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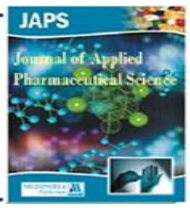


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# Acute and subacute oral toxicity evaluation of *benincasa hispida* extract in rodents

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**ABSTRACT**

The toxicity studies were carried out a 50% aqueous ethanolic extract of *Benincasa hispida* ( *B. hispida* )in rodents.The acute toxicity study, *B. hispida* was found to be well tolerated upto 2000mg/kg, produced neither mortality nor in behavior in mice. In subacute toxicity study, *B. hispida* at dose level of 200 and 400 mg/kg did not produce any significant difference in their body weight, food and water intake when compared to vehicle treated rats. It also showed no significant alteration in hematological and biochemical parameters in experimental groups of rats apart from a decrease in aspatate transaminase, alanine transaminase and alkaline phosphate content at the dose of 400 mg/kg. Histopathological study revealed normal architecture of kidney and liver of *B. hispida* treated rats. These results demonstrated that there is a wide margin of safety for the therapeutic use of *B. hispida* and further corroborated the traditional use of this extract as an anti hepatocarcinogenic agent.

**Keywords:** *Benincasa hispida*: Acute and sub acute toxicitv.

## INTRODUCTION

*Benincasa hispida* (Thunb) Cogn. (Family: Cucurbitaceae) is commonly known as *Bhuru Kolu* or *Safed Kolu* (Gujarati), *Petha* (Hindi), white pumpkin or wax gourd or ash gourd (English), and *Kushmanda* (Sanskrit). Fruits of this plant are traditionally used as a laxative, diuretic, tonic, aphrodisiac, cardiotonic, urinary calculi, blood disease, insanity, epilepsy, and also in cases of jaundice, dyspepsia, fever, and menstrual disorders (Kirtikar *et al*, 1975). The methanolic extract of the fruit is reported to possess antiulcer (Grover *et al*, 2001) anti-inflammatory, (Chandrababu S *et al*, 2002) antihistaminic, and antidepressant activities (Anilkumar D *et al*, 2002). Phytochemical review indicates the presence of triterpenes: alnusenol, multiflorenol, iso-multiflorenol, flavone, iso-vitexin, and sterols, lupeol, lupeol acetate, and beta-sitosterol. (Yoshizumi S *et al*, 1998). A number of cucurbitaceae plants (Yang X *et al*, 2007, Gurbuz I *et al*, 2000) have been shown to possess antiulcer and antioxidant activity, viz. *Cucurbita moschata* (Fruit), *Momordica charantia* (Immature fruits), *Cucumis melo* (Mature fruit), etc. despite the wide use of *B. hispida* in folk medicine, no study has been published in the scientific literature about its toxicological profile.

summarizes the acute and subacute oral toxicity of the 50% aqueous ethanolic extract of *B. hispida* in experimental animals.

## MATERIAL AND METHODS

### Plant material

The dried seeds of *Benincasa hispida* were received from Kondappanaikanpatti Salem, Tamil Nadu. Dr. Marimuthu Govt. Arts and Science College, authentified plant. The seeds were coarsely powdered and extracted thrice with 50% aqueous ethanol, the yield of the extract 7.8% w/w was stored in a refrigerator at $4^0$C, until use for the biological testing.

### Animals

Wistar albino rats (140-160g) and swiss albino mice (25-30g) were used for this experiment. They were procured from Sri Venkateswara Enterprises, Bangalore, India. They were housed in polypropylene cages at temperature of (22 ± 2) $^0$Cand 50-60% relative humidity, with a 12 h light/dark cycle respectively, for one week before and during the experiment. The animals were given a standard rodent pellet diet (Hindustan Lever Ltd, Bangalore, India) and drinking water. Food was withdrawn for overnight before the experiment though water was allowed ad libitum and allocated to different experimental groups. The study protocol was approved from the Institutional Animal Ethics Committee (Ref. No.: IAEC/Pcology/08/2006) constituted in accordance with the rules and guidelines of the CPCSEA (Committee for the purpose of Control and Supervision of
Experiments on Animals), India.

### Acute toxicity study

Acute oral toxicity of the 50% aqueous ethanolic extract of *B.hispida* was evaluated in swiss albino mice either sex (25-30g), as per OECD guideline (organization for economic co-operation and development, guideline-423). Twenty four animals were equally divided into four groups (n=6) as per sex. The extract was administered in 0.3% carboxyl-methylcellulose (CMC) suspension at doses of 100, 500, 1500 and 2000mg/kg by oral route. After administration of the extract, the animals were observed continuously for the first four hours for the death to acute toxicity. 0ne-tenth and one-fifth of the maximum tolerated dose of the extract (2000mg/kg) tested for acute toxicity was selected for the subacute toxicity study i.e., 200 and 400mg/kg.

### Subacute toxicity study (Anderson *et al*, 1993; Loeb *et al, 1989)*

Twenty four rats (140-160g) were randomly assigned into three groups (n=8 or n=4/sex), four females and four males were housed separately as per sex in each group. Treatment were administered orally once a day for 4 weeks. The group I rats served as control, group II and III received *B.hispida* at doses of 200 and 400 mg/kg (one-tenth and one-fifth of the maximum tolerated dose) respectively. All rats were observed daily for physiological and behavioral changes. Rat that died during the test period was tested pathologically, and all animals were examined at the end of the test period.

### Observation and examination methods

Clinical signs were observed at least once a day during 28 days of dosing. Body weights, water and food intake were measured once a week by using digital feeding and drinking analyzer. They were fasted on overnight prior to blood collection by retro-orbital technique on 29$^{th}$ day of study. Hematological parameters included: red blood cell (RBC) count, hemoglobin (HB), leukocyte (WBC) count, neutrophil, eosinophil, basophil, and lymphocyte and monocyte counts. For biochemical analysis, blood was centrifuged at 3000 rpm for 10 min. serum was separated and assayed by using diagnostic kits for glucose, creatinine, blood urea nitrogen (BUN)( D.Webster *et al*, 1977), total bilirubin (TB), aspartate transaminase (AST)and alanine transaminase (ALT) ( Reitman *et al*.1957,*)* alkaline phosphate (ALP) ( Kind *et al*,1954),total cholesterol (TC)( Hawk *et al*,1954), total protein (TP) and albumin. After blood collection, animals were sacrificed for isolation of kidney and liver to observe histopathological study.

### Histopathological study

Histopathological investigation of the organs was done according to the method described by Lamb (G.M. Lamb *et al*, 1981). The organ pieces (3-5 µm thick) were fixed in 10 % formalin for 24 h and washed in running water for 24 h. Samples were dehydrated in an autotechnicon and then cleared in benzene to remove absolute alcohol. Embedding was done by passing the cleared samples through three cups containing molten paraffin at 50 °C and then in a cubical block of paraffin made by the "L" moulds. It was followed by microtome and the slides were stained with Haematoxylin-eosin.

### Statistical Analysis

Values were expressed as mean ±SEM. The statistical analyses of variance were done by ONE WAY ANOVA forward the Dunnett's test using software version. $P< 0.05$ was considered as the level statistical significance.

## RESULT AND DISCUSSION

Mice administered with *B.hispida* up to 2000 mg/kg did not show any kind of abnormal behavior, during initial 4 h after drug administration. No mortality was observed during 24 h after treatment with *B.hispida* in either sex. No significant difference either in control or *B.hispida* treated group of both sexes were noticed in body weight, food and water intake (data not shown). As summarized in **(Table-1)**, extract treatments did not significantly change the level of RBC, HB, WBC and lymphocyte.

**Table. 1:** Effect of *B.hispida* on hematological parameters of rats in subacute toxicity(mean±SEM,n=8).

| parameters | Control group | *B.hispida* treated group (200mg/kg) | *B.hispida* treated group (400mg/kg) |
|---|---|---|---|
| RBC (million/mm$^3$) | 8.34±0.35 | 8.93±0.87 | 9.37±1.13 |
| WBC (million/mm$^3$) | 7.17±1.34 | 7.49±1.37 | 6.79±0.93 |
| HB (g/dl) | 15.22±1.02 | 15.92±0.97 | 15.82±1.08 |
| Neutrophils (%) | 24.61±2.13 | 23.93±3.74 | 25.79±3.64 |
| Eosinophils (%) | 1.37±0.55 | 1.55±0.62 | 1.32±0.45 |

| | | | |
|---|---|---|---|
| Basophils(%) | 0.18±0.07 | 0.16±0.07 | 0.17±0.06 |
| Lymphocyte(%) | 70.82±6.53 | 68.96±6.54 | 67.34±6.28 |
| Momocyte (%) | 2.03±0.48 | 1.43±0.39 | 1.45±0.32 |

It is apparent from **( Table- 2)** that level of glucose, creatinine, BUN, TB, TC, TP and albumin were not altered while AST,ALT,ALP content was slightly decreased in group III rats, however it was within the normal range.

**Table. 2:** Effect of *B.hispida* on blood chemistry values of rats in subacute toxicity (mean±SEM,n=8).

| parameters | Control group | *B.hispida* treated group (200mg/kg) | *B.hispida* treated group (400mg/kg) |
|---|---|---|---|
| Glucose (mg/dl) | 72.54±6.27 | 67.42±7.53 | 64.32±8.43* |
| Creatinine (mg/dl) | 0.91±0.06 | 0.85±0.05 | 0.92±0.03 |
| BUN (mg/dl) | 19.27±1.76 | 18.44±1.09 | 17.76±1.83 |
| TB(mg/dl) | 0.69±0.05 | 0.67±0.05 | 0.63±0.07 |
| AST (U/L) | 135.4±7.32 | 125.3±6.33 | 121.3±6.17 |
| ALT(U/L) | 38.21±3.56 | 33.11±2.65 | 30.25±2.39* |
| ALP(U/L) | 79.35±4.43 | 70.66±4.32 | 65.38±4.36 |
| TC(mg/dl) | 56.86±5.77 | 57.51±5.68 | 55.95±5.21 |
| TP (g/dl) | 8.41±0.23 | 7.84±0.23 | 7.48±0.78 |
| Albumin (g/dl) | 2.66±0.08 | 2.64±0.07 | 2.66±0.06 |

*P<0.05 compared with respective control group.

Histopathological features of control and *B.hispida* treated (200 and 400mg/kg) rats revealed the absence of pathological lesion in kidney and liver. The use of herbal medicine as alternative treatments have been increasing world wild and medicinal plants may have biological activities that are beneficial to mankind. (Chopra R *et al*, 1956) Since no reports on toxicity and safety profile of *B.hispida* extract is yet available, it was considered necessary to have some information on toxicity potential of extract tested to maximize their benefits. Observation made during our efforts in the oral acute toxicity studies showed the lack of mortality and toxicity upto oral treatment of 200mg/kg body weight, which suggests that the *B.hispida* is practically nontoxic at single dose. For subacute toxicity study the higher dose level of 400 mg/kg ($1/5^{th}$ of the maximum tolerated dose) and low dose level of 200mg/kg ($1/10^{th}$ of the maximum tolerated dose) were selected. The 50% aqueous ethanolic extract of *B.hispida* at the doses used did not produce any marked changes in experimental groups rats, as evidenced by the absence of toxic symptoms, no changes in water/food ingestion and body weight. Analysis of blood parameters is relevant to risk evaluation as the changes in the haematological system have higher predictive value for human toxicity , when the data are translated from animal studies(Olson H *et al*, 2000, Hossain S *et al*,2012, Jain M *et al*,2011, Thirumalai T *et al*,2012). No significant alteration in the haematological parameters of treated rats can be attributed to the plant extract. The normal values of the renal biochemical parameters such as blood urea nitrogen and creatinine suggest that the extract does not produce any sort of disturbance in renal function, as has been found in case of various plant extracts and hence is safe on its chronic use in various diseases. Subacute administration of *B.hispida* did not cause any significant change in serum glucose level TC, TB, TP and albumin. However, liver enzymes (AST, ALT and ALP) were decreased at higher dose but the magnitude is too small to have biological relevance. The increase levels of AST, ALT and ALP in blood are associated with structural and functional dysfunction of hepatocellular membrane damage of hepatic cells (Burger C *et al*,2005). These observations of decrease in the level of liver enzyme might be due to the presence of hepatoprotective agents in the extract. These results are in support of previous studies and decrease of hepatic enzyme levels need to be further investigated (Choon Sik Jeong *et al*,2003). Therefore ongoing research work is directed towards identifying an herbal anti-hepatocarcinogenic agent.

## CONCLUSION

Present observation indicate for the first time that 50% aqueous ethanolic extract of *B.hispida* have a broad safety margin in experimental animals commonly used in *invivo* experimental and preclinical pharmacological studies. In Future clinical research.

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