 and 200 mg/kg did not alter blood urea level, NPN and serum uric acid content indicated the compound has no nephrotoxic action. However, the MECB and MEBR at the doses of 400 mg/kg increased the level of non-protein nitrogen and decreased the uric acid level.

Creatinine is the least variable nitrogenous constituent of blood. The value is increased in early nephritis and in chronic hemorrhage nephritis with uremia. Similarly, increased blood content of creatinine has been reported in renal injury, subsequent to trauma or anuria, in traumatic injuries to the muscle and in muscular dystrophy. But in the present study, there is no significant alteration in creatinine level in drug-treated animals.

Effect of plant extracts on hematological parameters were also studied, and it has been observed that the red and white blood cell counts as well as hemoglobin content were not altered at the low and median doses (100 and 200 mg/kg). At the dose of 400 mg/kg, lymphocyte count decreased and neutrophil count was increased.

From the above investigation, it can be concluded that MECB and MEBR at the doses of 100 and 200 mg/kg did not alter the hematological parameters, liver and kidney functions significantly. However, at the higher dose (400 mg/kg) of the MECB- and MEBR-treated groups exhibited significant alteration of hematological profile and hepatorenal functions and metabolism. From the above discussion, it is clear that MECB and MEBR up to the dose of 200 mg/kg body weight are free from toxication. The chronic toxicity at 400 mg/kg b.w. may be due to the presence of various chemical constituents present in the extracts. Further work has is needed to find out the exact mechanism of toxicity. After clinical trials it can be considered as a good antineoplastic agent.

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