Toxicological studies of *Momordica charantia* Linn Seed extracts in Male Mice

Estudios Toxicológicos de los Extractos de la Semilla de *Momordica charantia* Linn en Ratones Macho

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**SUMMARY:** An attempt to find out the male contraceptive molecule of plant origin, the extracts of seeds of *Momordica charantia* were tested in male mice. Petroleum ether, chloroform and ethanolic extracts of *Momordica charantia* were administered at the dose level of 25mg/100gm body weight to the albino mice for 48 days intraperitoneally. All the extracts showed antispermatogenic effect as the number of spermatogonia, spermatocytes, spermatids and spermatozoa were decreased. The increase in the weight of epididymis, prostate gland, seminal vesicle and vas deferens indicates clearly the androgenic property of these extracts. After subjecting to preliminary phytochemical screening ethanol extract showed positive tests for alkaloids, flavonoids, glycosides, phenols, tannins, oils and fats. Out of the three extracts tested, the ethanol extract seems to be more potent in its contraceptive and androgenic activities.

**KEY WORDS:** *Momordica charantia* Linn; Toxicological; Androgenic; Testis; Spermatogenesis.

**INTRODUCTION**

Hormonally active chemicals that are capable of inducing adverse effects on reproduction and/or carcinogenesis in wildlife as well as human beings are featured as “endocrine disruptors” (ED). The National Academy of Science and the U.S. Environmental Protection Agency (EDSTAC) recommend various animal studies to clarify the characteristics of those hormonal active compounds. Although herbal medicines are effective in the treatment of various ailments very often these drugs are unscientifically exploited or improperly used.

*Momordica charantia* Linn. (Cucurbitaceae) grows in tropical areas, including countries like India, China, Japan, East Africa, and South America where it is used as food as well as the medicinal plant. It's a slender, climbing annual vine with long-stalked leaves and yellow, solitary male and female flowers borne in the leaf axils. The fruit looks like a warty gourd, usually oblong and resembling a small cucumber. The young fruit is emerald green, turning to orange-yellow when ripe. At maturity, the fruit splits into three irregular valves that curl backwards and release numerous reddish-brown seeds. Biological actions of various extracts of the plants or its constituents include antidiabetic (Ahmed *et al*., 1998; Mastuda *et al*., 1999; Raza *et al*., 2000), anti ulcerogenic (Gürbüz *et al*., 2000), antimitogenic (Guevara *et al*., 1990), antioxidant (Scartezzini & Speroni, 2000), anti-tumour (Lee-Huang *et al*., 1995a), immunomodulatory (Spreafico *et al*., 1983). Two proteins known as α- and β - monorcharin which are present in the seeds, fruits and leaves have shown to inhibit the human immune deficiency virus (HIV) *in vitro* (Zhang, 1992; Lee-Huang *et al*., 1995b). Leaf extracts of bitter melon have demonstrated broad-spectrum antimicrobial activity. Various extracts of the leaves have demonstrated *in vitro* antibacterial activities against *E. coli*, *Staphylococcus*, *Pseudomonas*, *Salmonella*, *Streptobacillus* and *Streptococcus*. Further it is also reported that alcoholic extracts of whole plant of *M. charantia* cause infertility in dogs (Bhargava, 1988). Many *in vivo* clinical studies have demonstrated the relatively low toxicity of all parts of the bitter melon plant when ingested orally. However, toxicity and even death in laboratory animals has been reported when extracts are injected intravenously. Other studies have shown extracts of the fruit and leaf (ingested orally) to be safe during pregnancy. The seeds, however, have demonstrated the ability to induce abortions in rats and mice, and the root has been documented...
as a uterine stimulant in animals. The fruit and leaf of bitter melon have demonstrated an in vivo antifertility effect in female animals. Our earlier studies have reported the estrogenic and antiovulatory activities in female rats (Sharanabasappa & Saraswati, 2001; Sharanabasappa et al., 2002). The M. charantia is known for a variety of biological actions no systematic investigation for its male reproductive activities and toxicological studies has been done. Therefore in the present investigation an attempt was made to assess the effect of various extracts of seeds of M. charantia on spermatogenesis, development of its accessory reproductive organs and their androgenic nature in male mice.

MATERIAL AND METHOD

Plant material and preparation of the extract. In the present study the seeds of Momordica charantia were collected from the fields of north Karnataka region in the months of November and December 2002. This plant was authenticated at the Department of Botany, Gulbarga University, Gulbarga, India (Voucher specimen No. HGUG-905). Seeds were shade dried and powdered. 500g powder was subjected to soxhlet extraction successively and separately from nonpolar to polar solvents i.e. petroleum ether, chloroform and ethanol. The extracts were concentrated to dryness in a flash evaporator under reduced pressure and controlled temperature (40-60ºC). The petroleum ether extract yielded yellow greasy extract (140g), chloroform extract yielded light yellow extract (10g) and ethanol extract yielded dark brown gummy extract (10g). All the extracts were prepared in Tween-80 (1%) suspended in distilled water for their complete dissolution.

Phytochemical study. The presence of various phytochemical constituents in petroleum ether, chloroform and ethanol extracts of M. charantia were carried out as described by Harborne (1973). Dragendorff’s reagent was used for alkaloids, Libermann-Buchard reagent for steroids, Shinoda test for flavonoids, Keller-Kiliani test for glycosides and ferric chloride reagent for phenolic.

Animals. Twenty four colony bred male albino mice of Swiss strain 60-70 days old were used for the experiment (Animal ethics Reg.No. 34800/2001/CPAE/A dated 1-9-08-2001). Six animals per group (4 groups) were housed in polycarbonate cages with soft rice husk bedding in a room controlled for 12-h light-dark cycle, ventilation (air exchange 18 time/hour), temperature (23-25ºC) and relative humidity (50-60%) during the study. The cages and husk bedding were exchanged twice in a week. The animal had given balanced food and water ad libitum.

Different extracts of M. charantia seeds were administered intraperitoneally for 48 days at the dose level of 25mg/100gm body weight. Group I received 0.1 ml Tween-80 (1%) and served as control. Group II, III and IV received petroleum ether, chloroform and ethanol extracts in Tween-80 (1%) respectively.

Histological studies. Animals were sacrificed by cervical dislocation on 49th day. Both the testis and accessory organs like caput epididymis, cauda epididymis, vas deferens seminal vesicle and prostate were dissected out, made free from surrounding fat and connective tissue and weighed to the nearest mg. The organs from one side of each animal were fixed in Bouin’s fluid and processed for histological studies. The diameter of testis and seminiferous tubule was made from randomly chosen 20 sections from each group appearing round at cross section by using ocular and stage micrometer. The spermatogenic elements like spermatogonia, spermatocytes and spermatids were counted. The sperm count from cauda epididymis done by using haemocytometer (Kempinas & Lamano-Carvalho, 1987).

Biochemical studies. Organs (testis and epididymis) from other side were processed for biochemical estimations like protein (Lowry et al., 1951), glycogen (Caroll et al., 1956), cholesterol (Peters & Vanslyke, 1946), acid phosphatase and alkaline phosphatase (Bessy et al., 1946).

Hematology studies. Total Red blood corpuscles (RBC) and white blood corpuscles (WBC) were assessed by cell counter method (haemocytometer) and hemoglobin content is assessed by using haemocytometer in control and extract treated animals.

Statistical analysis. All the values were statistically analysed and means of different groups were compared using Student’s t-test (Snedecor & Cochran, 1967). The values were judged as significant at P<0.01 and P<0.001.

RESULTS

The results of phytochemical screening of three extracts are shown in Table I. The petroleum ether extract showed positive tests for steroids, oils and fats. The chloroform extract showed the positive tests for steroids, alkaloids, oil and fats. The ethanol extract showed positive tests for alkaloids, flavonoids, glycosides, phenols, tannins, oils and fats.

Means of the initial (before treatment) and final (after treatment) body weights as well as the percentages of body...
weight changes relatively to the initial body weights of control and extracts treated mice are presented in Table II. The control, both the doses of petroleum ether and ethanol extract treated animals gained weights at the end of the experiment and appeared to be non-toxic as observed by survival outcome. There is no change in morphological and behavioral pattern. Whereas, both the doses of chloroform extract treated animals showed comparable body weight loss as compared to control ones and appeared to be presence of toxic compounds in the extract.

Petroleum ether, chloroform and ethanol extracts of M. charantia have decreased the weight, diameter of testis and also diameter of seminiferous tubule. The number of spermatogonia, spermatocytes, spermatids and cauda epididymal sperm count were decreased with administration of all the three extracts. However ethanol extract seems to be more potent as compared to petroleum ether and chloroform extracts (Table III, V). All the three extracts effectively increased the weight of cauda and caput epididymis, vas deferens, seminal vesicle and prostate gland (Table III).

Table I. Phytochemical analysis of different extracts of M. charantia seeds.

<table>
<thead>
<tr>
<th>Chemical Classes</th>
<th>Petroleum ether</th>
<th>Chloroform extract</th>
<th>Ethanol extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steroids</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Phenols and Tannins</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Oil and fats</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+ : Positive test, - : Negative test.

Table II. Body weight changes (mean ± S.E of 6 mice) after treatment of M. charantia seed extracts for 7 days in male mice.

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Dose (mg/100g body wt.)</th>
<th>Initial body weight (g)</th>
<th>Final body weight (g)</th>
<th>Percentage change</th>
<th>Survival, alive/ total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control -I</td>
<td>Tween– 80 (1%)</td>
<td>30.00±0.81</td>
<td>33.33±0.93</td>
<td>13.30</td>
<td>6/6 (100%)</td>
</tr>
<tr>
<td>Petroleum ether -II</td>
<td>25</td>
<td>30.03±0.70</td>
<td>33.20±0.82</td>
<td>9.57</td>
<td>6/6 (100%)</td>
</tr>
<tr>
<td>Chloroform -III</td>
<td>25</td>
<td>30.30±0.84</td>
<td>28.60±0.63</td>
<td>-5.61</td>
<td>6/6 (100%)</td>
</tr>
<tr>
<td>Ethanol -IV</td>
<td>25</td>
<td>31.50±0.80</td>
<td>34.00±0.84</td>
<td>7.93</td>
<td>6/6 (100%)</td>
</tr>
</tbody>
</table>

Table III. Weight changes (mean ± S.E of 6 mice) in testis and accessory reproductive organs of mice treated with extracts of M. charantia seeds (25mg/100g body weight for 48 days).

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Testis</th>
<th>Caput epidymis</th>
<th>Cauda epidymis</th>
<th>vas deferens</th>
<th>Seminal Vesicle</th>
<th>Prostate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control -I</td>
<td>719.10±1.28</td>
<td>142.90±0.45</td>
<td>143.73±1.26</td>
<td>94.33±1.19</td>
<td>594.02±1.01</td>
<td>80.54±1.25</td>
</tr>
<tr>
<td>Petroleum ether -II</td>
<td>709.56±1.92</td>
<td>148.00±1.70</td>
<td>145.33±1.72</td>
<td>101.33±1.52</td>
<td>598.67±1.80</td>
<td>116.67±1.21</td>
</tr>
<tr>
<td>Chloroform -III</td>
<td>671.41±1.12*</td>
<td>189.71±1.26*</td>
<td>152.17±1.32*</td>
<td>97.51±1.17</td>
<td>615.80±2.16*</td>
<td>82.35±2.14*</td>
</tr>
<tr>
<td>Ethanol -IV</td>
<td>625.87±1.60**</td>
<td>236.04±2.78**</td>
<td>209.00±1.25**</td>
<td>116.00±1.26**</td>
<td>649.26±1.23**</td>
<td>134.87±1.3**</td>
</tr>
</tbody>
</table>

*P < 0.01, **P < 0.001, when compared to control.
Decrease in protein, glycogen and acid phosphatase content but increase in cholesterol and alkaline phosphatase of the testis is observed (Table IV). The biochemical contents like protein, glycogen and cholesterol of caput and cauda epididymis show significant increase (Table VI).

There is no appreciable change in red blood corpuscles, white blood corpuscles and hemoglobin content is found in petroleum ether and ethanol extract administered animals when compared to control. In chloroform extract administered animal showed decrease in hemoglobin content observed.

DISCUSSION

The studies on a few plant extracts like Carica papaya (Lohiya et al., 2000, 2008), Morinda lucida (Raji et al., 2005), Colebrookia oppositifolia (Gupta et al., 2001), Melia azedarach (Sharanabasappa et al., 2003), Crotolaria juncea (Vijayakumar et al., 2004) have shown antispermatogenic effects in male rats and mice.

In the present study petroleum ether, chloroform and ethanol extracts of M. charantia have reduced the weight of
indicates inhibited synthesis of testosterone a potent androgen, cholesterol in the testis of treated rats in the present study after the treatment of Hall, 1968). Reduced glycogen content of the testis observed which is an energy supplier to the tubular cells (Means & spermatogonia in testis, serves as a source of glucose, androgenic in nature. Therefore, it can be stated that though the extracts of M. charantia are antispermaticogenic and they are potent androgenic in nature.

In conclusion, the antispermaticogenic effects of M. charantia seeds seem to be mediated by disturbances in testicular somatic cell functions (Leydig and Sertoli cells) involved in the histophysiological events of spermatogenesis.

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RESUMEN: En un intento por descubrir la molécula de anticoncepción masculina de origen vegetal, fueron probados los extractos de semillas de Momordica charantia en ratones machos. Extractos de éter de petróleo, cloroformo y etanol de Momordica charantia fueron administrados en dosis de 25mg/100g de peso corporal a ratones albinos de 48 días por vía intraperitoneal. Todos los extractos mostraron un efecto antiespermaticogenic, con reducción del número de espermatozonias, espermatocitos, espermatídicas y espermatozoides. El aumento de peso del epidídimo, próstata, vesículas seminales y conductos deferentes indica claramente la propiedad androgénica de estos extractos. Después de someter el extracto de etanol a la detección preliminar fitoquímica se observaron resultados positivos para alcaloides, flavonoides, glucósidos, fenoles, taninos, aceites y grasas. De los tres extractos probados, el extracto de etanol parece ser más potente en sus actividades de anticonceptivas y androgénicas.

PALABRAS CLAVE: Momordica charantia Linn; Toxicológico; Androgénicos; Testículo; Espermaticogénesis.
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