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Toxicological evaluation of *Bauhinia variegata Linn*. for estimating the neurological, hematological and physical alterations in the albino mice

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Abstract

The objective of present study is to establish the safety standards and the evaluation of potential toxicity of *Bauhinia variegata Linn*. Dried stem bark extract of Bauhinia variegata were evaluated for toxicity studies. For acute toxicity study healthy swiss albino mice, divided in various groups, were administered the sample extract orally in increasing dose. For the sub- acute toxicity studies, the animals of the acute toxicity studies were administered daily for a period of 15 days. The parameters of the acute toxicity studies were tabulated after 48 and 72 hrs of the dose administration and those of the sub acute study were tabulated after 15 days of the dose administration. The toxicity studies did not show any mortality up to a dose of 3000 mg/kg and no significant changes were observed. As no toxicity was observed, *Bauhinia variegata Linn*. extract could be continued to use in folk medicines.

Key Words: Bauhinia variegata Linn., acute toxicity, sub acute toxicity.

Introduction

From the ancient time, Herbal plants are widely used for the treatment of varous human disorders all over the world because of the presence of active constituents of therapeutic value. According to World Health Organization (WHO) more than 80% of the world's population still relies on traditional medicine for their primary health care needs (Hassan et al.,2009).Among the hundreds of medicinal plant, *Bauhinia variegata Linn*. is one of them, and its value in medicine is known since ancient ages. *Bauhinia variegata Linn.* is a member of the family Leguminosae. It is a medium sized tree with hairy branches. The various parts of the tree like flowers, flowers bud, stem bark, stem, leaves, seeds and roots are popular in various systems of medicines like ayurveda, unani and homeopathy in India for the cure of variety of disease (Sahu& Gupta, 2012).Phytochemical screening of the stem bark and leaves of *Bauhinia variegata Linn*. showed that the plant contained various active constituents like carbohydrates, resins, saponins, terpinoids, alkaloids, steroids, flavonoids, tannins, proteins and cardiac glycosides (Kumar et al., 2015). Because of these active constituents this plant is used as folk medicines for treatment of various pathological diseases. Various pharmacological studies showed that this plant exerted antidiabetic activity, anti-inflammatory activity, anti-tumour activity, hepatoprotective effect, antibacterial activity, haemagglutinating activity, haematinic activity, antimicrobial activity, immunomodulatory activity and antiulcer activity (Esmail&Snafi, 2013, Sahu& Gupta, 2012).

In recent year, there are an upsurge in the clinical uses of indigenous drug because of their efficacy and free from serious toxic effects. Moreover, constant increase in the antibiotic resistance strains and various side effects caused by the synthetic drug has prompted scientists to look for herbal immune-modulators to treat various infections. Herbal drugs are believed to enhance the natural resistance of the body against infection and their immune-modulatory activity have been reported in numerous plant extract (Patwardhan et al., 1990)

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Materials and Methods:

Chemicals: The chemicals used were of analytical grade. Tween 80, petroleum ether, chloroform, ethanol were the chemicals used in the study.

Animals and their maintenance:

Healthy adult Swiss albino mice of either sex of weight range 25-30 g, housed in the animal house of the Department of Pharmaceutical Sciences were used. Animals were fed with standard diet[8]. Animals were maintained under standard conditions of temperature (25°C ± 5°C) and relative humidity (55 ± 10%), and 12/12 hr light/ dark cycle. They were housed in standard polypropylene cages with wire mesh top husk bedding. All the experimental procedures and protocols used in the study were reviewed by the Institutional Animal Ethics Committee and were in the accordance with the guidelines of the CPCSEA, Ministry of Forest and Environment, Government of India.

Extraction of the plant material:

Plant materials were washed with water and shade dried. The dried bark was crushed to coarse powder by the grinder. The powdered material was defatted with petroleum ether (60-80 ºC) and then successively extracted in Soxhlet apparatus with alcohol, petroleum ether and chloroform as a solvent. Extract obtained was passed through the Whatman filter paper No.1 and the solvent was evaporated with the help of a distillation unit and the spongy mass so obtained was dried in a desiccator (Kumar et al., 2015).The extract was concentrated for further studies on water bath at 40 ºC (Jangra et al., 2012).

Toxicity Study:

Principle of the Test: Acute toxicity refers to those adverse effects occurring following oral administration of a single dose of a substance or multiple given within 24 hours. For the acute toxicity evaluation the guidelines of the Organization of Economy and Cooperation development (OECD) were largely accepted and followed.

Procedure:

Healthy Swiss albino mice of either sex (n=6, 3 females and 3 males), weighing 20-25 g, divided in each group, were fasted for 18 hrs overnight were used for the study. Animals were divided in to the thirteen different groups including one control group. The animals of group I to IV were administered the alcoholic sample extract and the group V to VIII received petroleum ether extract and the groups IX to XII received Chloroform extract orally in increasing dose of 250,500,1000 and 3000 mg/kg body weight respectively. While the control group received vehicle only.Then the animals were continuously observed for 2 hrs for any gross behavioral, neurological or autonomic toxic effects and for any lethality after 24 to 72 hrs. After administration, Irwin's test was conducted, where the animals were observed for the gross behavioral changes(Kumar et al., 2015)

For this following list was employed:

Behavioral profile (Ukwuani et al.,2012)**:**

Awareness: Alertness, visual placing, sterotyphy and passivity.

Mood: Grooming, restlessness, irritability and fearfulness.

Neurological profile:

Motor Activity: Spontaneous activity, reactivity, touch response, pain response, startle response, tremor, gait, grip strength, pinna reflex, corneal reflex.

Autonomic profile:

Writhing, defecation, urination, pilo erection, heart rate, respiratory rate.

In the sub-acute toxicity study, all the animals (divided in each group) of acute toxicity studies were administered daily for a period of 15 days. Additional observations include change in skin and fur, eyes and mucous membranes, and also somatomotor activity and behavior pattern. Attentions were given to observations of tremors, convulsions, salivation, diarrhoea, sleep and coma. The following parameters were particularly observed and tabulated:

Body Weight:

Individual weights of animals were determined shortly before the test extract was administered and at least weekly thereafter(Sadashiv,2011).

Food consumption:

Food consumption was determined every day by considering food given and food left in each group.

Hematological parameters:

On 15th day of the experiment, animals were anaesthetized and the blood sample was collected from retro orbital plexus. Various hematological parameters such as Bleeding time (min), Clotting time (sec), Total WBC/mm^3, Total RBC/mm^2, and

Hemoglobin (mg/dl) were estimated using various standard methods(Gandhare&Rajkapoor,2013).

Data analysis:

The data of the change in body weight and food consumption of the extract treated group was compared with that of the normal and vehicle treated group. The level of significance was analysed by the application of the paired t – test.

Results and Discussion:

The acute toxicity studies of the *Bauhinia variegata Linn* did not show any mortality up to a dose of 3000 mg/kg body weight when administered orally. No toxic symptoms or death were observed in any of the groups. The acute toxicity studies carried out for 48 hrs showed normal motor reflexes and behavioral patterns. The groups I to XII all showed normal responses that were similar to the control group. Moreover, no mortality was seen in any group, at any dose. Similar results were observed when the acute toxicity study was carried out for 72 hrs, that is, no lethality in any group, at any dose.

In case of sub acute toxicity study the body weight increased in all the groups, both treated and controls (Table I). However, there was significantly ($P < 0.05$) more gain in body weight in the control group than in treated groups (Fig. 1). Similar result was obtained for feed intake in all the groups (Fig. 2 & 3).

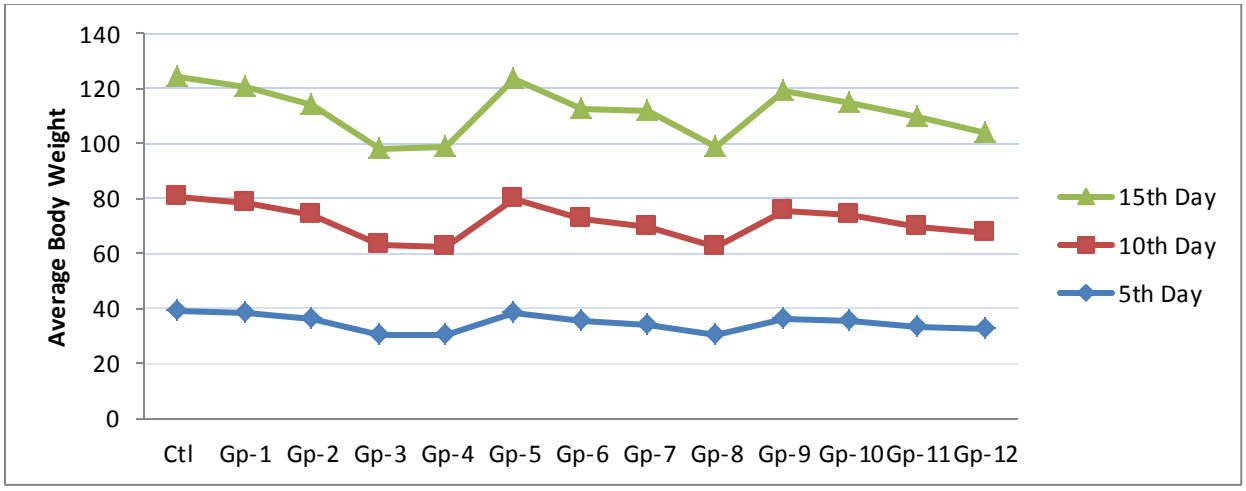

Fig. 1: Effect of the extracts of *Bauhinia variegata Linn.*on the body weight of mice for 15 days
X—Axis: Groups Y—Axis: Average Weight (g) of the mice

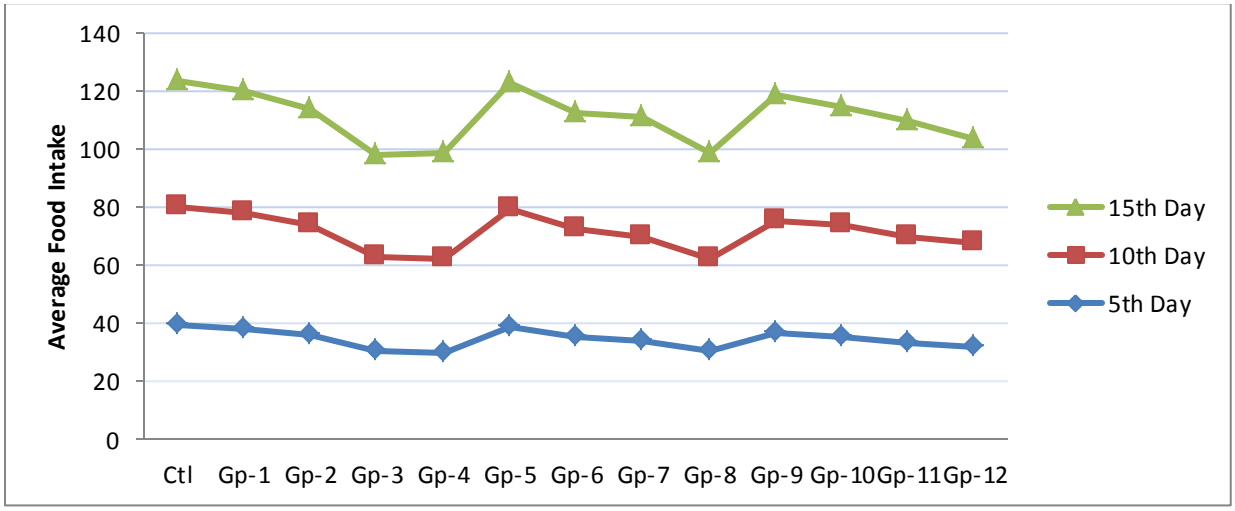

Fig. 2: Effect of the extracts of *Bauhinia variegata Linn.*on the feed intake of mice for 15 days
X—Axis: Groups Y—Axis: Feed intake (gm) of the mice

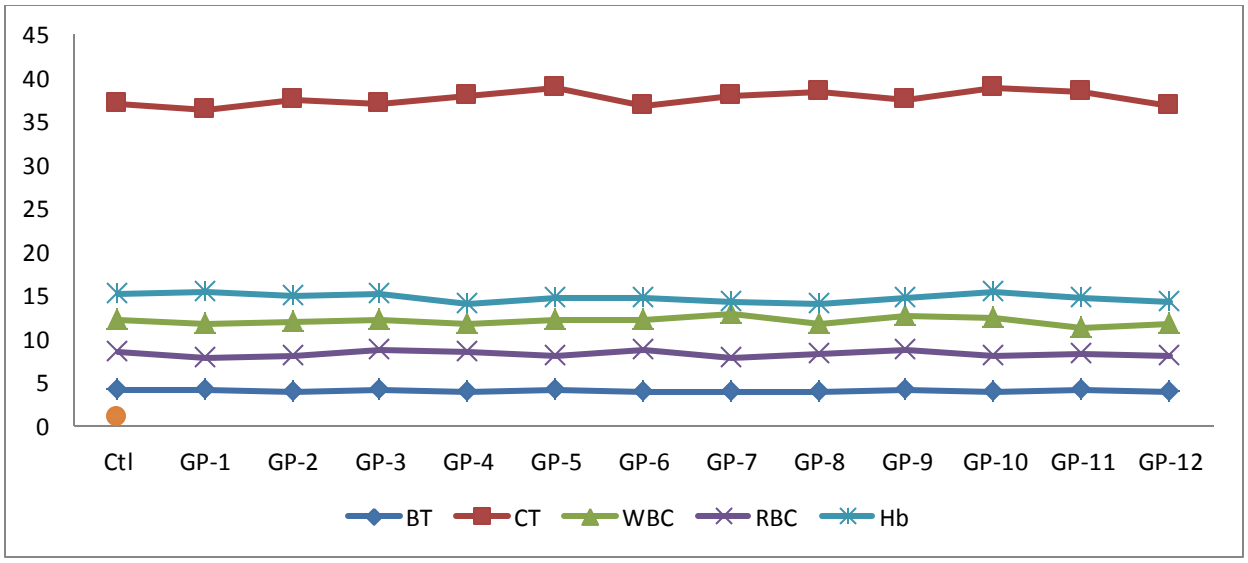

Fig. 3:Effect of the extracts of *Bauhinia variegata Linn.*on Hematological Parameters

X—Axis: Groups Y—Axis: Hematological Parameters range Value

Group I-IV: Alcoholic extract treated group Group V-VIII: Pet. Ether extract treated group Group IX-XII: Chloroform extract treated group

Table1 : Sub-Acute toxicity studies for the estimation of Hematological parameters

S.No	Group (n=6)	Dose (mg/kg)	Bleeding Time(min)	Clotting time (sec)	WBC (x 10^3 uL^{-1})	RBC (x 10^6 uL^1)	Hemoglobin (mg/dl)
1.	Ctrl Gp	---	4.06±0.07	37.03_±0.02	12.3±1.6	8.52±0.57	15.1±0.21
2.	Gp-I(Al. Ext.)	100	4.05±0.12	36.4±0.61	11.8±1.3	7.92±0.11	15.30±0.22
3.	Gp-II(Al. Ext.)	500	3.86±0.22	37.45±0.80	11.9±1.1	8.03±0.27	14.9±0.19
4.	Gp-III(Al. Ext.)	1000	4.1±0.36	37.09±0.11	12.2±1.0	8.75±0.02	15.07±0.22
5.	Gp-IV(Al. Ext.)	3000	3.9±0.81	37.94±0.38	11.8±2.0	8.60±0.11	14.03±0.41
6.	Gp-V(P.ether Ext.)	100	4.11±0.52	38.88±0.54	12.3±5.0	8.02±0.25	14.6±0.60
7.	Gp-VI(P.ether Ext.)	500	3.90±0.33	36.77±0.21	12.2±1.3	8.66±0.32	14.7±0.32
8.	Gp-VII(P.ether Ext.)	1000	4.02±0.60	37.83±0.05	12.8±2.0	7.93±0.42	14.32±0.28
9.	Gp-VIII(P.ether Ext.)	3000	3.88±0.71	38.35±0.31	11.7±2.0	8.22±0.40	13.96±0.11
10.	Gp-IX(Ch. Ext.)	100	4.03±0.03	37.39±0.55	12.6±1.0	8.70±0.34	14.8±0.42
11.	Gp-X(Ch. Ext.)	500	3.93±0.53	38.81±0.64	12.4±2.1	8.10±0.40	15.5±0.50
12.	Gp-XI(Ch. Ext.)	1000	4.11±0.27	38.39±0.91	11.3±3.0	8.22±0.41	14.71±0.03
13.	Gp-XII(Ch. Ext)	3000	4.02±0.30	36.83±0.87	11.7±2.0	8.06±0.11	14.22±0.33

S.No	Group (n=6)	Dose (mg/kg)	Average body weight			Average food intake		
			5th Day	10th Day	15th Day	5thDay	10thDay	15thDay
1.	Ctrl Gp	---	23.85 ± 0.07	24.14 ± 0.04	25.38 ± 0.12	39.13 ± 3.9	41.25 ± 3.1	43.58 ± 3.4
2.	Gp-I (Al. Ext.)	100	23.94 ± 0.10	24.55 ± 0.09	25.10± 0.02	38.27 ± 3.3	40.02 ± 2.9	42.11 ± 3.6
3.	Gp-II (Al. Ext.)	500	24.62 ± 0.04	25.63 ± 0.02	26.61 ± 0.11	36.22 ± 3.3	37.81 ± 2.3	40.2 ±1.1
4.	Gp-III (Al. Ext.)	1000	23.72 ± 0.17	24.16 ± 0.09	25.81 ± 0.04	30.44 ± 3.8	32.44 ± 3.5	35.20 ±2.3
5.	Gp-IV (Al. Ext.)	3000	24.63 ± 0.12	24.98 ± 0.16	25.43 ± 0.03	30.12 ± 3.3	32.44 ± 2.9	36.22 ±3.1
6.	Gp-V (P.ether Ext.)	100	24.77 ± 0.71	24.83 ± 0.80	25.63 ± 0.04	38.49 ± 1.8	41.33 ± 2.1	43.31 ±1.8
7.	Gp-VI (P.ether Ext.)	500	24.16 ± 0.10	25.32 ± 0.09	26.13 ± 0.01	35.55 ± 3.7	36.71 ± 2.4	40.22 ± 2.8
8.	Gp-VII (P.ether Ext.)	1000	23.52 ± 0.02	24.26 ± 0.06	25.17 ± 0.11	34.21 ± 2.5	35.46 ± 3.2	41.85 ± 2.4
9.	Gp-VIII (P.ether Ext.)	3000	24.58 ± 0.06	25.18 ± 0.03	26.48 ± 0.07	30.15 ± 2.8	32.28 ± 2.9	36.33 ± 2.7
10.	Gp-IX (Ch. Ext.)	100	24.33 ±0.03	25.31 ± 0.16	26.07 ± 0.04	36.44 ± 3.1	39.22 ± 2 .9	43.12 ± 2.5
11.	Gp-X (Ch. Ext.)	500	25.13± 0.06	25.81 ± 0.03	26.01 ± 0.41	35.59 ± 3.2	38.18 ± 3.4	41.17 ± 3.6
12.	Gp-XI (Ch. Ext.)	1000	24.04 ± 0.16	25.77 ± 0.08	26.39 ± 0.13	33.42 ± 3.9	36.32 ± 3.3	40.17 ± 3.5
13.	Gp-XII (Ch. Ext)	3000	24.41 ± 0.13	25.16 ± 0.31	26.44 ± 0.22	32.16 ± 3.6	35.32 ± 3.4	36.11 ± 2.9

Table2 : Sub-Acute toxicity studies for the estimation of Hematological parameters

The values are mean ± SEM, n= 6, when compared with control group.
p = 0.001, (One way ANOVA followed by Dunnett's, Multiple comparison test).

Among the Hematological parameters mixed results were obtained. The groups I to XII showed no significant changes in the Bleeding time and Clotting time that was almost comparable to the control group. On the other hand, the parameters like WBC, RBC and Hemoglobin were found to be increasing in groups II to XIII when compared to the control group (Table II).

Conclusion:

The results showed that the extracts of *Bauhinia variegata Linn*. did not cause much change in the neurological, hematological and physical parameters of the albino mice. Thus, the present study may explain the continous use of *Bauhinia variegata Linn*. extract in folk medicines.

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