



Studies of *Anthocephalus cadamba* leaf extract on haematological and biochemical parameters of albino rats

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ABSTRACT

The present work has been designed to study the effect of hot aqueous leaf extract of *Anthocephalus cadamba* (Roxb.) Miq, Rubiaceae, on haematological and biochemical parameters of wistar albino rats. Different group of rats were administered orally with doses like 125mg/kg, 250mg/kg and 500mg/kg body weight of leaf extract. Treatment with different doses of leaf extract significantly ($p < .05$) increases the level of haemoglobin (Hb), packed cell volume (PCV), red blood cells (RBC) and white blood cells. The levels of albumin, urea, creatinine and bilirubin in wistar albino rats fed with different doses of leaf extract were not significantly different from control. Various doses of aqueous leaf extract significantly ($p < .05$ and $.01$) reduced the levels of glucose, total cholesterol, alanine amino transferase (ALT) and aspartate amino transferase (AST). Glucose, total cholesterol, ALT and AST decrease in accordance with the dose given.

Keywords: *Anthocephalus cadamba*, haematological parameters, biochemical parameters

INTRODUCTION

Anthocephalus cadamba commonly known as Kadam used in the treatment of various ailments dating back to 'charak samhita' and 'sushrut samhita'. Many Indian literatures revealed the use of this plant [1, 2] for treating skin disease, leprosy, dysentery, blood diseases, anaemia, urine complaints, leprosy, fever, colitis, menorrhagia and in improvement of semen quality. Analgesic, anti-inflammatory, antipyretic [3], anthelmintic [4], antimicrobial, wound healing, antioxidant [5], antihepatotoxic [6], diuretic, laxative [7] activity of *Anthocephalus cadamba* has been investigated. Phytochemical screening of various parts of this plant revealed out the presence of saponins [8, 9], alkaloids-cadamine and isocadamine [10], quinoline [11], secoiridoid glucoside [12], triterpenes [13]. Researchers conclude alternation in haematological and biochemical parameters after oral intake of many medicinal plants [14, 15]. Much work has not so far been done over haematological and biochemical effect of this plant. Therefore present study was designed to investigate the effect of hot aqueous extract of *Anthocephalus cadamba* leaves over haematological and biochemical parameters of wistar albino rats in order to correlate with its various pharmacological activities.

EXPERIMENTAL SECTION

Plant Material

Fresh leaves of *Anthocephalus cadamba* were collected from vrindavan, Mathura (U.P). The plant was identified and authenticated by Dr. A.K.Agrawal, Dept. of Microbiology, BSA College, Mathura (U.P). After authentication fresh leaves were collected in mass, washed, dried under shade and grinded to obtain coarse powder.

Preparation of Extract

Extraction of coarsely powdered material (200-250 gm) of shade dried leaves was accomplished with triple distilled water in a soxhlet apparatus at 100^oc for 2-3 hours. The extracted solution was evaporated to get a dark brownish crystals (percentage yield: 17-21, w/v) and was stored at 4^oC for further use.

Experimental animals

Wistar albino rats (24) of either sex weighing 80-120 gm were obtained from central animal house, GLA University, Mathura with GLAIPR/CPCSEA/IAEC/2014/Biotech02. Animals were randomly divided into 4 groups with six rats in each group. Group I i.e. control was fed with rat pellets and water ad libitum. Group II, III and IV were fed with 125 mg/kg, 250 mg/kg and 500 mg/kg body weight doses of aqueous leaf extract respectively for 21 days with rat pellets and water ad libitum.

Experimental design

At 22 day blood from all animals were collected from retro orbital plexus into ethylenediamine tetraacetic acid (EDTA) coated blood collection tubes. Serum was used for biochemical studies. Quantitative analysis of haematological parameters such as haemoglobin (Hb), packed cell volume (PCV), red blood cells (RBC) and white blood cells (WBC) was done by an automated haematology analyzer, Nihon-Kohden.

Albumin, creatinine, urea, glucose, total cholesterol, alanine aminotransferase (ALT), aspartate amino transferase (AST) and bilirubin were estimated using span diagnostic kit. Reading was taken with UV-visible double beam spectrophotometer.

Statistical Analysis

Statistical analysis of data was done with one way analysis of variance (ANOVA) using SPSS version 20.0 software and DMRT at $p < .05$ and $.01$ to determine significant differences among treatment means. Values are expressed as mean \pm SEM.

RESULTS AND DISCUSSION

The result of effect of hot aqueous extract of *A. cadamba* leaves is given in Table 1. Various doses of extract caused significant ($p < .05$) increase in Hb, RBC and PCV as compared with control. More significant ($p < .01$) increase in Hb, RBC and PCV was found at 250 mg/kg and 500mg/kg body weight (bwt) doses with respect to control. TLC significantly ($p < .05$) increase particularly at 500 mg/kg bwt dose of extract. Literatures revealed out the facts that oral intake of medicinal compounds or drugs can change the normal range of haematological parameters. These changes could either be beneficial or harmful [14, 15, 16, 17]. In this study, significant increase in Hb, RBC and PCV (Fig-1, Fig-3 and Fig-2) at various doses of leaf extract might be due to enhanced erythropoietin synthesis. [18] reported the presence of steroids, glycosides, alkaloids, flavonoids and phenolic compounds during phytochemical investigation of aqueous extract of *Anthocephalus cadamba* leaves. Detected biological ingredients may be responsible for its hematopoietic effects. Result of effect of aqueous leaf extract of the *P. nigrescens* on erythrocyte indices [19] agrees with the findings of the present study. Possible cause of enhanced Hb, RBC and PCV due to active biological ingredients needs to be further investigated.

TLC was found to be significantly increased ($p < .05$) at 500mg/Kg bwt (Fig-4), which gives us the impression of immunomodulatory activity of *A. cadamba* leaf extract, which needs to be further studied.

On biochemical parameters, the result of effect of hot aqueous extract of *A. cadamba* is given in Table 2. With respect to control significant ($p < .01$) decrease in glucose concentration at the dose of 125 mg/kg, 250 mg/kg and 500 mg/kg body weight was found. Dose dependent decrease in glucose concentration was found (Fig-5). Present study depicts glucose lowering (hypoglycemic) activity of hot aqueous extract of leaves of *A. cadamba*. Flavonoids and

phenols are found to be effective antihyperglycemic agents [20, 21]. [22] reported antidiabetic activity of leaves of *Anthocephalus indicus* in alloxan induced diabetic rats. Presence of more than one antihyperglycemic bioactive principle and their synergistic effects may be responsible for antidiabetic effect of aqueous extract of leaves. Further study needs to be carried to identify compound(s) responsible for antidiabetic property.

Table-1: Effect of Anthocephalus cadamba leaf extract at various doses on haematological parameters of wistar albino rats

Parameters (haematological)	Groups			
	Group-I (Control)	Group-II (125 mg/kg bwt)	Group-III (250 mg/kg bwt)	Group-IV (500 mg/kg bwt)
Hb (gm/dl)	11.72 ^a ± 0.11	11.73 ^a ± 0.10	12.15 ^b ± 0.14	12.30 ^b ± 0.17
PCV (%)	32.43 ^a ± 0.42	34.43 ^b ± 0.72	34.65 ^b ± 0.45	34.80 ^b ± 0.70
RBC (x 10 ⁶ /mm ³)	6.29 ^a ± 0.06	6.71 ^b ± 0.11	6.84 ^b ± 0.18	6.84 ^b ± 0.18
TLC (x 10 ³ /mm ³)	6.28 ^a ± 0.29	6.55 ^a ± 0.18	6.70 ^a ± 0.22	7.48 ^b ± 0.22

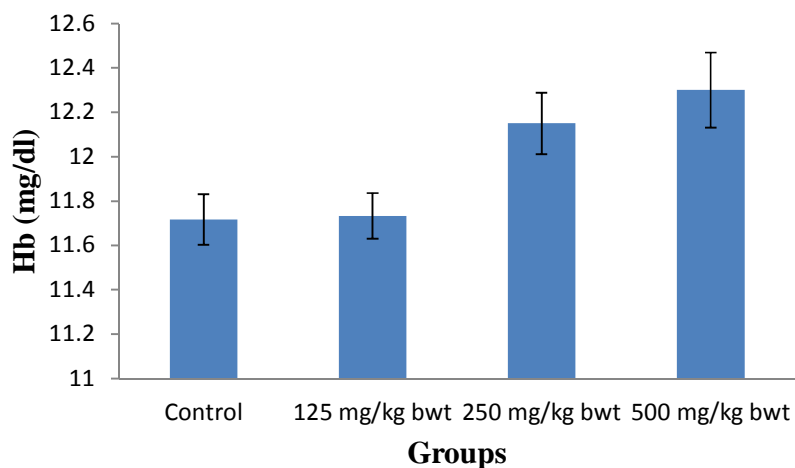
The values represent the mean ± SEM of six rats. Results are significant at $p < .05$ as per one way ANOVA followed by DMRT. Same superscript within different groups at each parameter indicate no significant difference between the values whereas ^b $p < .05$ indicates statistically significant difference in comparison with control group.

Table-2: Effect of Anthocephalus cadamba leaf extract at various doses on biochemical parameters of wistar albino rats

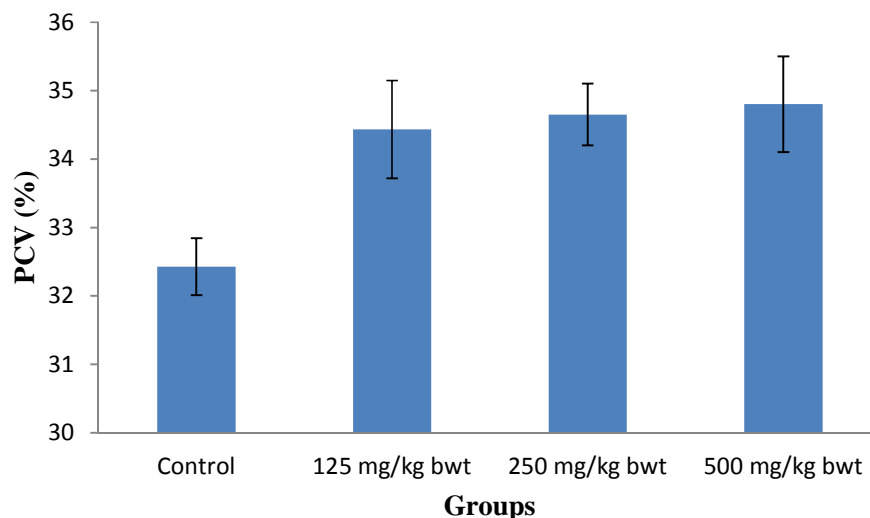
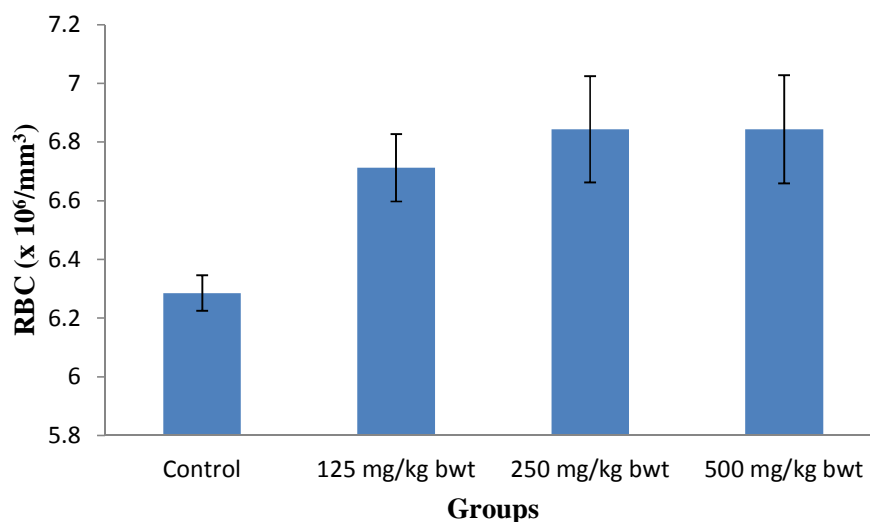
Parameters	Groups			
	Group-I (Control)	Group-II (125 mg/kg bwt)	Group-III (250 mg/kg bwt)	Group-IV (500 mg/kg bwt)
Glucose (mg/dl)	101.5 ^b ± 1.18	98.83 ^b ± .98	97.0 ^b ± 1.12	92.0 ^a ± 2.79
Urea (mg/dl)	36.03 ± 1.84	39.33 ± 4.00	37.03 ± 3.12	32.73 ± 4.04
Creatinine(mg/dl)	0.79 ± 0.05	0.72 ± 0.03	0.76 ± 0.02	0.75 ± 0.02
Cholesterol (mg/dl)	109.83 ^c ± 1.85	106.17 ^{bc} ± 1.72	103.17 ^b ± 1.75	97.66 ^a ± 1.70
Albumin (g/dl)	3.47 ± 0.25	3.22 ± 0.22	3.05 ± 0.11	2.96 ± .09
Bilirubin (mg/dl)	0.71 ± .30	0.80 ± 0.02	0.78 ± 0.02	0.73 ± .04
AST (IU/L)	75.84 ^c ± 1.40	67.71 ^b ± 2.30	66.52 ^b ± 3.72	57.20 ^a ± 2.90
ALT (IU/L)	23.63 ^b ± 0.90	20.97 ^a ± 0.72	19.93 ^a ± 1.09	18.45 ^a ± 0.52

The values represent the mean ± SEM of six rats. Results are significant at $p < .05$ and $.01$ as per one way ANOVA followed by DMRT. Same superscript within different groups at each parameter indicates no significant difference between values whereas ^b $p < .05$ and ^c $p < .01$ indicates statistically significant difference in comparison with control group.

Fig-1: Effect of various doses of aqueous leaf extract of Anthocephalus cadamba on Hb level of rats in comparison with control



No significant differences in albumin, creatinine, bilirubin and urea levels of albino rats fed with different doses of aqueous extract of leaves was observed when compared to control. Study suggested probable non toxic effect of aqueous extract of *A. cadamba* leaves over kidney of wistar albino rats since it does not cause any significant changes in creatinine level.

Fig-2: Effect of various doses of aqueous leaf extract of *Anthocephalus cadamba* on PCV level of rats in comparison with control**Fig-3: Effect of various doses of aqueous leaf extract of *Anthocephalus cadamba* on RBC level of rats in comparison with control**

Significant ($p < .05$, and $.01$) decrease in total cholesterol was found in 125mg/kg, 250mg/kg and 500mg/kg fed albino rats as compared with control. Dose dependent effect was found (Fig-6). [23] suggested lipid lowering activity of *Anthocephalus indicus* root in hyperlipidemic rats. Present study gives an impression about cardioprotective efficacy of this plant. Hence there is a need to work over lipid lowering activity of aqueous extract of this plant.

The activity of AST in treated groups were found to be significantly ($p < .05$ and $.01$) less as compared to control. The effect was dose dependent (Fig -7). Whereas significant ($p < .05$) decrease was found in ALT activity at 125mg/Kg, 250mg/Kg and 500 mg/Kg fed albino rats with respect to control (Fig-8). Whenever the lever cells are damaged, the AST and ALT activity in plasma is increased [24]. Result of present study indicates that there was no necrotic effect of aqueous extract of *Anthocephalus cadamba* leaves on the lever. Antihepatotoxic effect of *A. cadamba* is due to presence of chlorogenic acid. Phytochemical analysis and identification of components responsible for lowering AST and ALT activity needs to be further investigated.

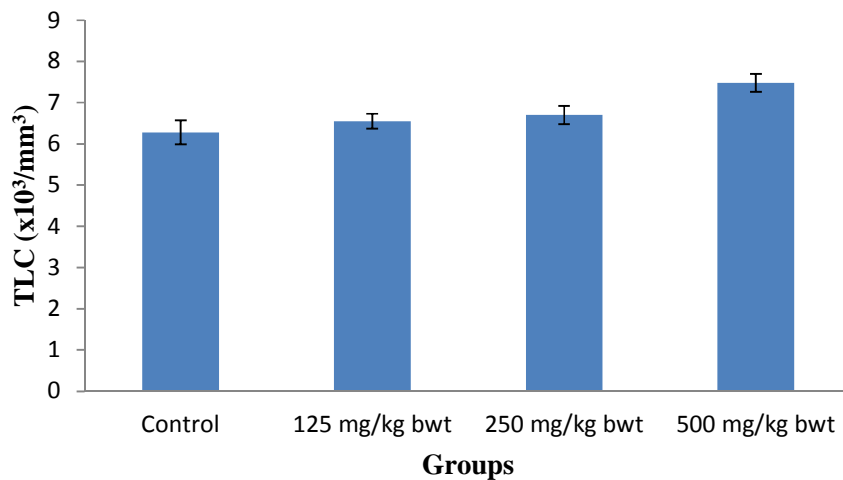
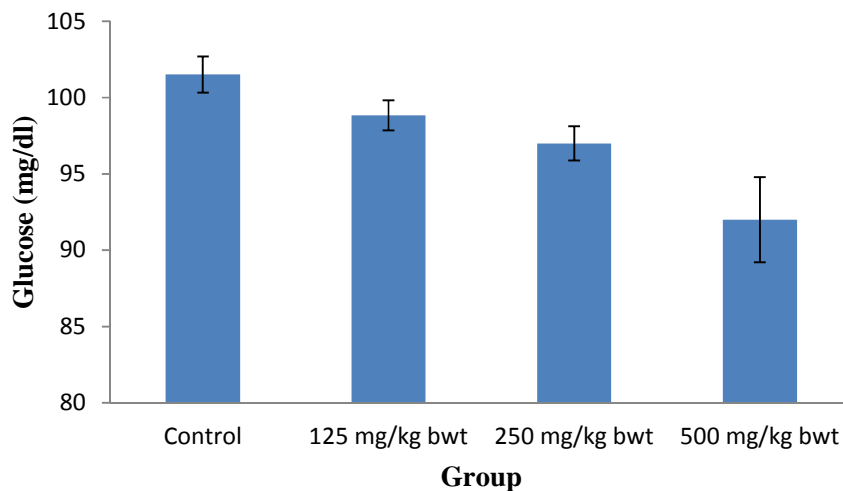
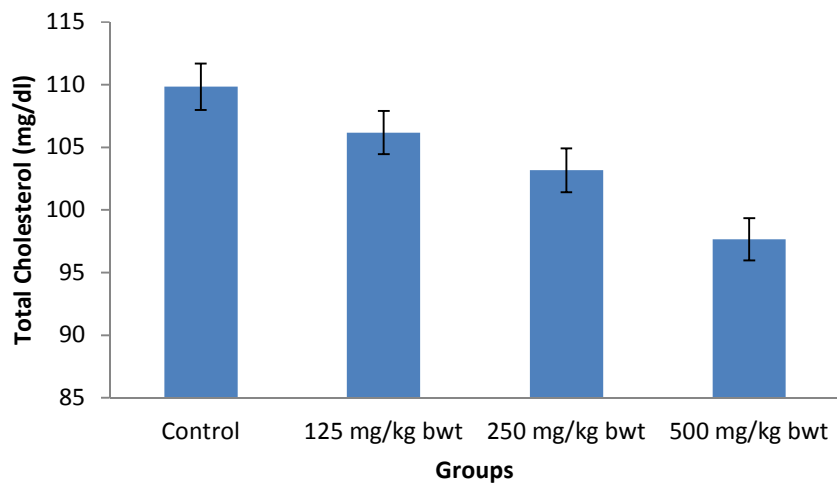
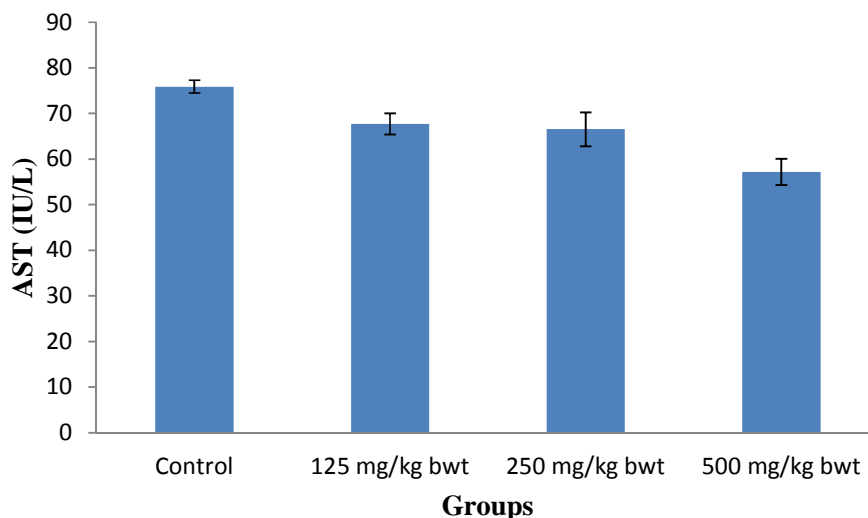
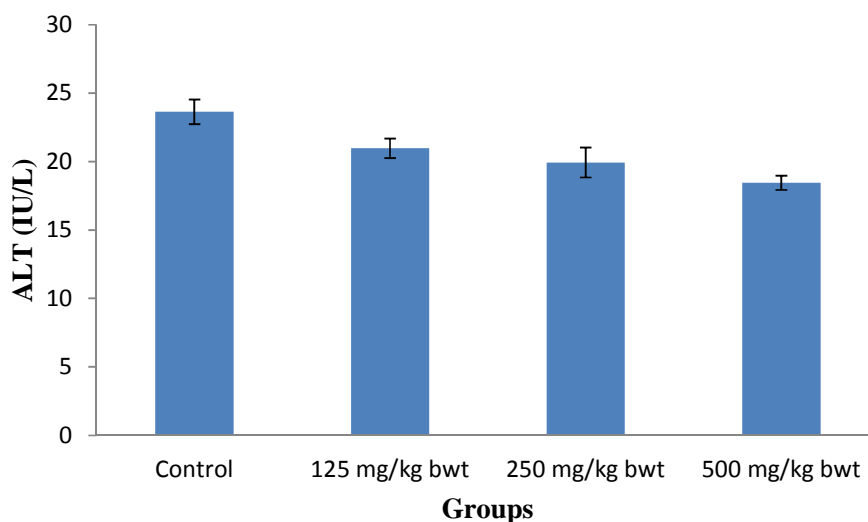
Fig-4: Effect of various doses of aqueous leaf extract of *Anthocephalus cadamba* on TLC level of rats in comparison with controlFig-5: Effect of various doses of aqueous leaf extract of *Anthocephalus cadamba* on Glucose level of rats in comparison with controlFig-6: Effect of various doses of aqueous leaf extract of *Anthocephalus cadamba* on total cholesterol level of rats in comparison with control

Fig-7: Effect of various doses of aqueous leaf extract of *Anthocephalus cadamba* on AST level of rats in comparison with control**Fig-8: Effect of various doses of aqueous leaf extract of *Anthocephalus cadamba* on ALT level of rats in comparison with control**

CONCLUSION

Present study concludes about hematopoietic, hypoglycemic, hypolipidemic, hepatoprotective and probable immunomodulatory activity of aqueous extract of leaves of *A. cadamba*.

Acknowledgement

The authors are thankful to GLA University for providing necessary facility.

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