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Chronic toxicity study of stem bark powder of *Kanchanara* (*Bauhinia variegata* L.) in albino rats

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Stem bark of *Kanchanara* (*Bauhinia variegata* L., Family-Caesalpiniaceae) is used in Ayurvedic system of medicine, either as a single drug or as ingredient of compound formulations. The present study was carried out to evaluate the toxicity of stem bark where its powder suspension was administered in Therapeutic Equivalent Dose (TED) (350mg/kg/day) in TED group and five fold of TED (1800 mg/kg/day) in TED× 5 group for sixty days in albino rats. Control group received distilled water. Parameters like body weight, weight of important organs, biochemical, hematological were studied along with histopathology of vital organs. *Kanchanara* at both the dose levels significantly increased the weight of spleen and thymus. Decreased HDL cholesterol and direct bilirubin were observed in both the treated group while decreased blood urea was observed in TED×5 group. Significant increase in platelet count and significant decrease in haemoglobin and lymphocyte count were observed in TED×5 treated group. In histopathological study, destruction of epithelial layer of stomach was observed in majority of the sections in TED×5 dose groups compared to control group. Thus, toxicity profile obtained from the present study showed that *B. variegata* bark is likely to produce toxic effect when administered in a five times dose in powder form.

Keywords: Ayurveda, *Bauhinia variegata*, Chronic toxicity, *Kanchanara,* Stem bark.

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Introduction

Prolonged usages of Ayurvedic medicines for the management of chronic disorders are common, considering the prevailing belief that they are natural and safe[1]. Hence, it becomes essential to prove the safety of drugs on prolonged use as well as at higher dose level. *Kanchanara,* botanically identified as *Bauhinia variegata* L., Caesalpiniaceae family is being used since *Samhita* period (3000 BC-200 AD)[2] for various therapeutic purposes. *Kanchanara* is one of the important ingredients of *Ushirasava*, *Chandanasava*, *Kanchanaraguggulu*, *Triphaladiguggulu* etc[3]. It is the drug of choice for *Gandamala* (multiple lymphadenitis)[4] which needs prolonged treatment. Alcoholic extract of stem bark of *B. variegata* produced hypothermia in mice and it also modulates amphetamine hyperactivity[5]. However, there has been no report of long-term toxicity study of this drug at conventional Ayurvedic therapeutic dose level. Keeping these points on focus, the chronic toxicity study of *B. variegata* stem bark powder was performed in rats.

Materials and Methods

Test drugs

Stem barks of *Kanchanara* (*B. variegata*) were collected from matured plant in its flowering season i.e. in the month of March 2011, from the periphery of Jamnagar. Its flowers are purple with one petal variegated in short racemes. Authenticity of the sample was confirmed by comparing their characters with the characters given in the authenticated books[6] and various floras[7] The sample was also authenticated by Pharmacognosy laboratory of IPGT&RA. The fine powder (120 mesh) of drug was used for the experimental study.

Animals

Wistar strain albino rats (*Rattus novergicus*) of either sex; weighing 200 ± 40 g were used for the study. The animals were obtained from the animal house (Registration No.548/2002/CPCSEA) attached to Pharmacology laboratory of IPGT & RA, Gujarat

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Ayurved University. The animals were housed in cage at 22 ± 3°C with constant humidity 50-70%, on a 12-h natural day and night cycle. They were fed with Amrut brand rat pellet feed supplied by Pranav Agro Industries and with drinking water *ad libitum*. The experiments were carried after obtaining permission from Institutional Animal Ethics Committee (IAEC), (Approval number: IAEC/09/2011/16).

Dose fixation

The dose of the test drug was calculated by extrapolating the human dose (4000 mg/kg)[8] to animals (360 mg/kg) based on the body surface area ratio by referring to the standard table of Paget and Barnes (1964)[9]. For overdose study, five times dose of the Therapeutic Equivalent Dose (TED) was selected (1800 mg/kg). Stock solutions were prepared in water with suitable concentration and administered to animals orally depending up on the body weight with the help of oral feeding cannula.

Experimental protocols

The toxicity study was carried out as per AYUSH guideline 170(Ref.10). The selected animals were divided into three groups with each group comprising three male and three female rats. The first group was kept as control and maintained with distilled water. The second group was administered orally with the therapeutic equivalent dose of test drug (TED group, 360 mg/kg) and third group was administered with five fold therapeutic equivalent dose of test drug (TED×5 group, 1800 mg/kg) for sixty consecutive days.

Parameters studied

Measurement of weight

Body weight of each animal was recorded on initial day, every 7th day and also before sacrifice for 60 consecutive days. On 61st day, the animals were sacrificed. Further, the animals were dissected and organs were separated and weighed accurately with a monopan balance and transferred immediately to a glass bottle containing 10% formalin for histopathological studies. Weight of important organs like liver, spleen, heart, kidney, thymus, uterus, testis, seminal vesicle and prostate were recorded after sacrificing at the end of the drug treatment period.

Haematological and serum biochemical parameters

On 61st day blood was collected by puncturing supra-orbital plexus by capillary tubes under ether anesthesia for estimation of haematological and serum biochemical parameters. To estimate haematological parameter 0.08 mL blood was mixed with 0.02 mL of EDTA (33.33 mg/mL) and fed to the auto analyzer (Sismes KX-21, Trans Asia). The parameters measured were; hemoglobin content, WBC count, neutrophil percentage, lymphocyte percentage, eosinophil percentage, monocytes count, PCV, RBC count, platelet count, MCV, MCH and MCHC. For estimation of biochemical parameters, serum was separated from collected blood and requisite quantity of serum was fed to the auto analyzer (Fully automated Biochemical Random Access Analyzer, BS-200; Lilac Medicare Pvt. Ltd., Mumbai) which was automatically drawn in to the instrument for estimating different parameters. Biochemical parameters measured were blood sugar[11], serum cholesterol[12], serum triglyceride[13], HDL cholesterol[14], blood urea[15], serum creatinine[16] serum glutamic pyruvic transaminase (SGPT) [17], serum glutamic oxaloacetic transaminase (SGOT)[18], serum total protein, serum calcium[19], serum albumin and serum globulin[20], serum alkaline phosphatase[21], total bilirubin[22], direct bilirubin[23]and uric acid[24].

Histopathological study

The histopathological slides of different organs like liver, spleen, heart, kidney, intestine, testis, ovary and uterus were prepared by referring to standard procedure of Raghuramulu *et al*[25]. The slides were viewed under trinocular research microscope (Carl Zeiss MicroImaging Standort Göttingen–Vertrieb Deutschland Knigsallee 9-2137081 Gottingen Germany) at various magnifications to note down the changes in the microscopic features of the tissues studied.

Statistical analysis

Results were presented as Mean ± standard error mean (SEM), difference between the group was statistically determined by paired and unpaired Student'sdft test for paired and unpaired data, respectively with the level of significance set at $P<0.05$. The level of significance was noted and interpreted accordingly.

Results and Discussion

No mortality and apparent gross behavioral changes could be observed in any of the study

groups during the 60 days dosing period. Normal progressive increase in body weight was observed in control and TED×*5* group whereas significant decrease (P<0.05) in the body weight was observed in TED group (Table 1).

Out of nine organs studied, weight of spleen and thymus were significantly increased (P<0.05) at both the dose levels of test drug. Increase in both the immunological organs may be attributed to immune-stimulation, as reported earlier[26]. However, histopathological examination of these organs showed normal cytoarchitecture, hence the cause may be other than increased cellullarity. Weight of liver was increased only in TED×5 (P<0.001) group as compared to control group (Table 2). Increased liver weight may suggest either stimulation of the organ or toxicity[27] but histopathological study revealed micro fatty changes in few sections of the liver. This may be the major reason for the observed weight increase. *Kanchanara* is rich in tannin[28] and high dose of tannin content causes hepatotoxicity[29]. Tannins also interfere with iron absorption through a complex formation with iron[30]. This may be responsible for the mild fatty changes observed in few liver sections from this group.

Table1—Effect of *Kanchanara* stem bark powder on body weight (in g)

Days	Control	TED (360 mg/kg)	TED×5 (1800 mg/kg)
Initial (week)	188.67 ±12.14	192.33 ±09.89	174.00 ± 5.09
1st	198.000 ±12.38	195.67 ±16.25	170.80 ± 7.71
2nd	205.00 ±16.68	200.00 ±15.49	184.40 ± 12.64
3rd	203.33 ±12.02	202.33 ±14.83	184.40 ± 22.60
4th	201.67 ±12.23	199.67 ±11.76	186.50 ± 22.11
5th	196.67 ±12.82	200.67 ±15.19	189.00 ± 22.22
6th	204.00 ±12.38	197.33 ±13.18	187.20 ± 25.65
7th	203.33 ±14.53	192.67 ±10.93	188.40 ± 20.23
8th	200.00 ±14.61	201.00 ±14.48	199.20 ± 23.79
Final weight	201.33 ±18.00	189.33 ±12.72	192.80 ± 20.73
% change	06.72 ↑	0.15 ↓	10.80 ↑

Data: Mean ± SEM, *P<0.05, ↑ - increase, ↓ - decrease

Table 2—Effect of *Kanchanara* stem bark powder on ponderal changes (in g)

Organs	Control	TED (360 mg/kg)	TED×5 (1800mg/kg)
Liver	2.78 ± 0.09	2.80 ± 0.13	3.39 ± 0.14**
Spleen	0.20 ± 0.01	0.25 ± 0.02*	0.24 ± 0.02*
Heart	0.31± 0.01	0.30 ± 0.01	0.33 ± 0.01
Kidney	0.72 ± 0.02	0.73 ± 0.02	0.77 ± 0.04
Thymus	0.13 ± 0.02	0.15 ± 0.01*	0.16 ± 0.01*
Uterus	0.20 ± 0.09	0.14 ± 0.03	0.11 ± 0.03
Testis	0.80 ± 0.27	1.03 ± 0.07	0.86 ± 0.14
Prostate	0.17 ± 0.01	0.13 ± 0.02	0.16 ± 0.02
Seminal vesicle	0.33 ± 0.04	0.33 ±0.07	0.48 ± 0.04

Data: Mean ± SEM, *P<0.05, **P<0.01compared to control group (unpaired t test)

Among the sixteen biochemical parameters studied, significant changes were observed in only three parameters (Table 3). There was decrease in HDL (P<0.01) at both the dose level. Earlier studies have shown antihyperlipidemic activity[31] of *Kanchanara*, the test drug may impair the transfer of cholesterol from both VLDL and tissue to HDL fraction and second it may be promoting the metabolism of this fraction by enhancing the activity of the key enzymes involved in HDL cholesterol metabolism. This may lead to significant decrease in HDL cholesterol in both the doses of *Kanchanara.* Blood urea was found to be decreased (P<0.05) in TED×5 group. The reason and significance of this decrease could not be decided on the basis of the present data. This may be due to decreased nitrogen turnover or decreased formation in liver. Direct bilirubin was also found to be decreased (P<0.05) at both the dose level. Elevated bilirubin has pathological significance, the reason for the decrease is not known. This may be an indirect indication of functional disturbance in the liver. The parameters which would have link to this are total RBC count and histopathology of liver which were not altered significantly.

Out of twelve hematological parameters studied; only two parameters were affected (Table 4). Interference with absorption and metabolism of iron may lead to decrease in Hb content as observed in TED×5group (P<0.05). Significant decrease (P<0.05) in lymphocyte count was observed in TED×5group and platelet count was found to be significantly increased (P<0.001) in this group. Total nine organs were examined in histopathology study and changes were observed in stomach only. *Kanchanara* at TED×5 dose levels showed erosion of the epithelial layer in majority of the sections in comparison to control group (Plate 1) but the sections of *Kanchanara* at TED dose group showed normal cytoarchitecture similar to that of control group.

Table 3—Effect of *Kanchanara* stem bark powder on serum biochemical parameters

Parameters	Control	TED(360 mg/kg)	TED×5(1800mg/kg)
Blood sugar (mg/dl)	100.75 ± 04.29	092.33 ±04.27	095.80 ±02.48
Cholesterol (mg/dl)	63.50 ±03.87	58.33 ±02.56	60.40 ±02.66
Triglyceride (mg/dl)	102.50 ±05.02	95.17 ±07.76	90.20 ±04.16
HDL (mg/dl)	41.12 ±03.99	19.00±00.77***	21.40 ±01.91**
Blood urea (mg/dl)	91.13±04.96	81.17 ±04.79	70.60 ±05.28*
Creatinine (mg/dl)	0.60 ±0.04	0.55 ±0.02	0.48 ±0.02
SGPT (IU/L)	72.70 ±00.58	57.83 ±04.40	53.20 ±07.83
SGOT (IU/L)	140.12 ±06.38	158.83 ±07.02	146.20 ±12.20
Albumin (g/dl)	3.75 ±0.11	3.52 ±0.10	3.38 ±0.15
Globulin (g/dl)	3.53 ±0.14	3.83 ±0.15	3.98 ±0.20
Total protein (g/dl)	7.40 ±0.16	7.35 ±0.13	7.36 ±0.17
Alkaline Phosphatase (IU/L)	277.50 ±34.19	183.00 ±24.88	262.40 ±55.98
Total bilirubin (mg/dl)	0.50 ±0.04	0.55 ±0.07	0.54 ±0.07
Direct Bilirubin (mg/dl)	0.19 ±0.01	0.12 ± 0.02*	0.12 ± 0.02*
Uric acid (mg/dl)	0.78 ±0.13	0.72 ±0.07	0.80 ±0.07
Serum calcium (mmol/dl	8.65 ±0.34	9.18 ±0.15	8.62 ±0.22

Data**:** Mean ± SEM, *P<0.05, **P<0.01,***P<0.001 (comparison to control group, unpaired t test)

Table 4—Effect of *Kanchanara* stem bark powder on various haematological parameters

Parameters	Control	TED(360 mg/kg)	TED×5(1800mg/kg)
Hb (g/dl)	15.69 ±00.29	15.17 ±00.24	14.50 ±00.17*
WBC(10^3/Cu mm)	7525.00 ±874.59	9816.67 ±730.49	9040.00 ±1866.71
Neutrophil (%)	19.25 ± 03.39	18.33 ±02.15	23.20 ±05.08
Lymphocyte (%)	75.75 ±03.47	75.83 ±02.10	71.4 ±04.73*
Eosinophil (%)	2.65 ±0.32	3.00 ±0.36	3.40 ±0.40
Monocyte (%)	2.37 ±0.26	2.83 ±0.40	2.00 ±0.00
PCV (%)	49.47 ±00.99	48.57 ±00.99	46.82 ±00.73
RBC(10^6/cu mm)	8.85 ±0.18	8.77 ±0.25	8.32 ±0.11
Platelet (10^3/µL)	1007.12 ±31.78	935.00 ±77.88	1336.80 ±61.38***
MCV (fl)	55.83 ±00.37	55.83 ±00.78	55.98 ±00.69
MCH (pg)	17.8 ±00.16	17.43 ±00.33	17.38 ±00.23
MCHC (g/dl)	31.77 ±00.25	31.17 ±00.23	31.02 ±00.15

Data**:** Mean ± SEM, *P<0.05, **P<0.01,***P<0.001 (comparison to control group, unpaired t test)

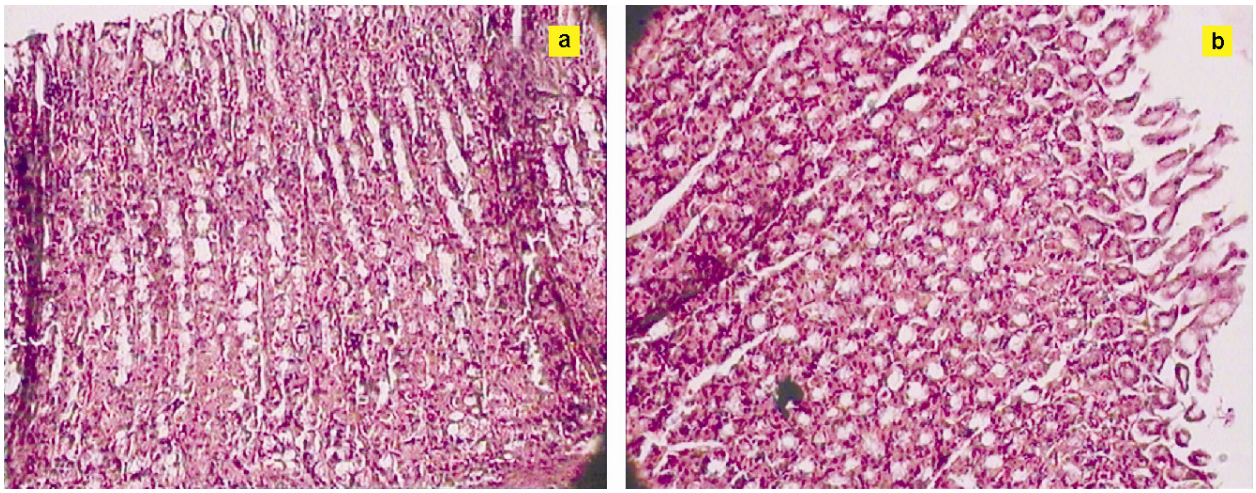

Plate 1(a-b)—Photomicrographs of representative sections of stomach of albino rats (1 × 200) magnification); a- Normal epithelial layer of stomach in control group; b- Destruction of epithelial layer of stomach in *Kanchanara* TED×5 group.

Conclusion

In conclusion it can be inferred that powder of bark of *Kanchanara* do not produce any toxicity at normal human therapeutically equivalent dose. At higher dose level gastric erosion of moderate intensity may occur and is not likely to be a major problem. Though significant decrease in HDL cholesterol and blood urea level was observed at higher dose level along with few hematological changes, the magnitude of changes is only moderate and hence may not have any serious pathological implications.

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