

# Effects of Chlorophytum Borivilianum Extract Against High Fat Diet Induced Obesity in Male Wistar Albino Rats

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## ABSTRACT

**Introduction:** the effect of Chlorophytum borivilianum extract against high fat diet induced obesity is unknown. Therefore, this study investigates activity of 250 & 500 mg/kg/day of Chlorophytum borivilianum extract in male Wistar Albino rats, on parameters like weight gain, Follicle Stimulating Hormone, Luteinizing hormone, and Testosterone. The objective is to determine the effects of Chlorophytum Borivilianum extract against high fat diet-induced obesity in male Wistar Albino rats.

**Materials and Methods:** it's an interventional, comparative, animal study of 60 days, conducted at KMCH college of pharmacy, Coimbatore. 20 male Wistar Albino rats were randomly divided into 5 experimental groups with 6 rats per group. At the end of experimental period, all rats were fasted overnight, weighted, and sacrificed within 24 hours by the mode of decapitation, and the blood was collected. Later, the organs were dissected out and fixed with formalin. The post-fixed tissue, slides were stained. Ethics committee approval was obtained initially. Statistical analysis was done by SPSS software.

**Results:** the Obese group showed an increase in body weight due to high-fat diet administration, whereas, the treatment group receiving Chlorophytum borivilianum extract showed reduction in body fat and improvement in the semen analysis when compared to control group.

**Conclusion:** when treated with Chlorophytum borivilianum, body weight as well as sperm function of the animals was significantly improved mediated through enhanced spermatogenesis, steroid genesis, and antioxidant mechanisms with histological studies. Thus, findings reinforce the advice recommending consumption of Chlorophytum borivilianum extract to modulate obesity and sperm morphology.

**Keywords:** Chlorophytum borivilianum; Wistar Albino rats; Obesity; Sperm morphology.

## Introduction

In today's time obesity and infertility are one of the gravest social complications. Obesity is a condition in which excessive fat accumulation triggers a disproportion in energy intake and expenditure due to alteration in healthy dietary habits<sup>1</sup>. Less physical activity also causes various disorders like infertility, Diabetes mellitus, hypertension, and cardiovascular problems. Infertility can also be caused by several factors such as genetic, environmental, bad habits like smoking, sedentary lifestyle leading to body hormonal and sexual disorders<sup>2</sup>.

Currently, no pharmacological treatments provide sustained weight loss without adverse effects. A variety of plant and their byproducts (extracts) are helpful to induce body weight reduction and also prevent diet-related obesity and infertility<sup>3,4</sup>. However, a previous study has reported that regular intake of antioxidants and vitamins such as vitamins A, B, C, and E improves testicular blood barrier stability and protects sperm DNA damage from endogenous oxidative stress resulting from the activities of highly reactive free radicals generated

within the body<sup>5</sup>.

The literature revealed that traditional medicine helps to nourish and stimulates the sexual tissues and ameliorates male reproductive health issues by various plant herb extracts such as Panax spp. (ginseng), Moringa oleifera and Lepidium meyenii (Maca) are reputed for their supposed aphrodisiac- and spermatogenesis-enhancing effects<sup>6</sup>.

Chlorophytum borivilianum belongs to the family 'Liliaceae' and the genus 'Chlorophytum'. It has various properties like antitussive activity, osteopenia, anti-diabetic activity, immune-modulatory activity, antioxidant activity, anti-stress property, anti-ulcer activity, anthelmintic activity, etc.<sup>7</sup>.

Recently anti-obesity and antioxidant properties of the plant Chlorophytum borivilianum have been explored and its relationship to the improvement of infertility has to be explored.<sup>8</sup> The present study is conducted with the intent to investigate the effects of Chlorophytum Borivilianum extract against high fat diet induced obesity in male Wistar Albino rats for the parameters, body weight management and sperm morphology.

## Materials and Methods

### Study design and population

It is an interventional, comparative animal study of 60 days, conducted at Kirupanandha Varier Medical College and Hospital, Tamilnadu. In the present study, a total of 20 male Wistar Albino rats were taken.

The study was conducted by the recommendations from the declaration of the institutional animal ethical committee no: KMCRET/RERC/Ph.D/28/2021 under proper care and use of animals.

### Group allocation

The animals were randomly assigned into five experimental groups with four rats per group:

**Group 1:** Control/Normal group (n=4)- Healthy male Wistar Albino rats

**Group 2:** High-fat diet (HF) group (n=4)- animals receiving high fat diet

**Group 3:** High-Fat diet (HF) + Sildenafil citrate (SC)- animals receiving standard drug, Sildenafil citrate (5mg/kg) for 28 days (n=4)

**Group 4:** High-Fat diet (HF) + Chlorophytum borivilianum (CB)- receiving low dose of CB (250mg/kg/day) for 28 days (n=4)

**Group 5:** High-Fat diet (HF) + Chlorophytum borivilianum (CB)- receiving high dose of CB (500 mg/kg/day) for 28 days (n=4)

At the end of the experimental period, all rats were fasted overnight, weighed, and sacrificed within 24 hours by the mode of decapitation, and the blood was collected through the retro-orbital puncture.

### Study Procedure

#### Plant material

Dried roots of Chlorophytum borivilianum were procured from the Ayurvedic store online. The dried roots were cut into small pieces and then shaded and ground into fine powder. The root powder (1000 g) of the powdered sample was exhaustively extracted with ethanol. The solvent was then distilled off, to give the solid extract, which was stored at 4°C. The solid extract was then dissolved in normal saline and used for study purposes.

#### Animal Model

Male Wistar Albino rats (250- 440g) obtained from the Central Animal House, Kirupanandha Varier Medical College and Hospital, were used for the study. The rats were housed in wire mesh cages under standard temperatures of 25 -29 ° C, 12h light, and 12h darkness cycles. Animals were fed with a pelleted standard rat diet with proper water ad libitum.

#### Histopathology Studies

The organs were dissected out and fixed with formalin. The post-fixed tissue was embedded with a Serial section by using a rotary microtome at 5

microns. The slides were stained with Haematoxylin and Eosin.

#### Weight Measurement

**Animal Body Weight:** Rat body weights were measured by using a digital Mettler weighing balance and values obtained were recorded a day before the commencement of the experiment, and also during the treatment period as well as at the last day of the experiment.

**Organ weights:** At the end of the experiment period, each animal was sacrificed, at weekly intervals of two, four, or twelve weeks respectively. Various parts of body organ tissues were removed and weight was calculated and recorded.

#### Analysing Sperm Motility, Viability, Counts, and Morphology

**Analysis of Sperm Cells:** After carefully separating the epididymis from the testes, the epididymis was minced in 0.1M phosphate buffer solution (1 mL). For obtaining the epididymal fluid, the minced tissues were swirled gently in the buffer solution for the proper diffusion of sperm cells. Sperm cell motility analysis, 10µL of the epididymal fluid was charged into a Neubauer chamber and the total number of sperms was estimated.

The number of sperms per cauda epididymis was calculated as follows:

$$\{\text{Mean count} \times 50\} \div \{0.01 \times 0.01\}$$

**Biochemical Analysis:** From each experimental animal, blood was obtained by a retro-orbital puncture after anaesthetization. Each blood sample was rotated at 2500 rpm for 10 minutes in a centrifuge at 10-25 °C. Serum samples were assayed for testosterone using the enzyme-linked immunoassay (EIA) technique.

#### Statistical Analysis

The statistical analysis was done by using SPSS software (21.0 version), the data is stated as Mean±SD in tabulated form. For data comparison One way ANOVA, followed by Dunnett comparison was performed. In all the cases p value ≤0.001\* is considered statistically significant.

#### Ethical approval

The ethical committee letter was obtained before the initiation of the study, (KMCRET/RERC/Ph.D/28/2021).

#### Results

In the Table showing body weight variations, where changes in control group rats and rats treated with ethanolic extracts of Chlorophytum borivilianum were noted, it was observed that, the Chlorophytum borivilianum extract did not show any significant

effect on the body weights of rats treated for 1st week (Group-1 (Control)- 122±1.23, Group-2 (HF group)- 125±1.42, Group-3 (HF + SC group)- 119±1.67, Group-4 (HF + CB group, Low dose)- 126±1.74 & Group-5 (HF + CB group, High dose)- 124±1.33) and 3rd week (Group-1 (Control)- 150±2.09, Group-2 (HF group)- 174±2.24, Group-3 (HF + SC group)- 170±2.84, Group-4 (HF + CB group, Low dose)- 174±3.11 & Group-5 (HF + CB group, High dose)- 175±2.29), respectively.

The body weight has decreased significantly in the groups treated for 4th and 6th weeks with Chlorophytum borivilianum extract (low and high doses) when compared to other groups. (Group-1 (Control)- 172±2.12, Group-2 (HF group)- 242±7.70, Group-3 (HF + SC group)- 207±5.79, Group-4 (HF + CB group, Low dose)- 212±11.03\* & Group-5 (HF + CB group, High dose)- 208±8.47\*)

However, normal body and organ weights were restored in rats which were allowed for a recovery period of 6 weeks. (Table 1)

In the table of reproductive organ weight, where, the effect of repeated daily oral treatments with 250-500mg/kg/day of CB ethanolic extract on the average testicular weight (TW), caudal epididymis, seminal vesicle and prostate gland of treated rats was done, it was observed that, there was a significant increase in weight of reproductive organs, Testis (Group-1 (Control)- 2.75±0.07, Group-2 (HF group)- 2.27±0.08, Group-3 (HF + SC group)- 3.05±0.04, Group-4 (HF + CB group, Low dose)- 2.27±0.05 & Group-5 (HF + CB group,

High dose)- 2.48±0.10\*) and Seminal vesical (Group-1 (Control)- 0.88±0.04, Group-2 (HF group)- 0.83±0.03, Group-3 (HF + SC group)- 0.76±0.03, Group-4 (HF + CB group, Low dose)- 0.88±0.02 & Group-5 (HF + CB group, High dose)- 0.96±0.02\*) when treated with Chlorophytum borivilianum extract (high dose).

Whereas, the weight of other reproductive organs namely, Caudal epididymis and Prostate gland were not increased much and were found to be similar to the Standard drug weight (Sildenafil citrate (SC)). (Table 2)

In the table of biochemical assay, it can be clearly noticed that, repeated treatments with Chlorophytum borivilianum extract (high dose), resulted in significant dose related increase in the circulating serum Follicle-stimulating hormone (FSH), (Group-1 (Control)- 0.34±0.01, Group-2 (HF group)- 0.27±0.04, Group-3 (HF + SC group)- 0.29±0.04, Group-4 (HF + CB group, Low dose)- 0.27±0.07 & Group-5 (HF + CB group, High dose)- 0.36±0.03\*), increase in the circulating serum Luteinizing hormone (LH), (Group-1 (Control)- 0.11±0.32, Group-2 (HF group)- 0.14±0.01, Group-3 (HF + SC group)- 0.11±0.12, Group-4 (HF + CB group, Low dose)- 0.12±0.17 & Group-5 (HF + CB group, High dose)- 0.27±0.02\*), as well as increase in the Testosterone levels, (Group-1 (Control)- 0.31±5.71, Group-2 (HF group)- 64.1±4.73, Group-3 (HF + SC group)- 85.4±8.17, Group-4 (HF + CB group, Low dose)- 102.0±4.23 & Group-5 (HF + CB group, High dose)- 207±9.18\*), when compared to Group-1 (control group). (Table 3)

**Table 1.** BODY WEIGHT- Changes in control group rats and rats treated with ethanolic extracts of Chlorophytum borivilianum.

Visiting days/ weeks	Group - 1 (Control) (Mean±SD)	Group - 2 (HF group) (Mean±SD)	Group - 3 (HF + SC group) (Mean±SD)	Group - 4 (HF + CB group, Low dose - 250 mg/kg/day) (Mean±SD)	Group - 5 (HF + CB group, High dose - 500 mg/kg/day) (Mean±SD)
1st day (1st week)	122±1.23	125±1.42	119±1.67	126±1.74	124±1.33
21st day (3rd week)	150±2.09	174±2.24	170±2.84	174±3.11	175±2.29
42nd day (4th to 6th week)	172±2.12	242±7.70	207±5.79	212±11.03*	208±8.47*

HF- High fat, SC- Sildenafil citrate, CB- Chlorophytum borivilianum. p<0.001\* considered as statistically significant and is represented as \*

**Table 2.** REPRODUCTIVE ORGAN WEGHT- Effect of repeated daily oral treatments with 250-500mg/kg/day of CB ethanolic extract on the average testicular weight (TW), caudal epididymis, seminal vesicle and prostate gland of treated rats.

Organs estimated for weight	Group - 1 (Control) (Mean±SD)	Group - 2 (HF group) (Mean±SD)	Group - 3 (HF + SC group) (Mean±SD)	Group - 4 (HF + CB group, Low dose - 250 mg/kg/day) (Mean±SD)	Group - 5 (HF + CB group, High dose - 500 mg/kg/day) (Mean±SD)
Testis	2.75±0.07	2.27±0.08	3.05±0.04	2.27±0.05	2.48±0.10*
Caudal epididymis	0.19±0.18	0.16±0.18	0.11±0.13	0.19±0.17	0.11±0.14
Seminal vesicle	0.88±0.04	0.83±0.03	0.76±0.03	0.88±0.02	0.96±0.02*
Prostate gland	1.04±0.04	1.24±0.30	1.04±0.11	0.94±0.08	1.02±0.09

HF- High fat, SC- Sildenafil citrate, CB- Chlorophytum borivilianum. p<0.001\* considered as statistically significant and is represented as \*

**Table 3.** BIOCHEMICAL ASSAY- To estimate the serum biochemical analysis between the control and treatment groups.

Parameters	Group - 1 (Control) (Mean±SD)	Group - 2 (HF group) (Mean±SD)	Group - 3 (HF + SC group) (Mean±SD)	Group - 4 (HF + CB group, Low dose - 250 mg/kg/day) (Mean±SD)	Group - 5 (HF + CB group, High dose - 500 mg/kg/day) (Mean±SD)
Follicle-stimulating hormone (FSH) (mIU/mL)	0.34±0.01	0.27±0.04	0.29±0.04	0.27±0.07	0.36±0.03*
Luteinizing hormone (LH) (IU/L)	0.11±0.32	0.14±0.01	0.11±0.12	0.12±0.17	0.27±0.02*
Testosterone (ng/dL)	0.31±5.71	64.1±4.73	85.4±8.17	102.0±4.23	207±9.18*

HF- High fat, SC- Sildenafil citrate, CB- Chlorophytum borivilianum.  $p \leq 0.001^*$  considered as statistically significant and is represented as \*

In the table of sperm cell characteristics, in the group treated with Chlorophytum borivilianum extract (low and high dose), there was an increase in the sperm count, (Group-1 (Control) - 77.4±0.39, Group-2 (HF group)- 41.6±0.68, Group-3 (HF + SC group)- 68.5±2.56, Group-4 (HF + CB group, Low dose)- 68.2±1.49 & Group-5 (HF + CB group, High dose)- 71.4±1.06\*),

increase in the motility (Group-1 (Control)- 80.3±0.65, Group-2 (HF group) - 61.9±1.19, Group-3 (HF + SC group)- 72.2±2.12, Group-4 (HF + CB group, Low dose)- 69.4±2.56 & Group-5 (HF + CB group, High dose)- 80.7±1.05\*),

and also increase in the viability percentages, (Group-1 (Control)- 85.5±0.55, Group-2 (HF group)- 61.8±4.64, Group-3 (HF + SC group)- 82.4±3.56, Group-4 (HF + CB group, Low dose)- 73.4±0.84 & Group-5 (HF + CB group, High dose)- 89.8±0.27\*), when compared to Group-2 (HF group) and Group-3 (HF + SC group) significantly.

The abnormalities in the sperm cell characteristics were minimal in the group treated with Chlorophytum borivilianum extract (high dose). (Group-1 (Control)- 21±0.57, Group-2 (HF group)- 38.5±0.45, Group-3 (HF

+ SC group)- 27±1.57\*, Group-4 (HF + CB group, Low dose)- 30.5±0.60 & Group-5 (HF + CB group, High dose)- 17.7±2.64\*). (Table 4)

In the table of biochemical assay, where the estimation of testicular reduced glutathione levels of SOD and catalase activities were done along with, GPX, GSH and LPO.

In the testicular tissue SOD, (Group-1 (Control)- 0.14±0.01, Group-2 (HF group)- 0.12±0.011, Group-3 (HF + SC group)- 0.18±0.04, Group-4 (HF + CB group, Low dose)- 0.16±0.36 & Group-5 (HF + CB group, High dose)- 0.20±0.22\*), CAT (Group-1 (Control)- 0.33±0.01, Group-2 (HF group)- 0.89±0.80, Group-3 (HF + SC group)- 0.36±0.85, Group-4 (HF + CB group, Low dose)- 0.138±0.36 & Group-5 (HF + CB group, High dose)- 0.143±0.29\*), and LPO activities (Group-1 (Control)- 0.76±0.01, Group-2 (HF group)- 0.51±0.61, Group-3 (HF + SC group)- 0.76±0.14, Group-4 (HF + CB group, Low dose)- 0.73±0.02 & Group-5 (HF + CB group, High dose)- 0.95±0.58\*) were significantly increased, when treated with Chlorophytum borivilianum extract (high dose).

In the testicular tissue GPX, (Group-1 (Control)- 0.39±0.06, Group-2 (HF group) - 0.22±0.23, Group-3

**Table 4.** SPERM CELL CHARACTERISTICS- Effects of CB extracts on sperm characteristics in rats treated for 2 weeks and their recovery groups. Estimating, Motility (%), Viability (%), Sperm Counts (million/ml) and abnormality.

Study groups	Sperm Count (x106/ mL) (Mean±SD)	Motility (%) (Mean±SD)	Viability (%) (Mean±SD)	Abnormalities (%) (Mean±SD)
Group-1 (Control)	77.4±0.39	80.3±0.65	85.5±0.55	21±0.57
Group - 2 (HF group)	41.6±0.68	61.9±1.19	61.8±4.64	38.5±0.45
Group - 3 (HF + SC group)	68.5±2.56	72.2±2.12	82.4±3.56	27±1.57*
Group - 4 (HF + CB group, Low dose - 250 mg/kg/day)	68.2±1.4	69.4±2.56	73.4±0.84	30.5±0.60
Group - 5 (HF + CB group, High dose - 500 mg/kg/day)	71.4±1.06*	80.7±1.05*	89.8±0.27*	17.7±2.64*

HF- High fat, SC- Sildenafil citrate, CB- Chlorophytum borivilianum.  $p \leq 0.001^*$  considered as statistically significant and is represented as \*

(HF + SC group)- 0.75±0.49, Group-4 (HF + CB group, Low dose)- 0.21±0.07 & Group-5 (HF + CB group, High dose)- 0.13±0.91\*), GSH (Group-1 (Control)- 0.18±0.52, Group-2 (HF group)- 0.32±0.63, Group-3 (HF + SC group)- 0.12±0.21, Group-4 (HF + CB group, Low dose)- 0.11±0.15 & Group-5 (HF + CB group, High dose)- 0.95±0.58\*), activities were not increased. (Table 5)

In our study, the effect of repeated daily oral treatments with 250-500mg/kg/day of Chlorophytum borivilianum extract on reproductive organ weight was done, it was observed that, there was a significant increase in weight of reproductive organs (Testis and Seminal vesical), when treated with Chlorophytum borivilianum extract (high dose- 500 mg/kg/day)<sup>13</sup>.

Table 5. BIOCHEMICAL ASSAY- Estimation of Testicular SOD, Catalase, LPO and Glutathione Levels Activities.

Parameters	Group - 1 (Control) (Mean±SD)	Group - 2 (HF group) (Mean±SD)	Group - 3 (HF + SC group) (Mean±SD)	Group - 4 (HF + CB group, Low dose- 250 mg/kg/day) (Mean±SD)	Group - 5 (HF + CB group, High dose- 500 mg/kg/day) (Mean±SD)
SOD (unit/min/mg protein)	0.14±0.01	0.12±0.011	0.18±0.04	0.16±0.36	0.20±0.22*
CATALASE (µmole of H2O2 consumed/min/mg protein)	0.33±0.01	0.89±0.80	0.36±0.85	0.138±0.36	0.143±0.29*
GPX (µmole of glutathione oxidized/min/mg protein)	0.39±0.06	0.22±0.23	0.75±0.49	0.21±0.07	0.13±0.91*
GSH (µmol/mg of protein)	0.18±0.52	0.32±0.63	0.12±0.21	0.11±0.15	0.10±0.13*
LPO (µmol/mg (of MDA nmol/gm))	0.76±0.01	0.51±0.61	0.76±0.14	0.73±0.02	0.95±0.58*

HF- High fat, SC- Sildenafil citrate, CB- Chlorophytum borivilianum. ps0.001\* considered as statistically significant and is represented as \*

### Discussion

Obesity is recognized as a metabolic disease with chronic effects probably due to various environmental as well as hereditary factors. In the recent years obesity in human population caused innumerable health hazards in which fertility was of one them<sup>9</sup>. Further studies have specified that a high fat percentage (high BMI) is also related with significant reduction in sperm concentrations<sup>10</sup>. Chlorophytum borivilianum (Safed Musli), is a highly valued Indian medicinal plant. This plant has been shown to possess anti-obesity, anticancer, antimicrobial, antifungal, antiulcer, antipyretic, antiarthritic and immunomodulatory activities<sup>11</sup>. The present study demonstrates the effect of Chlorophytum borivilianum extract against high-fat diet induced obesity in male Wistar Albino rats.

It was observed that that the body weight of the experimental animals on high-fat diet has been decreased when treated with low (250 mg/kg/day) and high doses (500 mg/kg/day) of Chlorophytum borivilianum extract, in the 4th and 6th weeks of the study. The significant weight reduction is supporting the fact that Chlorophytum borivilianum extract when given to obese rats on high-fat diet is helpful in reducing the body fat level leading towards normal body fat percentage<sup>12</sup>.

When the biochemical assay was conducted to estimate the serum biochemical analysis on experimental animals having repeated treatments with Chlorophytum borivilianum extract (high dose- 500 mg/kg/day), resulted in significant dose related increase in the circulating serum Follicle-stimulating hormone (FSH), Luteinizing hormone (LH) as well as increase in the Testosterone levels<sup>14</sup>.

The sperm cell characteristics were studied extensively with Chlorophytum borivilianum extract. It was demonstrated that low (250 mg/kg/day) and high doses (500 mg/kg/day) of Chlorophytum borivilianum extract significantly increased sperm count, motility and viability<sup>15</sup> as well. The abnormalities in the sperm cell characteristics were also found to be minimal with Chlorophytum borivilianum extract<sup>16</sup>.

The estimation of Testicular SOD, Catalase, LPO and Glutathione Levels activities were carried out, resulting in increase in SOD, CAT and LPO levels<sup>17</sup> and decrease in Glutathione levels<sup>18,19</sup>. When treated with Chlorophytum borivilianum extract.

### Conclusion

Chlorophytum borivilianum extract (Low dose- 250 mg/kg/day and High dose- 500 mg/kg/day) given to male Winstar Albino rats on high fat diet for 60 days

significantly improved sperm function like sperm motility, count, viability, and morphology, mediated via increased spermatogenesis, steroidogenesis, and biochemical and antioxidant mechanisms. This present study obtained results reinforce the advice recommending consumption of this plant product to modulate obesity and sperm morphology.

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