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Acute toxicity study of the nanoparticles of *Bauhinia variegata* Linn bark hydro-alcoholic extract in Wistar Albino rats

Research Article

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Abstract

Background: *Bauhinia variegata* Linn is a popular herb used as medicine in traditional health sciences in Southeast Asia. Traditional medicines if used in newer dosage forms such as nanoparticles, can help in reducing the dose, assuring quality, and reducing exploitation of natural resources. However, the safety of medicines needs to be established before use in humans. Objective: To evaluate the acute toxicity of nanoparticles of *Bauhinia variegata* Linn bark hydro-alcoholic extract (NBVBE) in Wistar Albino rats on the basis of OECD guidelines 423. Methodology: The study was conducted using six Wistar albino rats divided into two groups of three rats each. Test rats were given a single dose of 2000 mg/kg body weight of NBVBE orally, while the control group received feed and water ad libitum. Results: The data indicated no significant changes in weight, complete blood count, or renal and hepatic function when compared with control ($p > 0.05$). Histopathological examination shows some reversible changes such as steatosis and lymphoid hyperplasia in the rat's liver and intestine. LD50 for NBVBE is higher than 2000 mg/kg body weight. Conclusion: NBVBE is safe at 2000 mg/kg body weight. However, monitoring the status of the liver is necessary.

Key Words: *Ayurveda, Kanchanar,* Nanomedicine, Mountain ebony, Herbal medicine.

Introduction

Ayurveda is a holistic science that is present and serving the people since the ancient era. In present times, an amalgamation of this ancient treasure of knowledge with modern technology is necessary. Many researchers across the world are making attempts in this direction. Nanotechnology is one of such new technologies that have applications in science, engineering, and health (1). It is a convergence of disciplines such as chemistry, physics, material science, and biology (2). In the field of plant-based research, adding nano-based formulations has a variety of benefits namely – improved solubility and bioavailability, toxicity protection, increased pharmacological activity, improved stability, increased tissue macrophage distribution, prolonged delivery, and protection from physical and chemical degradation (3). As resources for plant-based medicines are limited and always face the danger of extinction replantation is not done. Hence, available resources need to be used wisely. So, a newer technique that may reduce the dose of medicine is a welcoming step.

Bauhinia variegata Linn is a medium-sized deciduous tree that is found throughout India, especially in the range of Himalayas at an altitude of 1800 meters (4). It is conventionally used in bronchitis, leprosy, inflammation, bacterial infection, liver disorders, diarrhoea, dysentery, skin disease, leprosy, intestinal worms, wounds, ulcer, fungal infections, ulcers (5). The stem bark is utilized for alliterative, antidiabetic, anticancer, tonic, and anthelmintic purposes, as well as obesity and ulcer treatment (6).

Due to the common belief that herbal medicines are free from toxic effects as they are obtained and used as found in nature. However, there is little evaluation of possible toxic manifestations caused by natural materials (7). Any new plant-based formulation or newer dosage forms need to be evaluated for possible harmful effects. One of the most extensively used approaches for testing acute oral toxicity effects of varied drugs is through animal studies. However, animal welfare concerns have led to the adoption that allows the use of a smaller number of animals in such studies (8).

Available data shows that the stem bark, its aqueous or hydroalcoholic extracts of *Bauhinia variegata* Linn are non-toxic at doses up to 2000 mg/kg to 5000 mg/kg. However, previous work done on nanoparticles of whole bark powder of *Bauhinia variegata* Linn has shown hydropic changes and steatosis in the liver of Wistar albino rats at the dose of

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2000 mg/ kg (9). Hence the present study was conducted to evaluate the possible toxic effect of the nanoparticles of *Bauhinia variegata* Linn bark hydro-alcoholic extract (NBVBE) in the Wistar albino rats by using behavioral patterns, blood parameters, and histopathology on the basis of OECD guidelines 423.

Objectives

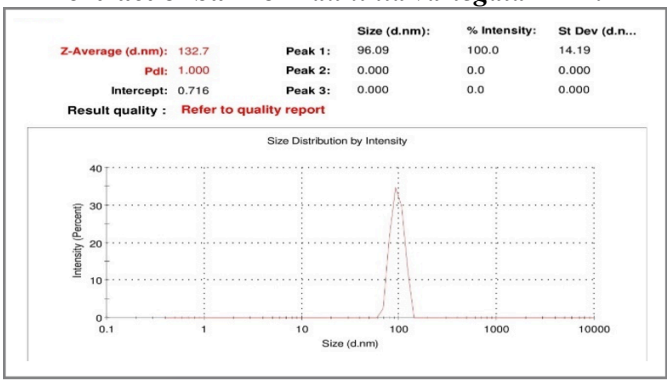
The objective of the study was to evaluate the safety nanoparticle of *Bauhinia variegata* Linn bark hydro-alcoholic extract in Wistar albino rats using OECD Guidelines- 423 on the basis of behavioral pattern, hemogram, hepatic and renal function test, and gross Necropsy.

Methodology

Collection of *Bauhinia variegata* Linn stem bark and preparation of extract

The *Bauhinia variegata* Linn stem bark was purchased from the authentic raw material supplier from the local market. The plant specimen was identified as the bark of *Bauhinia variegata* Linn by an in-house botanist. The samples were cleaned and air and shade dried at room temperature for two weeks. To make a raw powder, the stem bark was pounded and pulverized. Hydro-alcoholic extract of bark of *Bauhinia variegata* Linn was prepared with the help of the Soxhlet Extractor method. The raw powder was wrapped in filter paper and placed in a thimble. Solvent (Hydro-Ethanolic 1:1) placed inside the round bottom flask was subjected to heat. The solvent vapors started coming out and got condensed and collected in the thimble. Then after, the constituents of the drug were dissolved in the solvent. Then, the mixture traveled through the siphon, and after reaching the maximum point, it got collected in the round bottom flask due to the influence of gravity. This whole process marked the first cycle and like this, total of three cycles were done. In the end, the mixture was dried in the hot air oven and the final extract was collected in the dry form.

Figure 1: Particle size estimation of hydro-alcoholic extract of bark of *Bauhinia variegata* Linn.



Nano preparation

Dry hydro-alcoholic extract of *Bauhinia variegata* Linn has been processed with the help of the high-energy ball milling (HBEM) method to prepare nanoparticles. The process was repeated three times to achieve the desired particle size. Standardization of nano-size particles was done by using Zeta size (Malvern Instruments, UK) in the in-house pharmaceutical laboratory (9). Figure 1 shows that majority of the particles were within the acceptable range for nano-particles. (96.06 nanometer (nm)).

Ethical consideration

Permission was obtained from the Institutional Animal Ethical Committee of the study center, before beginning the experimental study (IAEC/Sangli/ 2020-21/04).

Experimental study

Experimental animals

For this experimental study, Wistar albino rats of both genders were procured from the authentic animal house (reference number – 2030/PO/RcBiBt/S/18/ CPCSEA). This experiment was carried out by taking healthy, young, adult male and female Wistar albino rats weighing between 150-250 gm. Identification was given by using yellow stain- Blank i.e. no marking, Marking over tail, Marking over head to each rat. Grouping was done by dividing animals into two groups, with 3 animals in each group.

Housing and Diet

Animals were housed in a sterilized polycarbonate cage and the temperature of the room was maintained at 22 ± 3°C with a relative humidity of 30% to 70%, 12-hour light and dark cycle. Animals were identified by marking with yellow stain. Each cage was identified with individual cage tags indicating study number, animal numbers, dose, group, route, species, gender, and experiment start, and end dates.

Drug Administration

Tube feeding has been used to administer the test drug in a single dosage to Wistar albino rats using a specially developed oral needle or gavage. Animals were made to fast for four hours before administration of the test dose. The weight of the animals was taken before the test dose administration. An oral test dose was administered at the dose of 2000 mg/kg. After administration of the test dose, the rat was made to fast for four hours.

Observation period

The animals were observed once during the first 30 min and the first four hours after administration of the test dose to them. The clinical signs, if any were recorded for consecutive 14 days. All the rats were checked twice morning and evening for any symptoms and mortality.

Clinical observations:

Clinical sign for observation was skin, fur, eyes, mucous membranes, respiratory, salivation, diarrhea, behavioral pattern, and autonomic and central nervous systems. Particular attention was directed to observations of tremors, convulsions, and coma. Death time if any was recorded. Same data was maintained for the next 14 days.

Biochemical analysis

SGOT, SGPT, ALP, Total Protein, Urea, Creatinine, and Uric acid were measured.

Haematological analysis

Blood samples were collected in EDTA containing tube from a retro-orbital. CBC parameter was measured- Haemoglobin, Total RBC, Packed cell volume (PVC), Mean corpuscular haemoglobin, Mean corpuscular haemoglobin concentration (MCHC), Platelet count, White blood cell (WBC), Neutrophil, Monocyte, and Eosinophils.

Histopathological study

The essential organs disconnected from the forfeited rat were fixed in 10% formalin, then, at that point, after handling inserted in paraffin wax. Paraffin blocks were made at 5 mm and stained with haematoxylin and eosin. The slides were examined under a light magnifying microscope and caught the amplified pictures of tissue structure for additional review.

Statistical analysis

The statistical significance between the groups was determined using a one-way ANOVA test, and the experimental data were provided as mean ± SD. Statistically, analysis was done by using the ANOVA test with, a significant value of ≤ P 0.05.

Results

With a limit test dose of 2000 mg/kg of Nano Kanchanar bark extract by using water as a vehicle, no mortality was observed. Special attention was given to the test animal during the first 30 minutes then every four hours till 24 hours. All the animals were observed for 14 days. On the 15th day, blood samples were collected. One rat from each group was sacrificed and vital organs were collected for histopathological examination.

Behavioural pattern

Behavioural observation of the test group did not show any changes in the first 30 minutes followed by after four hours, 24 hours, and 14 days of observation. The observations are outlined in table 1.

Table 1: Effect of NBVBE on the behavioural pattern in Wistar albino rats

S.no.	Clinical sign	Animal 1 (No marking)				Animal 2 (Marking over head)				Animal 3 (Marking over tail)			
		Day 1			Day 14	Day 1			Day 14	Day 1			Day 14
		First 30 minutes	4 hours	24 hours		First 30 minutes	4 hours	24 hours		First 30 minutes	4 hours	24 hours	
1	Skin changes	N	N	N	N	N	N	N	N	N	N	N	N
2	Fur changes	N	N	N	N	N	N	N	N	N	N	N	N
3	Lacrimation	N	N	N	N	N	N	N	N	N	N	N	N
4	Mucous membrane	N	N	N	N	N	N	N	N	N	N	N	N
5	Respiratory Sign	N	N	N	N	N	N	N	N	N	N	N	N
6	Salivation	N	N	N	N	N	N	N	N	N	N	N	N
7	Diarrhoea	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab
8	Behavioral pattern	N	N	N	N	N	N	N	N	N	N	N	N
9	Tremors	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab
10	Convulsions	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab
11	Coma	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab

[Abbreviations: N – Normal, Ab – Absent]

Effect of NBVBE on Body Weight

The mean body weight of rats from both groups increased during the first eight days of the study which reduced during the next seven days. But the changes were insignificant ($p > 0.05$) and the weight of rats from both groups was within normal limits It shows that the NBVBE has no adverse effect on the growth of rats (table 2)

Table 2: Effect of NBVBE on body weight of Wistar albino rat

Group	Day 1 Body Weight (gm)	Day 8 Body Weight (gm)	Day 14 Body Weight (gm)
Control	201 ± 16.64	228.33 ± 23.11	201.66 ± 7.23
Test (2000 mg/kg)	201.66 ± 8.50	228.66 ± 30.59	199.33 ± 10.06
P Value	0.954	0.989	0.761

[Values are expressed as mean ± standard deviation of six animals]

Effect of NBVBE on renal function test

There was an insignificant decrease ($p > 0.05$) in the mean uric acid levels in the test group when compared with the control. The changes observed in urea, and creatinine levels were also insignificant ($p > 0.05$). It shows that NBVBE does not have toxicity in the kidneys of rats. (Table 3)

Table 3: Effect of NBVBE on renal function test of Wistar albino rat

Parameter	Control Group	Test Group	P Value
Urea (mg/ dl)	43.31 ± 3.07	43.59 ± 3.86	0.881
Creatinine (mg/ dl)	0.9 ± 0.07	0.93 ± 0.23	0.674
Uric acid (mg/ dl)	0.87 ± 0.06	0.85 ± 0.26	0.963

Effect of NBVBE on liver function test

All the parameters of liver function showed insignificant difference ($p > 0.05$) change in S.G.O.T, S.G.P.T, alkaline phosphatase, and total protein was seen in the test group when compared with the normal control group. (Table 4)

Table 4: Effect of NBVBE on liver function test of Wistar albino rat

Liver Function Test	Unit	Control Group	Test Group	P Value
S.G.O.T	U/L	66.33 ± 22.27	66.66 ± 4.16	0.981
S.G.P.T	U/L	69.33 ± 11.59	69.33 ± 10.21	1.000
Alkaline Phosphatase	U/L	168.33 ± 25.69	168 ± 17.69	0.986
Total Protein	gm/dl	7.06 ± 0.83	7.06 ± 1.47	1.000

Effect of NBVBE on complete blood count

There were no significant changes ($p > 0.005$) seen in the haemoglobin, total leukocyte count, TEC, PCV, MCH, MCV, MCHC, Platelets, Neutrophil, Lymphocytes, and Monocyte of the test group when compared with the normal control group. (Table 5)

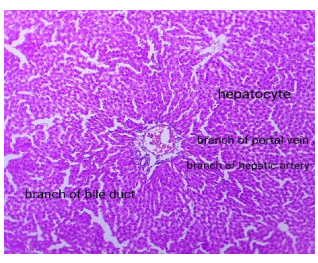
Table 5: Effect of NBVBE on complete blood count of Wistar albino rat

CBC	Unit	Normal Control	Test Group	P Value
Hb	gm/dl	14.63 ± 1.45	14.63 ± 1.30	1.000
Total Leukocyte Count	10^3/cu mm	6.43 ± 1.47	6.43 ± 1.44	1.000
TEC	10^6/cu.mm	8.2 ± 0.85	8.17 ± 0.99	0.970
PCV	%	43 ± 6.55	43 ± 6.08	1.000
MCH	pg	17.53 ± 1.25	17.53 ± 1.33	1.000
MCV	fl	50.43 ± 2.11	50.56 ± 4.52	0.965
MCHC	g/dl	34± 1.70	34 ± 2.61	1.000
Platelets	/ul	810966.66± 111461.03	810466.66 ± 166147.08	0.997
Neutrophil	%	24.23 ± 7.62	24.46 ± 9.12	0.975
Lymphocytes	%	69.36 ± 18.47	69.03 ± 15.47	0.982
Monocyte	%	2.06 ± 1.10	2 ± 1	0.942
Eosinophil	%	3 ± 1.64	3 ± 1.73	1.000

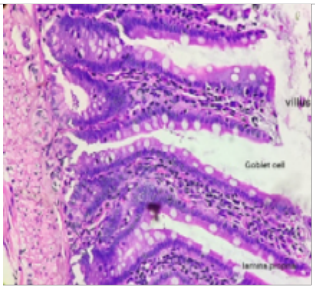
Histopathological changes

When histopathological slides of the liver and intestine of sacrificed rats were compared, it was observed that rats in the control group showed clear central vein and hepatocytes (figure 2 a and b), while in the test group steatosis was observed, while histopathology of intestine showed lymphoid hyperplasia (figure 2 c and d).

Figure 2:-(a)(b): Pictograph of Normal control rats with clear Central Vein (CV) hepatocytes and section of intestine. (c)(d) Pictograph of test group rats with steatosis and lymphoid hyperplasia



(a)



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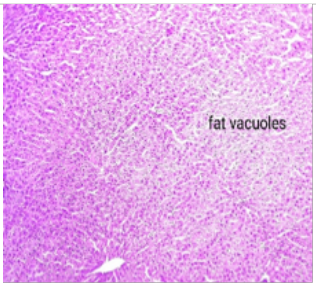
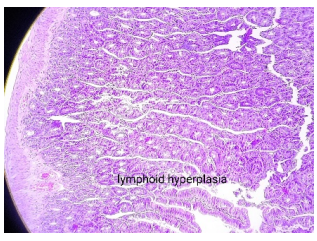


(c)



(d)

Discussion

The earth is an abundant source of medicinal herbs, containing enumerable active ingredients which are used to treat various disorders. Herbal medicine is frequently used as self-medication without any consultation. The world health organization emphasizes the importance of conducting scientific research to validate the safety and efficacy of herbal medicine before it is used for humans (10). To ensure the safety of the herbal drug, a preclinical toxicology study is necessary. Though the *Bauhinia variegatea* Linn bark is being used for a very long time to treat various disorders, it's necessary to evaluate its safety, especially when the form of the drug is changed to nanoparticles. Also, research conducted on nanoparticles of raw bark of the study herb at our study center showed steatosis and hydropic effect on the liver in an animal model. Hence the current study was conducted to investigate the acute toxicity of NBVBE in an animal model, according to OECD guidelines 423 (11)

During the whole acute toxicity study duration, there was no mortality seen. No changes were observed in behaviour, growth, feed, and water consumption. There was no noted variation in the weight of the rat after 14 days of the study noted. There is no noted change in the total protein of the test group compared with the control group. Total protein guides for diagnosis of liver and kidney disease. The result of the present study suggested that NBVBE is a non-toxic formulation, but it does have an effect on the liver of the animal rat. An increased level of ALT, AST, and Alkaline Phosphatase indicates liver injury (12,13). All parameters i.e., ALT, AST and Alkaline Phosphatase in the test group were within the normal limit in comparison with the control group.

Other parameters such as the level of serum creatinine, blood urea, and uric acid were not raised or decreased in the test group when compared with the normal group. Creatinine is a highly sensitive and specific test for the detection of abnormal kidney function test. Thus, it showed that NBVBE does not have any adverse effect on the kidney and its function. Blood parameter analysis is required for the assessment of Nano Kanchanar bark extract (14,15). NBVBE did not show an adverse effect on any of the blood indices. The non-significant effect of NBVBE on white blood count and lymphocytes suggests that the extract poses neither toxicity nor sub-acute inflammation to the experimental rat's general defence mechanism and immunity.

Previous studies done on *Bauhinia variegata* Linn. bark have not reported any histopathological changes such as steatosis or lymphoid hyperplasia in the liver and intestine in experimental or clinical studies (16). However, in the present study changes were observed in the histopathology of the intestine and liver. The intestine section showed lymphoid hyperplasia (figure 2 d). It may reflect an increased immune mucosal response to an antigenic stimulus, such as GMA or infection (17). Lymphoid hyperplasia may cause intestinal obstruction. The liver section shows Portal vein congestion and focal steatosis (figure 2 c). The intracellular accumulation of neutral fat within parenchymal cells is known as steatosis. The liver plays an important role in fat metabolism hence it is the most common site for fat accumulation. These fatty changes can be reversible or may produce irreversible cell injury and cell death. Fatty changes in the liver can occur due to excess fat or liver cell injury where fat cannot metabolize properly. The cause of liver cell damage due to excess fat deposition, starvation, chronic illnesses, and hypoxia. These causes also cannot be considered as the weight, food consumption, and all the blood parameters were normal. Then hepatotoxin or drug-induced liver cell injury can be considered here as one of the causes of focal steatosis. The focal steatosis in the initial stage is reversible but as the causative agent continues to happen these reversible changes can be shifted to irreversible liver cell damage (18). Further portal vein congestion might occur due to malignancy, chronic liver disease, or processes localized to the epigastrium and hepatobiliary system (19).

Some of the limitations of the study were that it was conducted only on three animals per group and only one animal was sacrificed as per the directions received from IAEC.

Conclusion

In Conclusion, Oral haematological were normal. But there were changes i.e. steatosis was seen over the liver which is reversible and lymphoid hyperplasia in the Intestine. Thus, while administering NBVBE its possible effect on the liver needs to acute administration of NBVBE is safe below the dose of 2000 mg/kg. As NBVBE did not show any mortality and behavioural changes also all the parameters, Liver function test, renal function test, and be monitored.

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