Original Article

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Pharmacology Section

# Effect of *Benincasa hispida* Fruit Dry Powder on Behaviour Changes and Antioxidants Levels in Adult Wistar Rats: An Experimental Study

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## ABSTRACT

**Introduction:** Chronic stress has been implicated to be the important aetiological factor involved in several pathologies. Antioxidants play major roles in the maintenance of good health in the cellular level. Various studies have shown the anxiolytic, muscle relaxant, antidepressant, and antioxidant effects of *Benincasa hispida (B.hispida)* (white pumpkin). There is no available literature exploring the effects of this *B.hispida* dry powder on food intake, body weight, behavioural changes and antioxidant levels in whole animal model.

**Aim:** This study aimed to understand the protective effects of the *B.hispida* dry powder in overcoming stress induction by analysing the pattern of food intake, behavioural changes and body weight and the change in blood levels of Superoxide Dismutase (SOD) and Malondialdehyde (MDA) in adult wister rats.

**Materials and Methods:** This experimental study was conducted in the Department of Pharmacology, PSG Institute of Medical Sciences and Research, Tamil Nadu, India, for the duration of three weeks during the month of March 2020. Six male wister rats were obtained from the central animal house and assessed for baseline values of parameters including body weight, food intake, behaviour changes, blood levels of SOD and MDA and they were used as controls (without treatment) later. Then another 12 animals, matched for age and weight were selected and divided into two test groups (six in each). The animals in test groups 1 and 2 were pretreated with dry powder preparation of *B.hispida* at a dose of 250 mg/kg and 500 mg/kg orally and once daily, respectively. The entire study population of 18 animals (control, group 1 and group 2) subjected to stress in a restrainer for two hours per day for seven days. The study parameters were repeated at the end of 24 hours and at the end of seven days. Results were analysed using Analysis of Variance (ANOVA) in Statistical Package for Social Sciences software version 24.0.

**Results:** The results were 500 mg/kg dose of *B.hispida* helped in overcoming stress induction by giving the statistically significant (p-value <0.05) results of increase in body weight, food intake pattern and normalised general behaviour after 24 hrs and 7 days of repeated stress induction. But no significant changes were seen in blood levels of SOD (p-value >0.05) and MDA (p-value >0.05).

**Conclusion:** Based on the previous literatures, it was stated as "sparing effect of the powder" that is, exogenous supplementation is likely to suppress its endogenous production of antioxidants. This mechanism could attribute to the potential beneficial effect of *B.hispida* on the outcomes, despite having no significant alterations in blood levels of SOD and MDA. Further studies are needed to confirm the effects *B. hispida* in overcoming stress induction.

**Keywords:** Body weight, Food intake, General behaviour, Restraint stress rat model

## INTRODUCTION

Oxidative stress is the result of an imbalance between oxidants and antioxidants. Physical or psychological stress induces change in hypothalamic-pituitary-adrenal axis which along with glucocorticoids releases Adrenocorticotropic Hormone (ACTH), Nor Epinephrine (NE), serotonin, dopamine, and acetylcholine. The metabolism of NE and dopamine lead to production of free radicals and Reactive Oxygen Species (ROS). Glucocorticoids increase the basal level of ROS in cells and also increase the toxicity of oxygen radical generators. Acute stress can be defined as any situation or new stimulus affecting the homoeostasis of any organism, thus inducing compensatory responses to restore previous stability. But chronic stress has been reported to exert negative effects and major etiologic factor involved in several pathologies like cardiovascular, immunological, neurodegenerative or neurobehavioural changes [1].

Studies have shown that restraint induced physical stress in turn lead to oxidative stress and decrease the levels of antioxidant such as Superoxide Dismutase (SOD), Catalase (CAT), Glutathione S Transferase (GST) and Glutathione (GSH) and proportionately increases the free radicals. Oxygen derived free radicals such as superoxide anions; hydroxyl radicals and hydrogen peroxide are cytotoxic and give rise to tissue injuries. Excessive amount of ROS is harmful because they initiate bimolecular oxidation which leads to cell death and creates oxidative stress. Research has implicated oxidative and free-radical mediated reactions in degenerative processes related to ageing and diseases such as cancer, coronary heart disease and neurodegenerative disorders such as alzheimer's disease. Stress is also well known to change body weight and food intake pattern and many studies have also shown that restraint induced physical stress suppresses body weight gain and food intake in rodents [2].

Antioxidants are important factor to maintain optimal cellular and human body health. Free radicals are stabilised or deactivated by antioxidants before they attack cells. Several epidemiologic studies revealed that consumption of foods containing high amount of antioxidant compound lowers the risk of human disease occurrence. But, fortification of food formulation by adding antioxidants adversely affect food quality attributes in spite of preventing the oxidative reactions. Several synthetic antioxidants are commercially available. By considering potential health risks and toxicity of synthetic antioxidants like Butylhydroxytoluene (BHT) and Butylhydroxyanisole (BHA), consumers started demanding the usage of natural antioxidants. Now researchers have drawn their attention towards the natural occurring antioxidants (plant materials) [3,4].

*Benincasa hispida* (white pumpkin) is found to have nutritional and medicinal purposes [5,6]. The plant was used medicinally in various complains such as gastrointestinal problems, respiratory disease, heart diseases, diabetes mellitus and urinary diseases [7]. *B. hispida* fruits contain volatile oils, flavonoids, glycosides, saccharides, proteins, carotenes, vitamins, minerals, ß-sitosterin and uronic acid. The pharmacological studies show the central nervous effects of the fruits like anxiolytic, muscle relaxant, antidepressant, in the treatment of alzheimer's disease and to minimise opiates withdrawal signs. It also has the properties of antioxidant, anti-inflammatory, analgesic, antiasthmatic, diuretic, nephroprotective, antidiabetic, hypolipidemic and antimicrobial [8-12]. Since this fruit is edible, easily available, and have got lot of medicinal values, so it becomes essential to check the antioxidant property. No studies have explored on the effects of *B. hispida* fruit dry powder preparation on combination of food intake, body weight, behaviour changes and antioxidant levels in a whole animal model. This study aimed to understand the protective effects of the *B.hispida* dry powder in overcoming stress induction by analysing the pattern of food intake, behavioural changes and body weight and the change in blood levels of SOD and MDA in adult wister rats.

## MATERIALS AND METHODS

This experimental study was conducted in the Department of Pharmacology, PSG Institute of Medical Sciences and Research, Tamil Nadu, India, for the duration of three weeks during the month of March 2020. The approval was obtained from the Institutional Animal Ethics Committee (338/2016/IAEC).

Eighteen adult male wistar rats weighing 150-250 gm were obtained from central animal house.

- **Control group** (n=6): Initially six animals were assessed for the base line values of the parameters (body weight, food intake, behaviour changes, blood levels of SOD and MDA) and then one week later, those six animals were used as control (without treatment) after getting adapted to their normal environment. This method was implemented for reducing the animal usage in the study.

The remaining 12 animals were randomly divided into two treatment groups; pretreated with dry powder preparation of *B.hispida* fruit (using hot air oven technique aseptically)

- **Group 1** (n=6): Dose 250 mg/kg body weight, orally and once daily.
- **Group 2** (n=6): Dose 500 mg/kg body weight, orally and once daily.

Then the control group, group 1 and group 2 animals were subjected to psychological stress using flat bottom rat restrainer for two hrs every day till seven days and the parameters (body weight, food intake, behaviour changes, blood levels of SOD and MDA) were assessed at the end of 24 hours for acute stress and again at the end of seven days of repeated stress induction [13].

Test parameter of body weight for control and test animals was measured in each group on day 1 and day 8 using a digital weighing machine. Food intake in grams was measured at the end of day 1 and day 7 using oxylet system. Food intake in dark/light phases cycle were measured and compared with controls. Behaviour changes were observed by open field test which assesses locomotor activity (quiet room) [14].

### Study Procedure

The apparatus comprised of a square wooden box (72×72×36 cm). The area of the open field divided into 16 squares (18×18 cm) with four inner squares in the centre and 12 squares in the periphery along the walls. On the day of testing, rats were allowed to adapt to the environment one hour prior to testing. After five minutes of acclimatisation in the open field apparatus, each animal from the respective treatment groups was placed individually in the centre of the open field and number of squares crossed, number of times it reared (animal stood on its hind legs), groomed (animal spent licking or scratching itself while stationary) and faecal pellets were observed for a period of five minutes.

**Estimation of serum SOD and MDA levels [15]:** End of 24 hrs and 8$^{th}$ day 1 mL of blood was withdrawn from rat tail vein for the analysis of SOD and MDA levels and was done under laboratory conditions.

**a. Estimation of SOD**

Reagents used were Tris buffer- 50 mL (containing 50 mM of Tris and 1 mM of EDTA), HCl- adjust pH at 8.5, Pyrogallol- 25 mg added to 10 mL of distilled H2O (20 mM conc.).

For blank: 2.9 mL Tris + 0.1 mL Pyrogallol- Mixed well. Absorbance A was read at 420 nm using colourimeter at 1 min 30 sec and Absorbance B at 3 min 30 sec.

For Sample: 2.8 mL Tris + 0.1 mL sample and 0.1 mL Pyrogallol were added and mixed well and the absorbance were read at 420 nm after 1 min 30 sec (Absorbance A) and after 3 min 30 sec (Absorbance B).

Calculation:

$$\text{SOD (in units)} = \frac{\text{Absorbance (A-B)}}{\text{Absorbance (A)}} \times 50$$

**b. Estimation of MDA**

Reagents used were Trichloroacetic Acid (TCA)- 40% (40 g in 100 mL), Thiobarbituric acid- 0.67% (0.67 g in 100 mL). Procedure was 0.5 mL sample+0.5 mL TCA+2 mL TBA added very slowly. The test tubes were kept in boiling water bath at 90-100 °C for 10 min. After 10 min, the tubes were brought back to the room temperature. Samples were centrifuged for 10 min at 3000 rpm. Separate the supernatant and readings were taken at 540 nm using colourimeter.

Calculation:

$$\text{MDA (in units)} = \frac{\text{Absorbance at 532 nm}}{1.56} \times 105$$

## STATISTICAL ANALYSIS

Data from the control and treatment groups were analysed by Analysis of Variance (ANOVA) in Statistical Package for Social Sciences software version 24.0.

## RESULTS

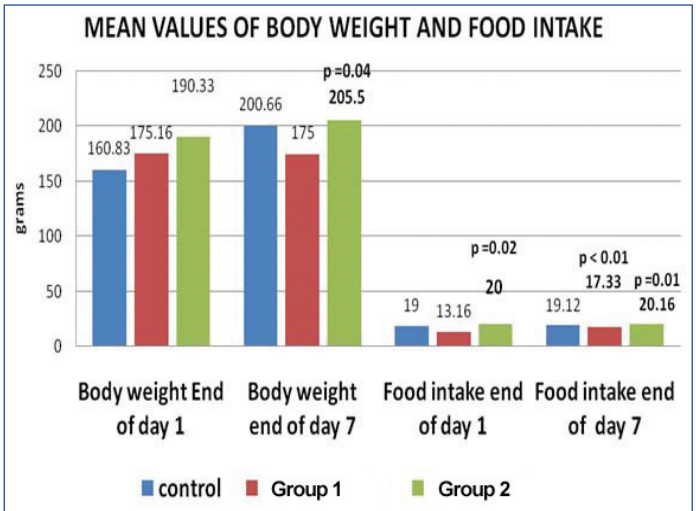
In this study, mean values of baseline parameters of 6 rats were: mean weight was 160.833±11.58 kg, mean food intake was 14.16±0.93 gm, mean rearing was 3.5 ±4.08, mean grooming was 13.5±7.71, mean number of squares crossed was 5.33±11.21, mean blood levels of SOD was 8.44±10.86 units/mL and mean MDA was 5.40±6.96 nmol/mL.

After the 1$^{st}$ day of stress induction in rats, changes were observed in the mean values of body weight, food intake pattern, behaviour changes, SOD and MDA levels in the both the treatment group of *B.hispida* (group 1 and group 2) compared to the control group and the baseline values [Table/Fig-1]. But more statistically significant values were seen in group 2 for food intake (p-value=0.02) [Table/Fig-2] and group 1 for grooming (p-value=0.01) [Table/Fig-3].

Similarly, at the end of 7$^{th}$ day of repeated stress induction, changes were observed in the mean values of body weight, food intake, behaviour changes, and SOD and MDA levels in both the treatment groups [Table/Fig-1]. But statistically significant changes in the body weight was observed at the dose of *B.hispida* 500 mg/kg (p-value 0.04) [Table/Fig-2] and food intake was observed in the treatment group of both *B.hispida* 250 mg/kg (p-value 0.00) and 500 mg/kg (p value 0.01) [Table/Fig-2]. In case of antioxidant levels, there were no significant changes in blood levels of SOD and MDA values, instead the antioxidant levels were reduced inspite of exploring good behavioural changes comparing to the control rats [Table/Fig-4,5].

| Parameters | 1st day of stress induction | | End of 7th day of stress induction | |
|---|---|---|---|---|
| | Mean±SD | p-value | Mean±SD | p-value |
| **Weight (grams)** | | | | |
| Control | 160.5±15.29 | 0.52 | 200.66±25.31 | <0.01 |
| Group 1 | 175.16±16.12 | 0.99 | 175±16.38 | 0.55 |
| Group 2 | 190.33±16.20 | 0.99 | 205±17.79 | 0.04 |
| **Food intake (grams)** | | | | |
| Control | 19.0±0.81 | 0.00 | 19.12±0.75 | 0.19 |
| Group 1 | 13.16±0.98 | 0.22 | 17.33±0.81 | <0.01 |
| Group 2 | 20±0.63 | 0.02 | 20.16±0.75 | 0.01 |
| **No. of rearing** | | | | |
| Control | 1.5±1.21 | 0.18 | 1.83±0.98 | 0.37 |
| Group 1 | 5.83±0.98 | 0.33 | 2.33±2.58 | 0.88 |
| Group 2 | 6.33±1.63 | 0.18 | 2.33±2.58 | 0.88 |
| **No. of grooming** | | | | |
| Control | 1±2.44 | 0.00 | 4.16±5.03 | 0.02 |
| Group 1 | 4.5±2.88 | 0.01 | 5.66±4.41 | 0.08 |
| Group 2 | 7.5±2.07 | 0.12 | 12.16±2.78 | 0.97 |
| **No. of squares crossed** | | | | |
| Control | 3±4.89 | 0.80 | 1±1 | 0.44 |
| Group 1 | 14.16±2.31 | 0.11 | 1.50±2.34 | 0.69 |
| Group 2 | 6.66±3.55 | 0.98 | 7.83±4.11 | 0.89 |
| **SOD (units/mL)** | | | | |
| Control | 8.12±15.61 | 1.00 | 4.59±9.21 | 1.00 |
| Group 1 | 8.85±3.30 | 1.00 | 2.73±1.47 | 0.54 |
| Group 2 | 2.47±1.51 | 0.70 | 2.80±2.67 | 0.55 |
| **MDA (nmol/mL)** | | | | |
| Control | 4.12±0.00 | 0.40 | 3.18±0.27 | 0.10 |
| Group 1 | 9.04±10.53 | 0.70 | 3.33±2.49 | 0.91 |
| Group 2 | 2.81±2.44 | 0.80 | 2.30±1.78 | 0.77 |

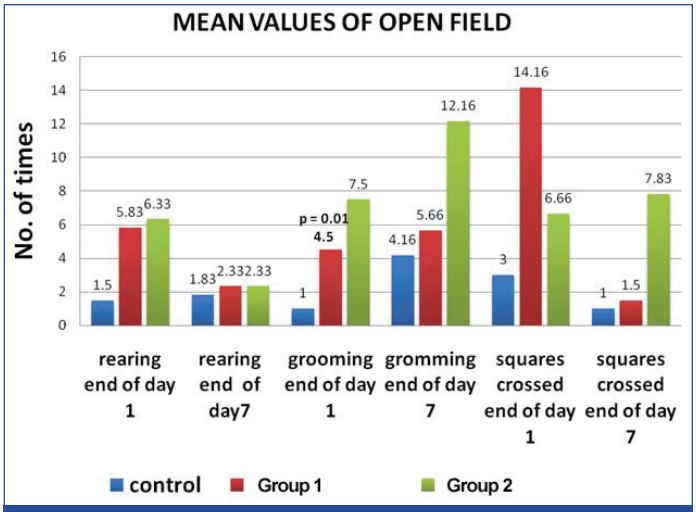
**[Table/Fig-1]:** Results of the test parameters of control group, Group 1 and Group 2 at the end of day 1 of stress induction and day 7 of stress induction. Antioxidant levels were measured using; SOD: Superoxide dismutase; MDA: Malondialdehyde; General behaviour pattern was assessed using Open field method (No of rearing, grooming, squares crossed)



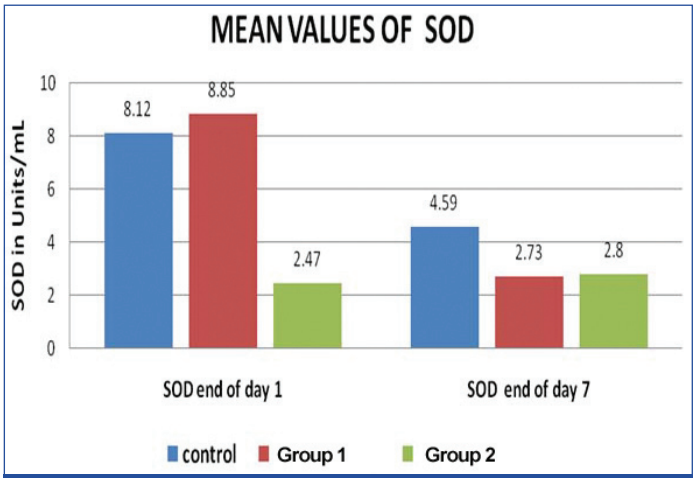

**[Table/Fig-2]:** Mean values of body weight and food intake pattern after stress induction at the end of day 1 and day 7.

## DISCUSSION

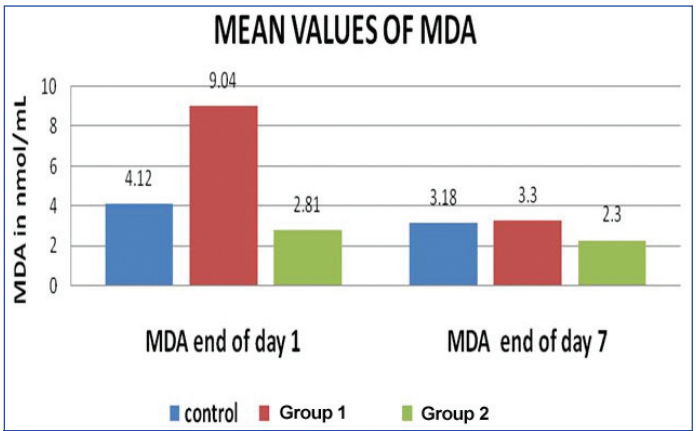
Oxidative stress depicts the existence of products called free radicals and reactive oxygen species, which are formed under normal physiological conditions but become deleterious when not being eliminated by the endogenous systems. Reactive oxygen species are major sources of primary catalysts that initiate oxidation in-vivo and in-vitro and create oxidative stress which results in numerous




**[Table/Fig-3]:** Mean values of open field test after stress induction at the end of day 1 and day 7.




**[Table/Fig-4]:** Mean values of SOD after stress induction at the end of day 1 and day 7.




**[Table/Fig-5]:** Mean values of MDA level after stress induction at the end of day 1 and day 7.

diseases and disorders, such as cancer, cardiovascular disease, neural disorders, ageing, and atherosclerosis [3].

Stress induces the modification of the body weight and food intake in animal models. Especially the restraint stress model effectively depicts the physical and psychological stress and is used as an animal model of depression. Many studies have shown that restraint stress suppresses body weight gain and food intake in rodents and also affects the behaviour patterns in animal models [2,6].

In the present study, pretreatment of *Benincasa hispida* at the doses of 250 and 500 mg/kg of body weight to the rat models have shown significant improvement in the body weight, food intake, and behaviour changes to some extent. In case of antioxidant levels, there were no significant changes in blood levels of SOD and

MDA values, instead the antioxidant levels were reduced inspite of exploring good behavioural changes comparing to the control rats. To explain this, previous studies of *Benincasa hispida* have proven that this reduction in the antioxidant levels could be attributed to the sparing effect of the powder [3,4]. It means exogenous supplement of antioxidants is likely to suppress the endogenous production which could be effectively revealed in the improved general behavioural changes of rats due to the pretreatment effect of *Benincasa hispida*. Also, the behaviour pattern observed was consistent with the study done by Al-Snafi AE, where neurological behaviour was compared between *B.hispida* and classical antidepressant drugs [16]. Hence, *Benincasa hispida* antioxidant effect has proven to support the cell system to overcome the depression produced by stress induction and also aided in normalising the food intake and body weight.

### Limitation(s)

The limitation of the study are basic parameters were included to assess the behaviour changes and restrain stress induction was given for 2 hrs/day for 7 days and so complete picture of development of stress model might not be achieved for assessment. The prophylactic role of *Benincasa hispida* fruit was assessed alone and compared with the control groups. Its effects can also be compared with standard drugs to understand whether its action is at central or peripheral level.

## CONCLUSION(S)

This study has shown the beneficial effect of *B.hispida* in stress induction whole animal model despite lack of significant change in antioxidant levels. Further studies with other specific models of stress induction would help to elucidate the various possible mechanisms of *B. hispida* in overcoming stress induction.

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