Research Article

Oral supplementation of ethanolic extract of *Bauhinia variegata* leaves did not disturbed the hematological and serum biochemical profile of adult albino mice

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Abstract

Flower buds, flowers, leaves, stem, seed, roots and stem bark of *Bauhinia variegata* (*B. variegata*) are used for pharmacological purposes in various systems such as Homeopathy, Unani, and Ayurveda but little information is available regarding their drug transportation in living systems. Present study was designed to report the effect, if any, of 150 mg/ ml solvent/ Kg body weight of *Bauhinia variegata* leaf extract on hematology and selected parameters of serum biochemical profile of female and male albino mouse. Seven week old albino mice were weighed and fed orally either with 150 mg/ ml solvent/ kg body weight of *Bauhinia variegata* leaf extract or saline solution for 17 days. Complete blood count and selected serological parameters were determined in both experimental treatments at the end of dose supplementation. Our results revealed that oral supplementation of 150 mg/ ml solvent/ kg body weight of *Bauhinia variegata* leaf extract for 17 days did not affected ($P > 0.05$) any of the studied parameters of complete blood count and serum biochemistry in both female and male albino mice. Hence, it is concluded that 150 mg/ ml solvent/ kg body weight of *Bauhinia variegata* leaf extract can be safely administrated through oral route in albino mice.

Keywords: *Bauhinia variegata*; Ethanolic leaf extract; Serum biochemistry; Complete blood count; Albino mice

Introduction

Plants are the most precious resource for an extensive range of derivative metabolites utilized as medicine, agrochemicals, biopesticides, food additives and flavors [1]. *Bauhinia variegata* L (Synonyms: *Phanera variegata* Benth) commonly known as mountain ebony, orchid-tree, poor-man's

orchid, camel's foot and Napoleon's hat belongs to the family Leguminosae is a very popular small deciduous ornamental tree grown for its scented flowers [2-4]. All parts of this plant (leaves, flower buds, flower, stem, stem bark, seeds and roots) are reported to be used in traditional medicine [5]. It is traditionally used in the treatment of

bronchitis, leprosy, and tumors. Infusion of the leaves is used as a laxative and for the treatment of piles [6, 7].

The phytochemical screening has revealed that *Bauhinia variegata* contains terpenoids, flavonoids, tannins, saponins, reducing sugars, steroids and cardiac glycosides [8-11]. A number of pharmacological studies has documented that *Bauhinia variegata* has anticancer, antioxidant, anti-hyperlipidemic, antidiabetic, antimicrobial, anti-inflammatory, nephro-protective, hepatoprotective, antiulcer, immunomodulating, molluscicidal and wound healing effects [7, 12-17]. Despite of its biological importance, limited information is available in literature regarding its potential effects on complete blood count and serum biochemistry. The aim of this study was to determine the effect of 150 mg/ ml solvent/ kg body weight of *Bauhinia variegata* leaf extract on complete blood count and on selected serological parameters in female and male albino mice.

Materials and methods

Subjects

In order to demonstrate the effect of *Bauhinia variegata* leaf extract on hematology and selected parameters of serum biochemistry seven week old albino mice of both genders (N = 28, male = 14 and female = 14) were used as experimental animals in this study. Breeding pairs of albino mice were donated by Department of Zoology, Quaid-e-Azam campus, Punjab University Lahore, Pakistan, reared and maintained at the animal facility of Bahauddin Zakariya University Multan in Bio- Park. Animals were kept in locally manufactured small rodent cages filled with wood chips. In breeding colony, standard mouse diet and water were available *ad libitum*. Room temperature was maintained at $22 \pm 1^{\circ}\text{C}$. The light/dark rhythm was maintained at 14:10. The room was illuminated with artificial light at an

intensity of about 200 Watt from 8 a.m. to 6 p.m. All experimental protocols and animal handling procedures were approved by ethical review board of Institute of Pure and Applied Biology, Bahauddin Zakariya University Multan, Pakistan.

Preparation of *Bauhinia variegata* leaf extract

The leaves of *Bauhinia variegata* were collected from different places in Multan, Pakistan. The leaves were thoroughly washed with distilled water and air dried under shade for about 15-20 days. The completely dried leaf samples were grounded to fine powder in a grinder (Waring, USA) and stored in air tight polythene bags at room temperature before further processing. The dried powder (200 grams) was soaked in 70% ethanol solvent for about 9 days in a brown colored amber bottle to avoid sunlight. The bottle with soaked material was shook 3-4 times a day and lid of bottle was opened for a few seconds to evaporate fumes produced and lid was tightly closed after that. Every third day, the material was filtered through a filtration assembly by using a Whatman filter paper to avoid impurities and residue were re-soaked in 70% ethanol solvent. On the 9th day the filtrate was passed through Rota vapor (Buchi Rota-vapor R-205, Switzerland) by keeping the temperature of chiller at 37°C until extract was separated from ethanol. The extract was further dried in an electric oven at 40°C until maximum ethanol was evaporated and then it took the form a dark green gummy residue. From this paste, 150 mg was taken and dissolved in 1 ml of distilled water to prepare the stock solution of *Bauhinia variegata* leaf extract following [18].

Experimental design

Following weaning, male and female mice were separated from their parents and were kept individually in cages until they were seven week old. At this point, animals were

divided into two groups. First group was orally supplemented with 150 mg/ ml solvent/ kg body weight of *Bauhinia variegata* leaf extract (N = 14), While the second group was orally supplemented with saline solution for 17 days.

Blood and serum collection

At the end of experimental treatment, blood was sampled either through cardiac puncture or from retro-orbital sinus under Isoflurane (3%) inhalation. For the analysis of serum biochemical parameters and for the determination of complete blood count in male and female albino mice, blood was divided into two parts respectively.

Hematological and serum biochemical profiling

Complete blood count (mean corpuscular volume, mean corpuscular hemoglobin concentration, packed cell volume, hemoglobin level, total red and white blood cell count, total lymphocytes count, total platelets count, red blood cell distribution width, platelets distribution width and large platelet concentration ratio) was determined in treated and untreated albino mice by using hematology analyzer FMI- 6180 (Jiangsu, China) following [19]. While

serum biochemical parameters [Cholesterol, Alanine transaminase (ALT), Aspartate transaminase (AST), Total protein, creatinine and triglycerides] were analyzed in serum samples by using Hitachi 902 automatic analyzer (Japan) following the instructions of diagnostic kit manufacturers following Qadir [20].

Statistical analysis

All the data is expressed as Mean $\pm$ Standard error of mean. Statistical package Minitab (version 17, Pennsylvania) was used for the statistical analysis of the results. Two sample t-test was used to compare all studied parameters of complete blood count and serum biochemistry between *Bauhinia variegata* and saline treated female and male albino mice.

Results

Effect of *Bauhinia variegata* on complete blood count of female and male albino mice

Analysis of the results revealed that all the studied parameters of complete blood counting varied non significantly ($P > 0.05$) when compared between *Bauhinia variegata* leaf extract treated and untreated female and male albino mice (Table 1).

Table 1. Comparison of various studied hematological parameters between adult albino mice (of both genders) treated with *Bauhinia variegata* (150 mg / ml solvent / kg body weight) or saline solution for 17 days. (Treated N = 7 for each treatment). All values are expressed as mean $\pm$ slandered deviation. P-value presents the results of 2 sample t-test conducted for each parameter between the two treated groups

Parameters	Female mice			Male mice		
	Saline treatment	<i>Bauhinia variegata</i> treatment	P - value	Saline treatment	<i>Bauhinia variegata</i> treatment	P - value
RBC ($\times 10^6 \mu\text{L}^{-1}$)	4.49 $\pm$ 1.68	4.74 $\pm$ 1.54	0.78	5.44 $\pm$ 0.8	4.94 $\pm$ 1.33	0.5
WBC ($\times 10^3 \mu\text{L}^{-1}$)	7.87 $\pm$ 2.6	9.99 $\pm$ 4.61	0.32	10.31 $\pm$ 4.52	9.89 $\pm$ 3.7	0.84
HGB (gdL ⁻¹)	9.07 $\pm$ 5.73	12.51 $\pm$ 4.29	0.23	12.74 $\pm$ 2.57	10.71 $\pm$ 3.43	0.24
HCT (%)	36.9 $\pm$ 13.7	39.5 $\pm$ 12.3	0.72	42.79 $\pm$ 6.67	36.84 $\pm$ 7.04	0.13
MCV (fl)	84.67 $\pm$ 8.69	83.60 $\pm$ 2.38	0.76	79.5 $\pm$ 11.0	76.9 $\pm$ 14.3	0.71
MCH (pg)	22.6 $\pm$ 11.2	26.31 $\pm$ 2.16	0.43	23.59 $\pm$ 3.84	22.63 $\pm$ 7.65	0.77
MCHC (gdL ⁻¹)	26.6 $\pm$ 12.3	31.47 $\pm$ 2.37	0.34	29.54 $\pm$ 1.62	28.89 $\pm$ 6.46	0.8
PLT ($\times 10^3 \mu\text{L}^{-1}$)	200.0 $\pm$ 67.0	260 $\pm$ 12	0.28	175.4 $\pm$ 88.2	235 $\pm$ 144	0.37
LYM (%)	39.5 $\pm$ 12.6	31.1 $\pm$ 12.8	0.24	31.6 $\pm$ 12.7	32.8 $\pm$ 16.3	0.88

MXD (%)	9.87 ± 3.69	10.06 ± 3.43	0.92	10.10 ± 3.04	8.06 ± 2.86	0.22
NEUT(%)	56.4 ± 14.3	58.9 ± 14.4	0.75	58.3 ± 13.6	59.5 ± 18.0	0.89
LYM (%)	2.48 ± 0.768	3.04 ± 2.47	0.59	2.77 ± 0.79	2.97 ± 1.93	0.81
MXD (%)	0.84 ± 0.445	1.09 ± 0.886	0.64	1.04 ± 0.588	0.74 ± 0.199	0.24
NEUT (#)	4.06 ± 1.80	5.86 ± 3.15	0.23	6.50 ± 3.74	16.17 ± 4.21	0.88
RDW (%)	34.6 ± 15.9	45.50 ± 3.94	0.16	42.69 ± 3.53	37.6 ± 15.6	0.43
PDW (%)	10.05 ± 0.38	11.37 ± 1.6	0.08	9.06 ± 4.72	5.84 ± 4.04	0.2
MPV (fl)	7.85 ± 0.229	8.229 ± 0.53	0.13	6.73 ± 3.15	5.04 ± 3.45	0.36

RBC: red blood cell, WBC: white blood cell, HGB: hemoglobin concentration, HCT: hematocrit, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, PLT: Platelet, LYM: Lymphocyte, MXD: Relative (%) content of the mixture of monocytes, basophils, and eosinophils, NEUT: Neutrophils, LYM: Lymphocytes, RDW: Red cell distribution width, PWD: Platelet distribution width, MPV: Mean platelet volume. P > 0.05 = Non significant.

Effect of *Bauhinia variegata* on serum biochemistry of female and male albino mice

Analysis of the results revealed that all the studied parameters of serum biochemical

profile varied non-significantly (P > 0.05) when compared between *Bauhinia variegata* leaf extract treated and untreated female and male albino mice (Table 2).

Table 2. Comparison of various studied serum biochemical parameters between adult albino mice (of both genders) treated with *Bauhinia variegata* (150 mg / ml solvent / kg body weight) or saline solution for 17 days treated (N = 7 for each treatment). All values are expressed as mean ± standard deviation. P-value presents the results of 2 sample t-test conducted for each parameter between the two treated groups

Parameters	Female mice			Male mice		
	Saline treatment	<i>Bauhinia variegata</i> treatment	P - value	Saline treatment	<i>Bauhinia variegata</i> treatment	P - value
ALT (IU/L)	140.1 ± 59.2	191.4 ± 15.2	0.07	139.8 ± 29.1	163.9 ± 41.8	0.24
AST (IU/L)	158 ± 135	185.1 ± 8.32	0.61	120.0 ± 35.3	119.0 ± 68.2	0.09
Total proteins (g/dl)	8.21 ± 2.39	9.66 ± 1.11	0.19	9.43 ± 2.16	8.67 ± 2.21	0.53
Triglycerides (mg/dl)	246 ± 139	333.3 ± 39.8	0.16	278 ± 109	293.9 ± 69.5	0.76
Creatinine (mg/dl)	0.357 ± 0.15	0.457 ± 0.18	0.29	0.429 ± 0.19	0.471 ± 0.32	0.76
Cholesterol (mg/dl)	67.1 ± 29.2	48.9 ± 18.0	0.19	78.0 ± 29.8	76.3 ± 59.6	0.95

Where ALT: Alanine transaminase, AST: Aspartate aminotransferase. P > 0.05 = Non significant (NS)

Discussion

Medicinal plants, as potential source of therapeutic aids, have attained significance in health system, for both humans and animals, all over the world not only in diseased condition but also for maintaining proper health [21]. The genus *Bauhinia* belongs to the family Caesalpiniaceae (formally Leguminosae) and several members of this genus like *Bauhinia manca*,

Bauhinia divaricata, *Bauhinia purpurea* and *Bauhinia variegata* are known for their medicinal importance [5, 22-25]. This study was conducted to determine the effect of 150 mg/ ml solvent/ kg body weight of *Bauhinia variegata* leaf extract on hematology and serum biochemical parameters of adult female and male albino mice. Our results revealed that all the studied hematological parameters varied non

significantly ($P > 0.05$) when compared between the *B. variegata* leaf extract treated and untreated albino mice of both genders, indicating that *B. variegata* has no effect on hematology and can be supplemented orally as well as intravenously as therapeutic agent with no side effects (Table 1). Our results are in agreement with Jacobo *et al.* [26] as they did not observed any significant change in red blood cell, white blood cell, platelet count and in hemoglobin concentrations when compared between rodents, of both genders, exposed to variable doses of *Bauhinia purpurea* leaf extract.

It was observed that all the studied serological parameters also varied non-significant ($P > 0.05$) when compared between *Bauhinia variegata* leaf extract treated female and male mice and their respective untreated control group (Table 2). Our results are in agreement with Jacobo *et al.* as they reported that all of the parameters in serum biochemistry analysis were within normal range in the *B. purpurea* treated adult female and male albino rats [26]. Pani *et al.* [27] has reported that the serum creatinine decreased significantly in *B. variegata* stem extract treated mice as compared with the control group [27]. Balamurugan and Muralidharan [28] has also reported that the oral administration of methanolic extract of *B. variegata* (200 mg/kg) induced obese animals resulted in decreased total cholesterol and triglycerides levels in serum [28]. Abduljassim *et al.* [29] reported that the treatment with *B. variegata* ethanolic leaf extract significantly reduced the total cholesterol and triglyceride in Plasma in diabetic mice [29].

Conclusions

In conclusion, we are reporting that 150 mg/ml solvent/ kg body weight of *B. variegata* leaf extract orally supplemented for 17 days to female and male albino mice did not affect the complete blood count and studied parameters of serum biochemistry and can

be safely administrated orally as therapeutic agent.

Authors' contributions

Designed the study: F Iqbal, Lab experiments: A Ullah, S Iqbal & M Qasim, Analyzed the data: Asmatullah, S Iqbal & M Qasim, Wrote the manuscript: F Iqbal.

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