Original Research Article

Histomorphological Observations In Reproductive Organs Of Wistar Rats Administered With Aqueous Leaf Extract Of *Momordica Charantia*

ABSTRACT

Effort to explore the adverse effect of the plant, *Momordica charantia* on mammals has remained inadequate despite previous attempt by earlier investigators. More glaring is the paucity of information on the histomorphological effect of the plant's aqueous leaf extract hence the need to determine possible alteration of tissue structures in reproductive organs of adult Wistar rats which may affect their functions.

This study aimed at determining the effect of *Momordica charantia* aqueous leaf extract (MCALE) on the reproductive organs of experimental adult Wistar rats.

Materials and Methods: Twenty five rats (male and female) weighing 180-200g were randomly divided into five (5) groups of five rats each. The experimental groups, A to D were fed on standard diet and administered with 100, 200, 400 and 800 mg/kg body weight /day of MCE orally using gavage for 30 days. Rats in the control groups were fed on standard diet and physiological saline orally. Organs were harvested, fixed in 10% neutral buffered formalin (ovaries) and Bouin's fluid (testes), embedded in molten paraffin wax, sectioned with a rotary microtome and

stained with the haematoxylin and eosin technique. Stained slides were examined using the Olympus microscope.

Results

Sections of ovaries administered 100mg/kg of the extract showed vesicular spaces in corpus luteum and enlarged blood vessels. Sections treated with 200 mg/kg revealed follicular cyst and mild vacuolation of zona granulosa. Sections of the ovaries administered 400 mg/kg revealed degenerative changes, follicular cyst, mild vacuolation and reduction of zona granulosa layer while those treated with 800mg/kg showed severe vacuolation of the zona granulosa layer.

Conclusion: *Momordica charantia* caused histomorphological changes in ovaries of Wistar rats which could cause hormonal imbalance and infertility in females. No histomorphological changes were observed in male testes.

Keywords: Histopathology, *Momordica charantia*, ovaries, Wistar rats

1. INTRODUCTION

Demand for local herbs as alternative to orthodox medicine by consumers in both developed and developing countries have been on the increase in recent times. Pharmaceutical companies too have heightened their interest in prospecting for medicinal plants (1). Historically, the healthcare industry globally has benefited from medical plants as 25 percent of modern medicines are reported to be directly or indirectly derived from plants. Close to 80 percent of inhabitants in third world countries are claimed to depend on medicinal plants for treatment of diseases (3).

Due to the increasing frustration in multi resistance to conventional drugs, interest in herbal alternatives have been upbeat especially in third world countries in Africa and Asia (4,5). The demand for herbal condiments have therefore been on the increase in these countries and some developed ones following the entrance of pharmaceutical companies prospecting for medicinal plants (6). Bitter melon, *Momordica charantia* has gained popularity in alternative medicine in countries like India, Malaysia, China, Bangladesh, Nigeria and it is widely distributed in tropical Africa (7,8). The plant is climbing, monoecious, and botanically classified as family Cucurbitaceae (4, 9). The plant has been variously named as karela, wild cucumber, balsam pear and bitter cucumber. Locally in Nigeria, famous names are ejirin in Yoruba, daddasu in Hausa, okwunuolo in Igbo and Ugbebhe in Esa, Edo State (9, 10).

Common ailments parts of the plant were used for are, diabetes and fungal infection. It is also traditionally known and used as antitumor, anticancer, anti-inflammatory, antiviral, and cholesterol lowering agent (11).

Cultural differences have been noticed in the use of *Momordica charantia*. While it is used as sub-continental diet in Asian countries like China, Indonesia and India, it is mostly used as herbal condiment in Nigeria by alternative medicine practitioners (5, 12). The leaves in particular are used in folk medicine as antimalaria, antihelmithics, anti-diabetic, anti-inflammatory, anti-indigestion and anti-rheumatism (13).

Phytochemically, saponins, glycosides, proteins, alkaloids, fixed oils, triterpenes, steroids, carotenoids, monterpenes, carbohydrates benzanoids, alkene C3, sterol, alkanol C5 and sesquiterpenes has been reported to be present in the plant. Others

are ascorbigens, carotene, anthocyanins, flavonoids, luteolin, and quercetin. Flavonoids and triterpenoids in particular has been described as powerful antioxidants which could be useful in alleviating nutritional risk factors like obesity associated with atherosclerosis, hypertension and cardiovascular complications (2, 7, 8,14).

Previous studies emphasized the effect of seed and fruit extract of the plant (15, 16). The few who investigated on effect of aqueous leaf extract only considered biochemical parameters (17, 18). This study investigated the possible histomorphological changes in ovaries and testes of adult Wistar rats treated with aqueous leaf extract of *Momordica charantia* so as to enrich literature on the cellular and tissue toxicity effect of the plant when consumed for a long time.

2. MATERIAL AND METHODS

2.1 PLANT COLLECTION

Fresh leaves of *M. charantia* (Class, Dycotyledonnae; Order, Cucurbitales; Family, Cucurbitaceae) were harvested in a local garden at Eyenkorin area in Ilorin, Kwara State and sent to the Department of Biological Science, University of Ilorin for identification with reference number, UILH/001/963/2020 and dried in open air away from sunlight for one week for phytochemical content preservation.

2.1.1 PLANT EXTRACTION

Two hundred and fifty grams (250g) of the dried leaves of *M. charantia* were soaked in 500 ml of distilled water in a corked flask for 48 hrs at room temperature with

intermittent shaking. The mixture was filtered, evaporated over water bath and kept inside hot air oven until a semi solid substance was obtained.

2.2 EXPERIMENTAL ANIMALS

A total of 25 Wistar rats (Class, Mammalia; Order, Rodentia; Family, Muridae) (mixed male and female) weighing between 180-200g with 3 female in each group were used in this study. The rats were allowed to acclimatize for 2 weeks where feed and water were administered without restrictions. The protocol for this study was approved by the Ministry of Agriculture and Natural Resources, Benin City, Edo State with reference number V.1041/55

2.2.1 PREPARATION AND ADMINISTRATION OF EXTRACT

The extract of *Momordica charantia* was weighed by using a digital balance which measured in grams. The weighed extract was transferred to well labeled, small sized plastic bottles. The bottles were labeled according to the predetermined dosage of *Momordica charantia*. The corresponding weights were placed in each bottle with matching labels and reconstituted in 1ml of distilled water. The rats were subsequently administered with the extract of *M. charantia* through oral gavage while giving them their normal feed and drinking water ad libitum.

2.2.2 EXPERIMENTAL DESIGN

Control group rats were fed with 1 ml of normal saline daily through oral gavage for 30 days.

Group A were given 100 mg/kg body weight of aqueous extract of *Momordia charantia* for 30 days. **Group B** rats were given 200 mg/kg body weight of aqueous extract of *Momordia charantia* for 30 days. **Group C** rats were given 400 mg/kg

body weight of aqueous extract of *Momordia charantia* for 30 days. **Group D rats** were given 800 mg/kg body weight of aqueous extract of *Momordia charantia* for 30 days. All the rats were sacrificed at the end of 30 days.

The animals were anaesthesized with chloroform and subsequently sacrificed. The ovaries and testes were harvested and immediately fixed in 10% Neutral Buffered Formalin (NBF) and Bouin's fluid respectively for 24 hrs.

2.3 TISSUE PROCESSING

Fixed tissues were processed in an automatic tissue processor machine (Leica, 2000), Frankfurt, Germany) and dehydrated in ascending grades of alcohol, cleared in xylene and impregnated in molten paraffin wax. Processed tissues were embedded in fresh molten paraffin wax and allowed to set. Trimmed tissue blocks were sectioned at 5µ and dried on a hotplate for 30min. Dried sections were taken to water and stained in Harris' haematoxylin and 1% aqueous eosin to demonstrate general tissue structure. Stained slides were dehydrated in ascending grades of alcohol, cleared in xylene and mounted in DPX. Sections were examined microscopically using x10 and x40 objective lenses.

2.3.1 HAEMATOXYLIN AND EOSIN STAINING TECHNIQUE

Sections of testes and ovaries were taken to water, hydrated in descending grades of alcohol and water. Hydrated sections were stained in Mayer's haematoxylin for 10 min, rinsed in water and differentiated in 1% acid alcohol. Sections were rinsed in water, allowed to blue for 10 min and counterstained in 1% aqueous eosin for 2 min. Stained sections were rinsed in water, dehydrated in ascending grades of

alcohol, cleared in xylene and mounted in Dibutylphthalate Polystrene Xylene (DPX) (19). Stained sections were examined for histopathological changes using Olympus Microscope.

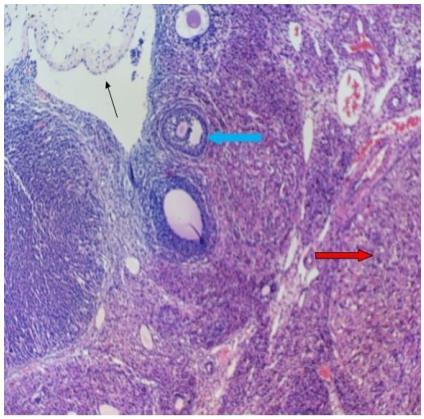
3. RESULTS

3.1OVARIES

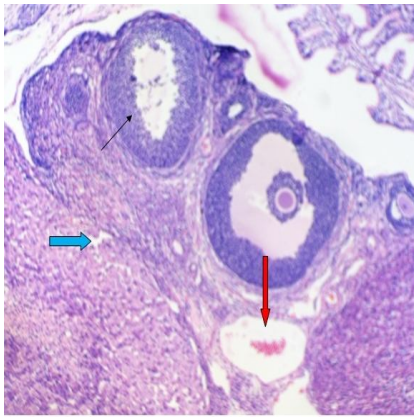
Sections of the ovary from Control group showed numerous follicles at various stages of maturation and corpus luteum dispersed in the peripheral cortical areas. The ovarian stroma is composed of spindle-shaped, fibroblast-like cells and delicate collagen fibres admixed with ground substance. The ovarian bursa is distinct with a wide space separating it from the cortical section. Blood vessels are seen anastomosing the luteal cells. The luteal cells are eosinophilic with large nuclei and moderate cytoplasm (Figure 1).

Sections of ovary of rats administered with 100 mg/kg body weight of *Momordica charantia* extract showed secondary follicles suspended in a dense connective tissue stroma, composed mainly of collagen connective tissues. The ovarian stroma surrounding a mature Graafian follicle is composed of spindle –shaped, fibroblast-like cells and delicate collagen fibres admixed with ground substance. The oocyte of the Graafian follicle was seen detached from the zona granulosa by a thick layer of cumulus oophorus but closely knitted by the corona radiata. The zona pellucida was clear and distinct while the follicular antrum was extensive and amorphous. The ovarian corpus luteum seems mature with eosinophilic cytoplasm and large nuclei with numerous vesicular spaces between the luteal cells. A wide inter-lutea oedematous blood vessel with few red blood cells was also visible (Figure 1).

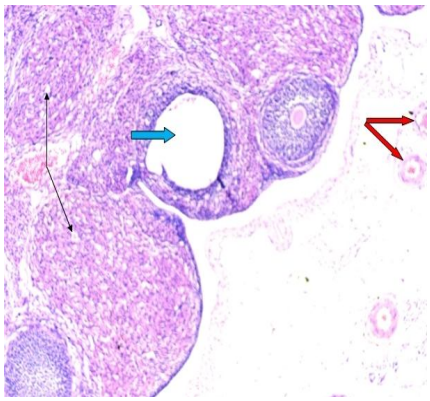
Sections of ovary of rats administered 200 mg/kg body weight of *Momordica charantia* extract revealed many corpus luteum with vesicular foci. One of the follicules seen presented with a large cystic space. Arteries with thick tunica intima and few red blood cells were identified in the inter-lutea borders (Figure 1). Sections of ovary of rats given 400 mg/kg body weight of *Momordica charantia* extract revealed corpus luteum with some degenerative changes. Follicles presented with cystic spaces and a occlusive zona granulosa with mild vacuolation (Figure 1). Sections of ovary of rats administered 800 mg/kg body weight of *Momordica charantia* extract revealed corpus luteum with some degenerative changes (Fig. 1).



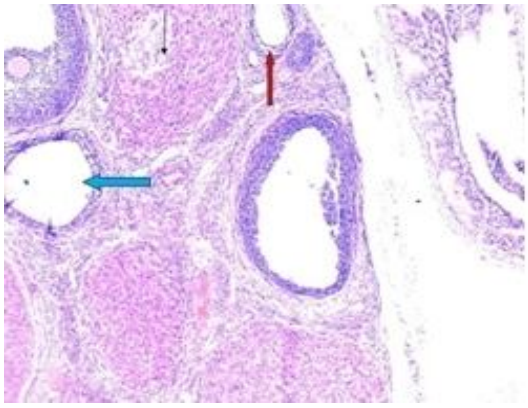
Control: Section of rat ovary showing normal Graafian follicle (blue arrow), Corpus luteum (red arrow) and ovarian bursa (black arrow). H&E. Mag x400



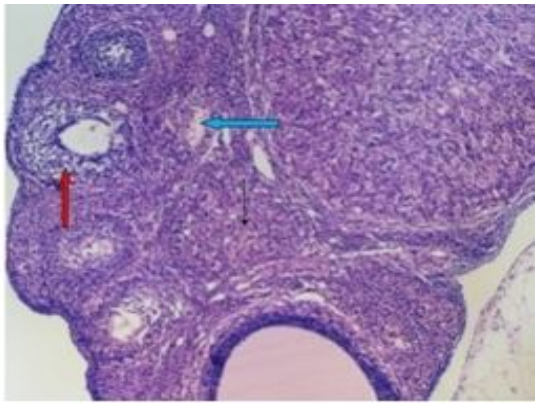
100mg/kg: Section of rat ovary showing Corpus luteum vesicular space (blue arrow) and enlarged blood vessel (red arrow). H&E Mag x400



200mg/kg: Section of rat ovary showing Corpus luteum with vesicular spaces (black arrow), follicular cyst (blue arrow) and thickened blood vessels (red arrow). H&E Mag x400

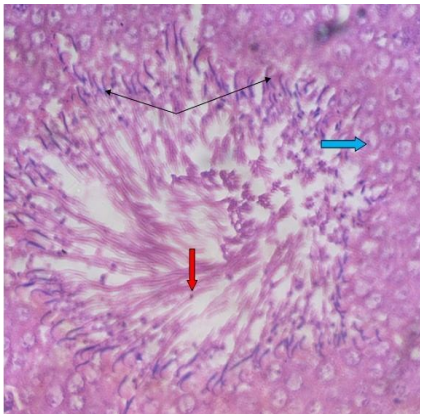


400mg/kg: Section of rat ovary showing degenerated corpus luteum (black arrow), follicular cyst (blue arrow) and mild vacuoulization of zona granulosa (red arrow). H&E. Mag x400

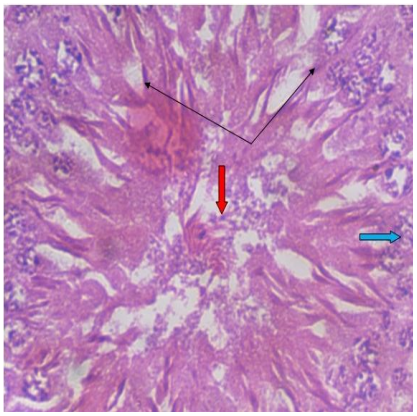


800mg/kg: Section of rat ovary showing degenerating corpus luteum (black arrow), few rbc (blue arrow) and severe vacuolization of zona granulosa (red arrow). H&E. Mag x400

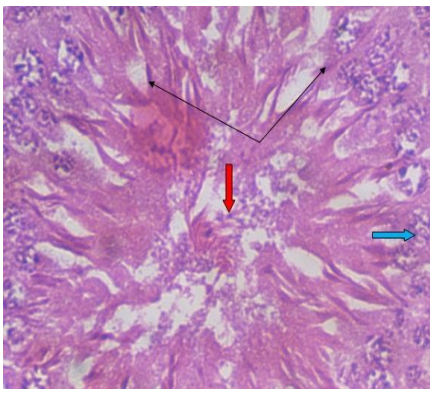
Figure 1 : Sections of rat ovaries for control group, and treatment at 100, 200mg/kg, 400mg/kg, and 800mg/kg body weight/day of the aqueous MCE respectively for 30 days.



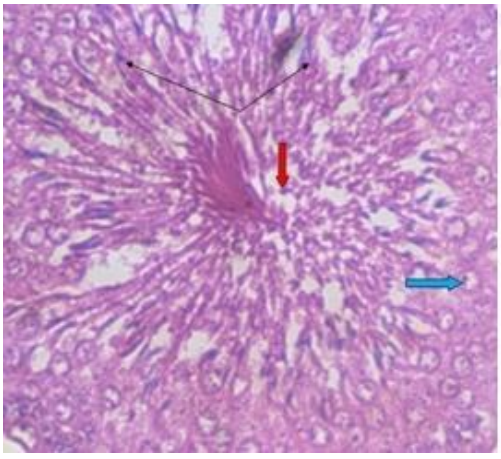
Control Group: section of testis showing secondary spermatocytes (Blue arrow), elongated spermatids (Black arrow) and spermatozoon (Red arrow). H&E: Mag x400
H &E (Mag. X 400)



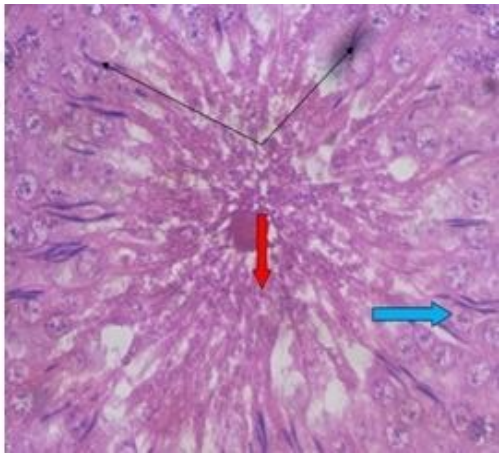
100mg/kg: section of rat testis showing active spermatocytes (blue arrow), elongated spermatids (black arrow) and mature spermatozoa (red arrow). H&E. Mag x 400.



200mg/kg: Section of rat testis showing active primary spermatocytes (blue arrow), elongated spermatids (black arrow) and mature spermatozoa (red arrow). H&E Mag x400



400mg/kg: Section of rat testis showing active primary spermatocytes (blue arrow), elongated spermatids (black arrow) and mature spermatozoa (red arrow) H&E. Mag x400



800mg/kg: Section of rat testis showing primary spermatocytes (blue arrow), elongated spermatids (black arrow) and mature spermatozoa (red arrow) H&E. Mag x400

Figure 2 : Sections of rat testes for control group, and treatment at 100, 200mg/kg, 400mg/kg, and 800mg/kg body weight/day of the aqueous MCE respectively for 30 days.

3.2 TESTES

Sections of testes from control rats showed testicular parenchyma with numerous seminiferous tubules bound by thin layer of basement membrane. Transverse sections of seminiferous tubules from control rats showed different phases of spermatogenesis. Sertoli cells lucked between primary spermatocytes were seen supporting germinal cells. Secondary spermatocytes were seen congregating at the center and forming majority of the stromal cells. The cells were lying in discrete layers and had large nuclei with coarse chromatin, peripheral nucleoli, some nuclear vacuoles and poorly stained cytoplasm. Also seen were elongated spermatids and small, mature spermatozoa (Fig. 2).

Sections of testis of rats administered 100 mg/kg body weight of *Momordica charantia* extract exhibited germ cells in various phases of spermatogenesis. The seminiferous tubules are well preserved in a clean stroma background. Primary spermatocytes were seen arising from the basal layer with some sertoli cells. Also seen were several secondary spermatocytes in various phases of development. Elongated spermatids and mature spermatozoa were visible. Sections of testes appeared normal when compared with the control (Fig. 2).

Sections of testes of rats administered 200 mg/kg body weight of *Momordica charantia* extract revealed germ cells in various phases of spermatogenesis. The testicular capsule was intact and the seminiferous tubules well preserved. There was evidence of progressive development of germinal cells from the basal layer to primary and secondary spermatocytes with prominent nuclei and eosinophilic cytoplasm. The numerous secondary spermatocytes in various stages of development, spindle shaped spermatids and mature spermatozoa were visible. The morphological presentation observed to be consistent with normal histomorphology of the testis when compared with the control (Fig. 2).

Sections of testes of rats administered 400 mg/kg body weight of *Momordica charantia* extract showed germ cells in various phases of spermatogenesis. The cells were seen lying in discrete layers with large, round, oval nuclei with condensed chromatin, peripheral nucleoli, nuclear vacuoles and poorly stained cytoplasm. Features of testes appeared normal (Fig. 2).

Sections of testes of rats administered 800 mg/kg body weight of *Momordica charantia* extract showed spematogonia in various stages of spermatogenesis. The seminiferous tubules were intact and well separated with thin, inter-seminiferous tubule basement membrane. The cells were observed to be well preserved with clear nuclear and cytoplasm differentiation. The nuclei stained purplish-blue and round to oval with condensed chromatin. Spindle shaped spermatids and dot-like spermatozoa were also seen. Features of testes appeared normal when compared with control (Fig. 2).

3.3. DISCUSSION

Observations noticed in this study showed that the oral administration of *Momordica charantia* leaf extract triggered alterations in the histomorphology of the female reproductive organ. Such changes has been attributed to hormonal imbalance especially levels of follicle stimulating hormone, FSH (9, 17, 20).

The activities of this hormone have been reported to depend on the hypothalamic-pituitary-ovarian axis integrity and that of the hypothalamus-pituitary-gonadal axis through the positive and negative feedback loops and products (21, 22, 23).

Hormonal imbalance could be due to elevated estradiol value which could be caused by stress, low fiber, nutrients deficiencies, high toxic burden, gut dysbiosis, elevated beta-glucuronidase, constipation, and methylation gene variants (24). It could also be due to high level of progresterone and prolactin (25, 26).

High levels of progesterone have been implicated in pregnancy, ovarian cyst, molar pregnancy, ovarian cancer or adrenal glands disorder (25) while high level of prolactin has been attributed to pituitary tumour, hypothyroidism, and disease of the hypothalamus (26).

Sections of ovaries of Wistar rats at the dose of 200 mg/kg body weight of Momordica charantia extract (MCE) revealed follicular cyst and arteries with thick tunica media. Such changes may be due to the high plasma levels of follicle stimulating hormone (FSH) and progesterone (22).

Follicle stimulating hormone (FSH) is produced by the gonadotropin releasing hormone (GnRH) from the hypothalamus-pituitary axis which in turn stimulates the granulosa cells of the ovaries to secret progesterone (8, 22).

The follicular cyst observed histologically in this study could have been induced by the phytochemical constituents in *Momordica charantia* especially, steroids. Activities of steroids have been implicated in the developmental status of the ovarian follicles (6, 27).

Ovarian sections of Wistar rats administered with 400 and 800 mg/kg body weight of MCE showed follicular cyst, mild vacuolation and severe vacuolation of granulosa cells layer, disorientation of interstitial cells, obliteration of theca cells and disorganization of the follicular morphology respectively. This is a more severe effect that could have resulted from high levels of FSH and progesterone (8, 21, 22).

Dosage of 100, 200, 400 and 800 mg/kg body weight of *M. charantia* leaf extract showed no negative histomorphological effect on the testes of the Wistar rats. This is an opposite effect on what was observed for female animals.

The transition from spermatocytes to fully mature spermatozoon was thorough and steady as observed in this study. The spermatozoa were also well formed and healthy. The histological virility of the spermatozoa suggests ability to boost libido by the administered extract.

An estimation of testosterone levels in the male animals could be a complementing investigation to reinforce this observation of ours as phytochemical substances in plants has been credited with aphrodisiac capability hence encouraging consumption in males (28, 29, 30). The testis is the male reproductive organ responsible for sperm cell production and the secretion of testosterone (31). Testosterone is secreted by Leydig cells of the testis and the stimulus for its production depends on the blood level of luteinizing hormone (LH) from the pituitary (32). The testis is also where spermatogenesis occurs and this function depends on endocrine control by the pituitary-testicular axis in concert with complex autocrine and paracrine interactions with Sertoli cells, germ cells, leydig cells, endothelial cells and testicular macrophages (33). Both the hypothalamus and pituitary controls the endocrine functions of the testis by the release of FSH and LH. Luteinizing hormone when released acts on Leydig cells to stimulate testosterone synthesis while FSH acts on Sertoli cells to regulate spermatogenesis (34). The libido effect of the plant's leaf extract should be subject to further studies which if well established could be a pharmacopeutical possibility for boosting male sexual performance.

4. CONCLUSION

Momordica charantia extract altered the histomorphological architecture of the ovaries in female Wistar rats which could lead to infertility and malfunctions while there was no alteration in the histomorphological architecture of the testes of the male animals. We suggest that profiling for *Momordica charantia* consumption should be part of clinical history review in females with problems of infertility especially in areas where there is abuse of consumption of the plant.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

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