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Momordica charantia L. extracts restores ovarian function in Estradiol-induced polycystic ovarian syndrome in rats

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Abstract: **Background**: This study investigated the therapeutic potential of aqueous fruit and seed extract of *Momordica charantia* L. (MC) as an alternative treatment for PCOS. **Objectives**: Evaluations included ovarian histopathology, fasting blood sugar, lipid profiles, antioxidant markers and hormone levels. **Methods**: Female Wistar rats induced with PCOS via a single 4.5 mg/kg dose of estradiol valerate were treated with MC extracts at 500 and 1000 mg/kg/day for 60 days. **Results**: The results showed that treatment with MC fruit and seed extract at the dose of 500 and 1000 mg/kg/day possesses potent anti-inflammatory and antioxidant activity in in-vitro models. MC fruit and seed extract showed no cytotoxicity in HeLa cell viability in the MTT assay and the highest antioxidant activity followed by DPPH compared with ascorbic acid ($p < 0.01$). The fasting blood sugar serum levels, total lipid profile, LH, FSH, estradiol and antioxidant enzyme levels were significantly restored after 60 days of treatment ($p < 0.01$). Further, MC extract significantly reduced body weight ($p < 0.01$) and ovarian weight ($p < 0.05$), restored the normal estrous cycle, resolved cysts in the ovaries and displayed positive effects on ovarian histopathology after 60 days of treatment. **Conclusion**: MC fruit and seed extracts is considered a potential treatment for PCOS in estradiol-induced female rats.

Keywords: Antioxidant; Bitter gourd; Estrous cycle; Hormone; Metformin

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INTRODUCTION

Polycystic ovary syndrome (PCOS) has been established as a major global health issue for women present with a wide range of symptoms in the reproductive, metabolic and psychological domains. This includes abnormal menstrual cycles, elevated levels of androgens and polycystic ovaries, which cause fertility problems, obesity and high risks of type 2 diabetes and cardiovascular diseases as stated by Sun and his team (Sun *et al*., 2019). PCOS affects women of reproductive age frequenting clinics with a prevalence of between 6% and 15 % depending on the diagnostic criteria used (Zhang *et al*., 2019).

With the change in perception of PCOS, the focus shifts towards finding different treatment options for this condition, especially those from natural sources. Of these, the therapeutic interest in *Momordica charantia* L. (Bitter gourd) has come up as one of the most promising approaches to coping with the complex issues associated with PCOS (Shukla and Kashaw, 2018).

Momordica charantia L. (MC) commonly known as bitter gourd has been used in folk medicine of Asian region for quite some time. The fruit and seeds act as sources of bioactive compounds, flavonoids, alkaloids and phenolic acids which have been recognized to possess antioxidant, anti-inflammatory agent together with hypoglycemic effects (Vinav *et al*., 2016). Findings from the previous studies also show that extract from *Momordica. charantia* L has favorable impact on lipid concentrations, insulin sensitivity and exhibits significant antioxidant and anti-inflammatory properties and therefore, could be useful in the management of diseases such as PCOS which is characterized by oxidative stress and metabolic dysfunction (Santomauro *et al*., 2021).

Additionally, it may have a significant anti-inflammatory effect which might be useful when treating the chronic inflammation, given that inflammation plays a role in one of the PCOS phenotypic changes (Desai *et al*., 2014). Wang and Ryu it has been suggested that compounds found in *Momordica charantia* L extracts might improve lipid profiles and lipid metabolism in the animal models of PCOs and hence this herb might equally be recommended for use in the management of metabolic conditions associated with PCOS (Wang and Ryu, 2015).

The current experiment was a preclinical experimental research to assess the therapeutic potential of *Momordica charantia* L. (bitter gourd) fruit and seed in a rat model of PCOS. This model was considered more suitable because it exhibits the hormonal dysfunctions and metabolic alterations similar to humans with PCOS, therefore it is ideal to analyze the therapeutic effects of natural compounds (Linares *et al*., 2013). The extracts' effects were determined through integrated *in-vitro* and *in-vivo*

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analyses, which included assessing antioxidant and anti-inflammatory activity *in vitro*, and evaluating hormonal, metabolic, and histopathological restoration *in vivo*. Such dual approach led to better understanding of the mechanisms of the possible therapeutic effects of the extracts and therefore it can be considered as a solid base for investigation of the efficiency of the preparations in the treatment of PCOS.

MATERIALS AND METHODS

Plant material and extraction

Bitter melon fruits and seeds (*Momordica charantia* L.) were identified, and a voucher specimen (Number MC-05-20) was deposited at the Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences. Five kilograms of fresh bitter melon fruits were purchased from a local market, washed and freeze-dried. The fruits and seeds were separated, resulting in 13.5 grams of fruit powder and 8.4 grams of seed powder per 100 grams of raw bitter melon. Aqueous extracts were prepared by dissolving 2 grams of fruit or seed powder separately in 20 ml of distilled water. These extracts were prepared fresh daily for sixty days. A preliminary phytochemical screening was conducted to identify the active phytonutrients in the fruit and seed extracts of *Momordica charantia* L.

Preliminary phytochemical screening

The fruit and seed extracts of *Momordica charantia* L. were subjected to qualitative phytochemical screening based on standard procedures described by Evans (Evans, 2009). The extracts were dissolved in their respective solvents and tested for the presence of alkaloids, carbohydrates, steroids, saponins, tannins and flavonoids.

In-vitro testing

List of equipment and reagents used

- *Equipment*: Grinding machines (IKA®; Germany), 96-well microplates (Thermo Fisher Scientific; USA), hemocytometers, incubators (37°C, 5% CO2; Eppendorf; Germany), microplate readers (Spectra Max Plus; Molecular Devices, USA), luminometer (Berthold Technologies; Germany), glass slides, Coplin jars, microscopes (Olympus Corporation; Japan) and Zeiss (Germany).
- *Reagents*: Mayer's and Dragendorff's reagents, α-naphthol, concentrated sulfuric acid, Fehling's reagent A and B, acetic anhydride, ferric chloride, MTT, DMSO, HBSS++, luminol, SOZ (Thermo Fisher Scientific, USA), crystal violet stain and glycerol (Sigma-Aldrich, Merck KGaA, Germany). Distilled water, ethanol, fetal bovine serum, penicillin, streptomycin and minimum essential medium (Gibco, Thermo Fisher Scientific, USA).

Cytotoxicity analysis via MTT assay

The MTT assay was performed to assess cytotoxicity, as safety is a prerequisite for *in-vivo* PCOS studies (Kumar *et al*., 2010). MC's non-toxicity supports its therapeutic potential. The cytotoxic properties of the extracts were evaluated using the MTT assay. HeLa cells were cultured and treated with 30 µg/ml/µm concentrations of the extract for a 48-hour incubation period. Subsequently, MTT reagent was added to each well, followed by a 4-hour incubation to facilitate formazan crystal formation. These crystals were dissolved using DMSO and absorbance was measured at 570 nm with a microplate reader to assess the cytotoxic potential.

Anti-inflammatory activity assessment

PCOS is characterized by chronic inflammation and oxidative stress (Desai *et al*., 2014, Sun *et al*., 2019). The assays validated MC's ability to mitigate these pathways, supporting its mechanistic role in PCOS management. The anti-inflammatory properties of the extracts were analyzed using a luminol-enhanced chemiluminescence assay. Whole blood samples were incubated with different extract concentrations in 96-well white plates. The levels of reactive oxygen species (ROS) were quantified using a luminometer and the results were reported as relative light units (RLU), based on the procedure outlined by Hefland *et al*., 1982 (Helfand *et al*., 1982).

Antioxidant activity assessment

Antioxidant potential was determined through the DPPH radical scavenging assay. Methanolic DPPH solution was combined with the extract and incubated in the dark at 37°C for 30 minutes. Absorbance was recorded at 517 nm and the free radical scavenging activity was calculated using standard protocols (Sowndhararajan and Kang, 2013).

Total phenolic content analysis

The total phenolic content of the extract was quantified using a spectrophotometric method. The absorbance was measured at 750 nm and the total phenolic content was determined using a gallic acid standard curve (Phuyal *et al*., 2020).

In-vivo studies

Six female Albino Wistar rats, weighing 180-200 grams each, were housed under controlled conditions (26–28°C, 12h light/dark cycle) following the National Institute of Health's guidelines to minimize animal discomfort.

Assessment of anti-inflammatory activity

Using the carrageenan-induced paw edema assay, anti-inflammatory activity was evaluated. Twenty-eight rats were divided into seven groups, each consisting of four Wistar rats. A sub-plantar injection of 0.1 ml of a 1% carrageenan solution induced edema in the right hind paw of each rat except group I. Group I control rats were non-inflamed and received a sub-plantar injection of physiological saline (0.9%). Group II did not receive any drug and was termed as the negative control group. Group III-VI received the aqueous extract of M. charantia Linn fruit and seeds at 500mg/kg and 1000 mg/Kg (low dose and

high dose respectively), while the VII group of animals received 10 mg/kg indomethacin as the standard drug respectively. Paw thickness was measured before and after carrageenan injection and the percentage of edema inhibition was computed (Amdekar *et al*., 2012). The percentage inhibition of edema was calculated by using the following formula:

Inhibition (%) =Vc−Vt/Vc×100
where Vc represents the mean paw volume increase in the control group and Vt represents the mean paw volume increase in the treated group.

Experimental design

Induction of PCOS

Polycystic ovary syndrome (PCOS) was induced in female Wistar rats via a single intramuscular injection of estradiol valerate (4.5 mg/kg), following the method described by Venegas *et al*. The estrous cycle and body weight were monitored to confirm successful PCOS induction (Venegas *et al*., 2019).

The study involved a total of 42 animals, with 7 groups containing 6 animals in each group. Group I served as the control group, while groups II to VII were the test groups in which polycystic ovary syndrome (PCOS) was induced. The success of induction was confirmed by the monitoring of the estrous cycle and the body weights at regular intervals during the experimental period. In order to evaluate the effects of therapy on the reproductive health of the rat, we closely observed the estrous cycle in female rats. Estrous cycles were monitored by daily inspection of vaginal lavage as described by Bingel and Schwartz (Bingel, Schwartz, 1969). The procedure classified the phases of the cycle, namely Proestrus, Estrus, Metestrus and Diestrus, by analyzing the detected cell types. Estrous cycle length was defined as the number of days from one proestrous stage to another (U.S. Environmental Protection Agency, 2011). This approach enabled us to monitor hormonal state and detect alterations in reproductive phases of rat caused by the treatment being used in the form of our herbal drug, offering valuable information on the impact of this treatment on female reproductive health.

Treatment protocol

After 60 days of PCOS induction, group II served as the untreated PCOS negative control group, while groups IV to VII received different extracts of *Momordica charantia* L. treatments as follows: Group III received the standard drug metformin HCl (350mg/kg), group IV & V received the fruit extract at doses of 1000mg/kg and 500mg/kg (high dose and low dose respectively) while groups VI & VII received the seed extract at doses of 1000mg/kg & 500mg/kg (High dose and Low dose respectively) . The treatments were administered orally once per day for sixty days. The doses of *Momordica charantia* L. fruit and seed extracts were determined based on the results of *in-vitro* studies and previous literature reports (Husna *et al*., 2013).

Assessment of treatment effectiveness

On the 60th day of treatment, blood samples were collected through cardiac puncture under ketamine anesthesia (100 mg/kg) (Joksimovic Jovic *et al*., 2021). Blood glucose levels were measured using a glucometer (Zhang *et al*., 2019), while hormonal analysis (LH, FSH, estradiol, superoxide dismutase [SOD] and glutathione) was conducted using ELISA kits (Health *et al*., 2006, Hafiane and Genest, 2015, Mäkelä *et al*., 2020, Tan *et al*., 2018, Zhang *et al*., 2019). Additionally, lipid profile parameters were analyzed through enzymatic assays.

Histological examination

Ovarian histopathology followed established PCOS protocols (Venegas *et al*., 2019), with cyst resolution and stromal normalization serving as key endpoints. Ovaries were harvested, weighed and preserved in 10% formalin for histological analysis. Tissue samples were embedded in paraffin, sectioned at a thickness of 4 µm and stained with hematoxylin and eosin (H&E). Microscopic analysis at 40x magnification was performed to evaluate follicular development, including pre-antral, antral and atretic follicles, as well as the presence of corpus lutea.

Statistical analysis

Data were reported as the mean ± standard error of the mean (SEM). Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test, with a significance level of $p<0.05$. All statistical analyses were performed using SPSS version 26.

Δ Indicates a significant difference ($p < 0.05$) and ΔΔ represents a highly significant difference ($p < 0.01$) when compared to the negative control group.

RESULTS

Plant characterization

Preliminary phytochemical testing was conducted on the extracts of *Momordica charantia* Linn. fruit and seed extracts, revealing the presence of alkaloids, saponins, steroids, tannins and polyphenols; the results are presented in (Table 1).

MTT assay

The results of the MTT assay demonstrated no cytotoxic effect when treated with fruit extract at a concentration of 30µg/ml/µm, as compared to the standard drug doxorubicin at the same concentration, which significantly reduced cell viability (103.2% cell growth inhibition percentage) (Table 2) indicating that fruit extract is safe for use. The seed extract was insoluble so we were unable to retrieve the percentages.

Table 1: Preliminary phytochemical analysis of *Momordica charantia* L. fruit & seed extracts

Type of extract	Alkaloids	Carbohydrates	Steroids	Saponins	Tannins	Flavonoids
Fruit extract	Present (+)	Present (+)	Present (+)	Present (+)	Present (+)	Abundant (++)
Seed extract	Present (+)	Present (+)	Present (+)	Present (+)	Abundant (++)	Present (+)

Assessment of phytochemicals in the Fruit & Seed extracts of *Momordica charantia* L.

Table 2: MTT assay for *Momordica charantia* L. fruit and seed extracts

Product	Concentration (µg/ml/µm)	Percentage inhibition	Inhibitory concentration (IC_{50}) ± standard deviation
MC Extract (Fruit)	30	2.3%	Not active
MC Extract (Seed)	30	Insoluble	-
Doxorubicin (Reference drug)	30	103.2%	0.8±0.15

Cell viability of HeLa cells after exposure to the *Momordica charantia* L. fruit & seed extracts vs Doxorubicin at a concentration of 30 µg/ml/µm.

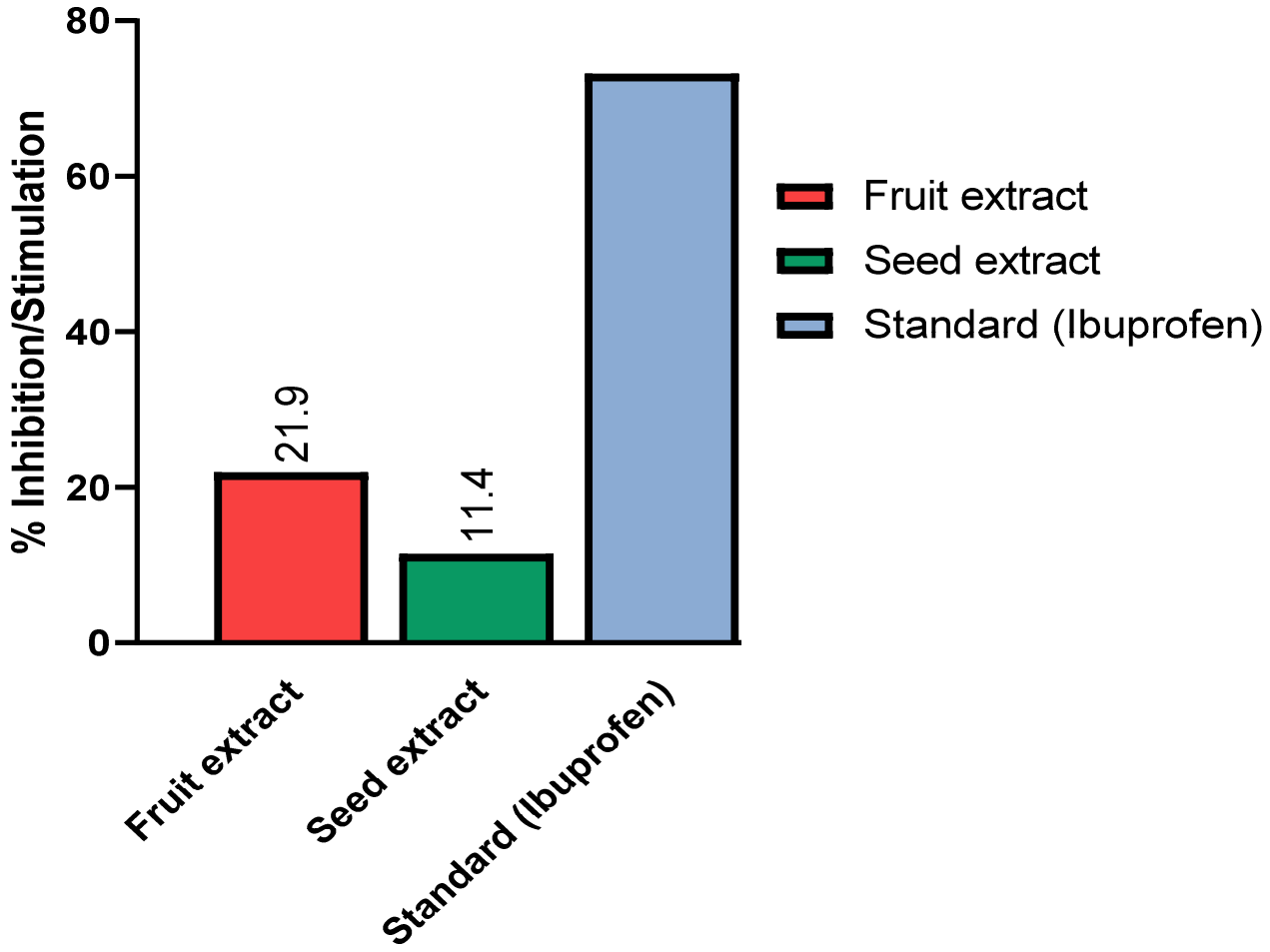

Fig. 1: Anti-inflammatory assay for *Momordica charantia* L. fruit and seed extracts
Comparative anti-inflammatory effects of 50 µg/ml *Momordica charantia* L. aqueous extract and 25 µg/ml ibuprofen

Anti-inflammatory assay

The luminol-enhanced chemiluminescence assay showed 21.9% and 11.4% inhibition for fruit and seed extracts (50µg/ml/µm), respectively, compared to the standard drug ibuprofen with 73.2±1.4% inhibition (25 µg/ml/µm) (Fig. 1).

Antioxidant assay

DPPH inhibition of fruit and seed extracts of bitter gourd was compared to the control ascorbic acid (Fig. 2). The concentration-dependent inhibition displayed a maximum of 83.6 ± 3.6% and 56.6 ± 1.43% for fruit and seed extracts, respectively, while ascorbic acid showed 98.21±2.15% inhibition at 200mg/ml.

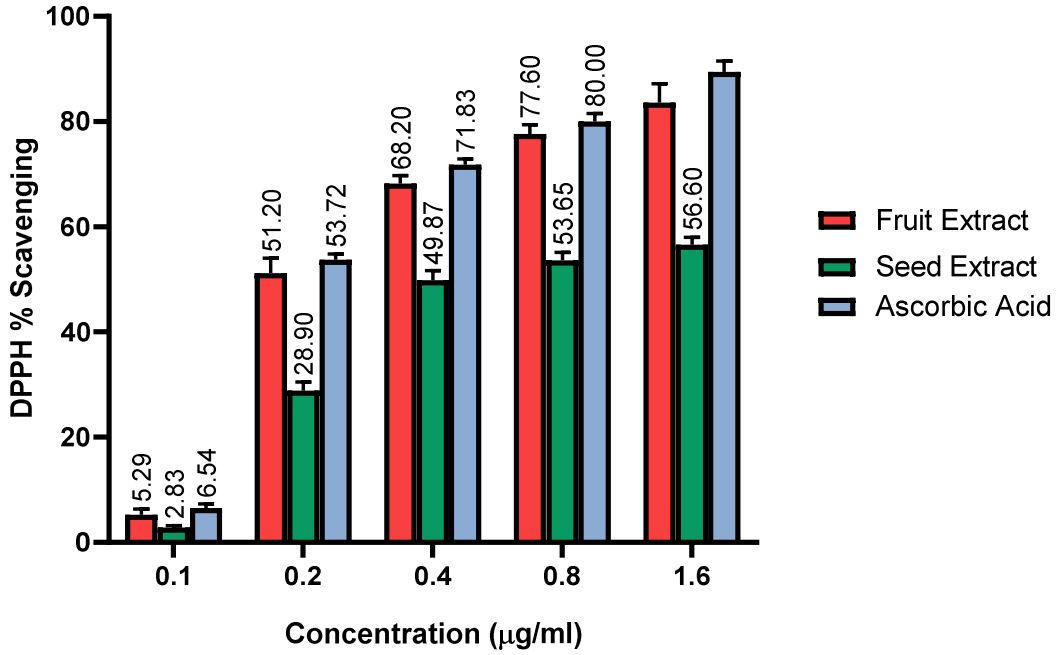

Fig. 2: Antioxidant activity of aqueous fruit & seed extracts of *Momordica charantia* L.
at concentrations of 0.1, 0.2, 0.4, 0.8 & 0.6µg/ml/µm as compared to Ascorbic acid standard drug at similar concentrations.

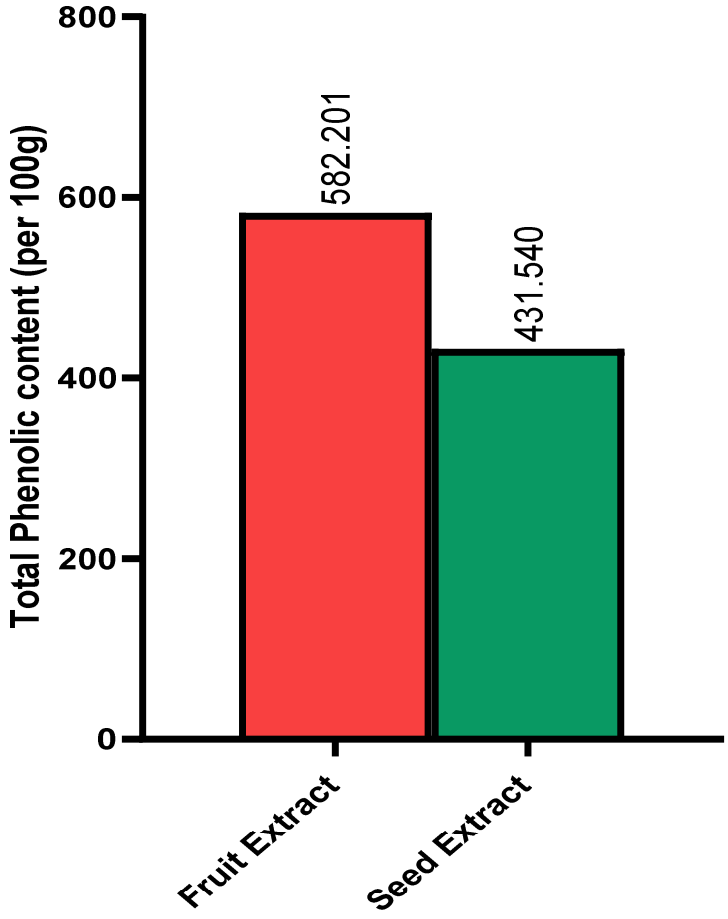

Fig. 3: Total phenolic contents (TPC) in 100-gram sample of each fruit & seed extracts of *Momordica charantia* L.

Total phenolic content

The Folin-Ciocalteu reagent was used to estimate total phenolic content, revealing that the fruit extract of *Momordica charantia* L. exhibited the highest total phenolic content (582.20mg/100g) among the two extracts (Fig. 3).

Impact on anti-inflammatory activity

In the carrageenan-induced paw edema test, the inhibition of paw edema was observed dose-dependently using MC fruit and seed extracts against the negative control group. The fruit extract at 1000mg/kg showed highly significant inhibition (p<0.01), but the 500mg/kg of fruit extract and both seed extracts decreased paw edema significantly at p< 0.05 level. One hour after the administration of indomethacin at 10 mg/kg, inhibition was highly significant, as shown in fig. 4.

Impact on body weight

The effects of MC fruit and seed extracts (500 mg/kg/day; 1000mg/kg/day) on body weight after 60 days of dosing are presented in figs. 5 (a) and 5 (b). The negative control group gained more weight than the control and treated groups. After 60 days of dosing, we observed a significant reduction (p< 0.01) in body weights at both low and high doses. The only difference observed at both doses was that the high-dose group exhibited a significant reduction in body weight after 30 days of dosing, while the low-dose group showed the same effect after 50 days of dosing.

Impact on rat estrous cycle

The estrous cycle in normal rats lasted an average of four to five days. In contrast, rats in Group II with PCOS demonstrated longer, regular, or irregular cycles. Rats that showed changes in phase sequence or stayed in the same phase for more than four to five days were deemed irregular (Fig. 6). After 60 days of treatment with high-dose MC fruit and seed extracts, the estrous cycles of the rats were restored to normal.

Impact on blood glucose

The effects of MC fruit and seed extracts (500 mg/kg/day; 1000mg/kg/day) on fasting blood glucose levels are presented in (Fig. 7). The negative control group significantly increased blood glucose levels compared to the control group. After 60 days of treatment, MC fruit and seed extracts at low and high doses exhibited a highly significant reduction (p< 0.01) in glucose levels compared with the negative control group. The metformin-treated group showed similar significant results to the MC fruit and seed high-dose groups.

Impact on hormonal parameters

The effects of MC fruit and seed extracts (500 mg/kg/day; 1000mg/kg/day) on LH, FSH and estradiol levels are shown in figs. 8 (a) and 8 (b). Elevated LH and estradiol, along with decreased FSH levels, were observed in a model of polycystic ovarian syndrome. *Momordica charantia* L. fruit and seed extracts at 1000mg/kg and 500mg/kg demonstrated highly significant effects (p<0.01) on LH, FSH and estradiol in all treated groups compared to the negative control group. The levels of LH and estradiol were decreased, while the levels of FSH were increased. The metformin standard group showed a decrease (p<0.01) in the levels of LH and estradiol, while the levels of FSH were increased (p<0.05) as compared to the negative control group.

Impact on biochemical parameters

The effects of MC fruit and seed extracts (500 mg/kg/day; 1000mg/kg/day) on the lipid profile are shown in (Table 3). Treatment with MC fruit and seed extracts at both low and high doses exhibited a significant drop (p <0.01) in total cholesterol (TC), triglycerides (TGs) and low-density lipoprotein (LDL) levels compared to the negative control group. The Metformin standard group also significantly reduced cholesterol, LDL and TGs (p <0.01) compared to the negative control group, similar to treated groups. Similarly, high density lipoprotein (HDL) levels were also prominently increased (p<0.01) after treatment with MC fruit seed extracts (1000mg/kg and 500mg/kg doses) versus the negative control group.

The effects of MC fruit and seed extracts on serum levels of SOD and glutathione are shown in (Table 4). In MC fruit and seed-treated groups, SOD levels were insignificant (p>0.05), while glutathione levels produced highly significant outcomes. Treatment with MC fruit and seed extracts at 1000 mg/kg doses showed promising outcomes in glutathione levels (p<0.01) when compared with the negative control group. Prominent results (p<0.05) were seen at a 500mg/kg dose versus the negative control. Likewise, the standard medicine metformin HCl group showed no effect on SOD levels; however, a significant increase in glutathione levels was seen (p<0.05), similar to the MC groups.

Impact on ovarian volume

Momordica charantia L. fruit and seed extracts impact ovarian volume, as shown in (Fig. 9). Treatment with MC fruit and seed extracts at 1000mg/kg doses showed a highly significant decrease (p<0.01) in ovarian volume compared to the negative control group and similar results were observed with the Standard Metformin group. In contrast, a 500mg/kg dose group demonstrated a significant reduction (p<0.05) in ovarian volume compared to the negative control group.

Impact on ovarian histopathology

Fig. 10 presents microscopic histopathological images of ovaries from adult female rats (HE × 40). Polycystic ovary sections of ovarian tissue show collagenized ovarian stroma. The standard drug metformin targets luteinized stroma. High-dose fruit (HDF) displays a section from ovarian tissue with luteinized stroma. High-dose seed (HDS) reveals ovarian tissue with luteinized stroma and no cysts.

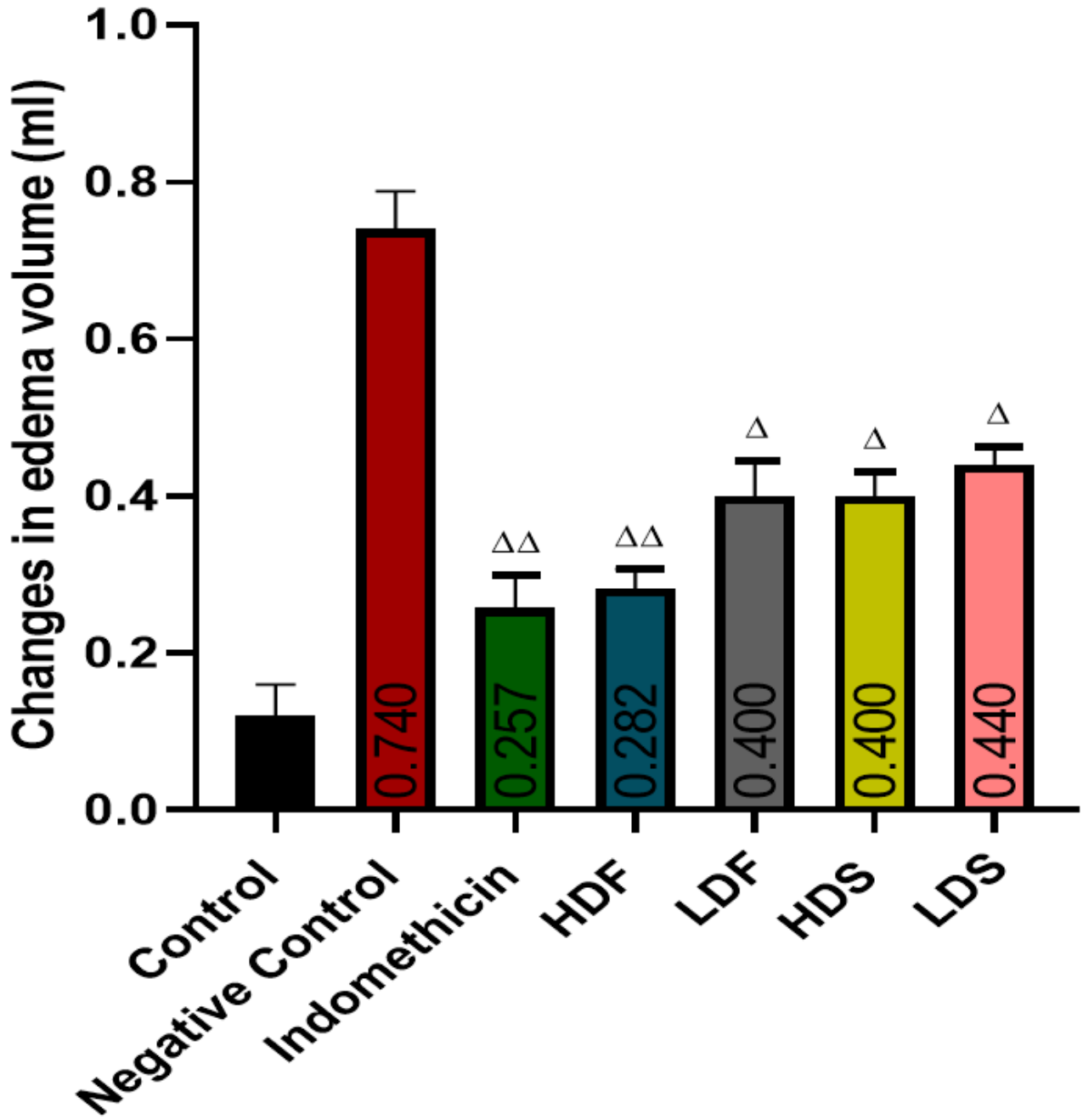

Fig. 4: Carrageenan induced anti-inflammatory activity of aqueous fruit & seed extracts of *Momordica charantia* L. at doses of 500mg/kg & 1000mg/kg as compared to indomethacin standard drug (10mg/kg) and 0.9% normal saline as the control group.

Table 3: Effect of MC fruit and seed extracts on lipid profile

	GROUPS								
	Control	NC (4.5mg/kg)	Standard (350mg/kg)	HDF (1000mg/kg)	LDF (500mg/kg)	HDS (1000mg/kg)	LDS (500mg/kg)	F Value	P Value
TC	82.3±0.31	140.5±2.04	96.0±0.62$^{\Delta\Delta}$	65.4±0.39$^{\Delta\Delta}$	76.5±1.57$^{\Delta\Delta}$	71.0±2.18$^{\Delta\Delta}$	72.7±1.53$^{\Delta\Delta}$	311.0	<0.01
TG	61.37±0.94	119.5±1.42	66.6±0.47$^{\Delta\Delta}$	61.11±0.19$^{\Delta\Delta}$	86.6±0.75$^{\Delta\Delta}$	60.3±0.27$^{\Delta\Delta}$	75.8±0.67$^{\Delta\Delta}$	903.3	<0.01
HDL	42.59±0.41	33.17±0.56	60.9±0.60$^{\Delta\Delta}$	57.6±0.30$^{\Delta\Delta}$	42.1±0.73$^{\Delta\Delta}$	57.2±0.40$^{\Delta\Delta}$	45.6±0.73$^{\Delta\Delta}$	245.4	<0.01
LDL	32.4±0.48	111.38±0.43	43.7±0.34$^{\Delta\Delta}$	35.03±0.12$^{\Delta\Delta}$	44.7±0.93$^{\Delta\Delta}$	34.0±0.62$^{\Delta\Delta}$	40.1±0.32$^{\Delta\Delta}$	2895.7	<0.01

The results are presented as each group's mean ± SD determinations (n = 6).

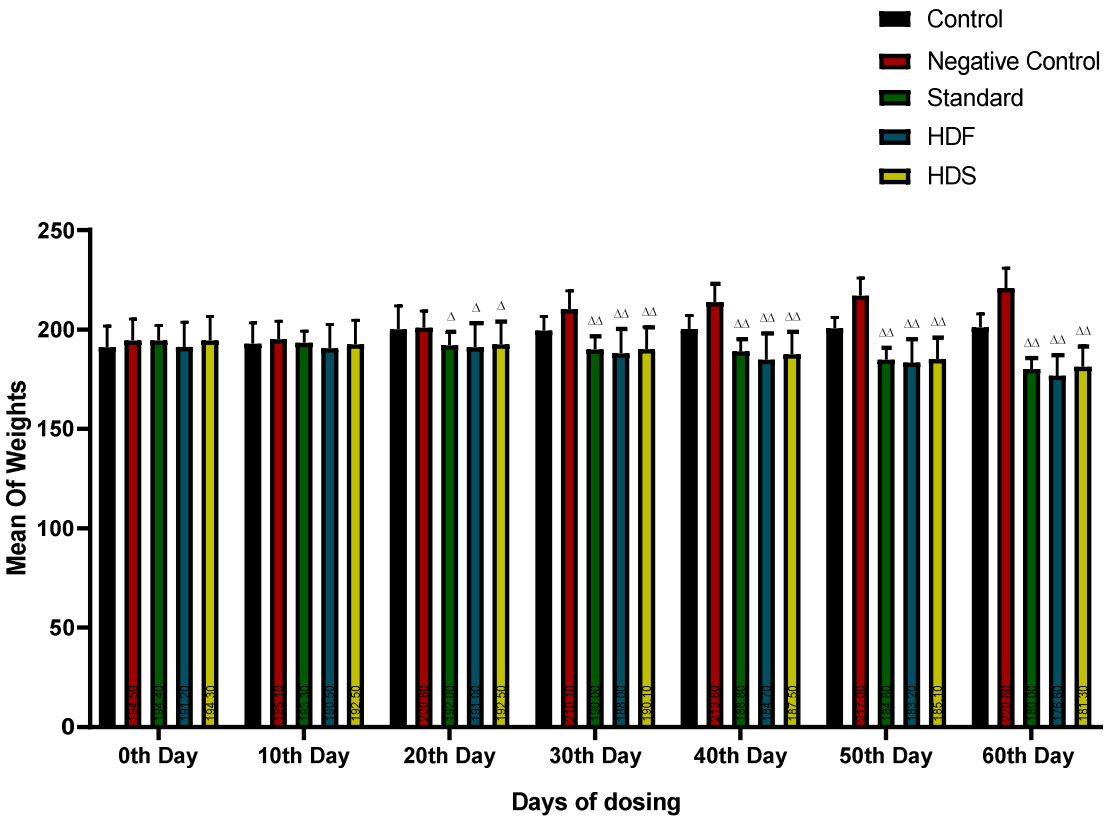

Fig. 5(a): Effect of high dose MC fruit and seed extracts on Body weight following 60 days of dosing. The results are presented as the mean ± SD determinations for each group (n = 6).

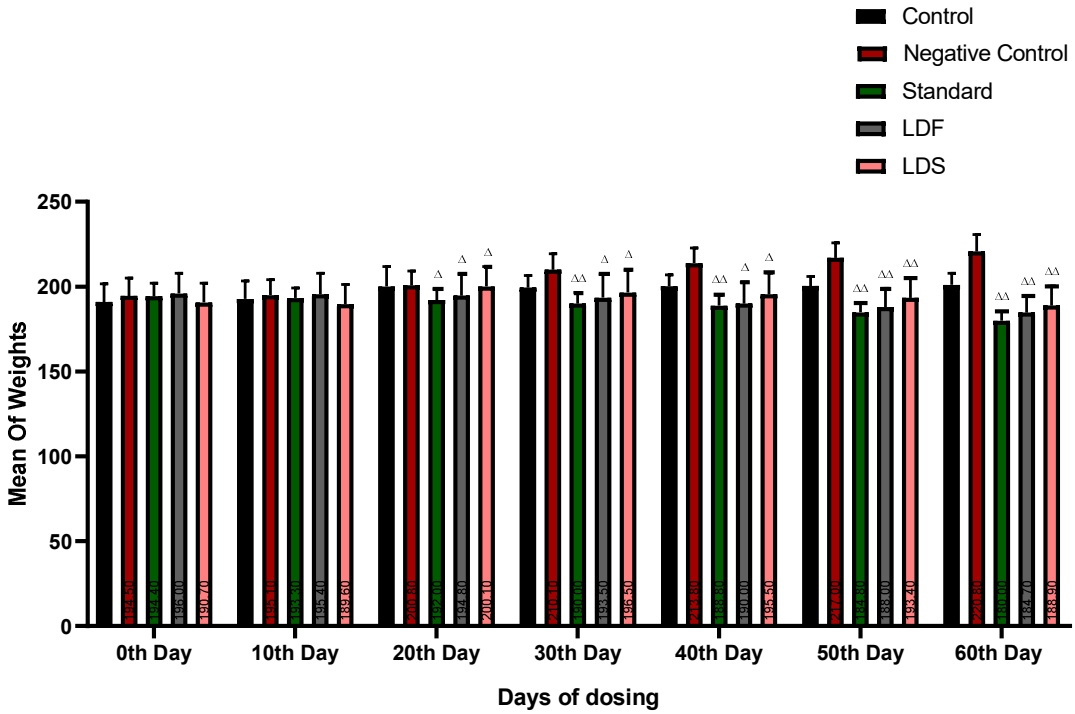

Fig. 5(b): Effect of low dose MC fruit and seed extracts on body weight following 60 days of dosing. The results are presented as the mean ± SD determinations for each group (n = 6).

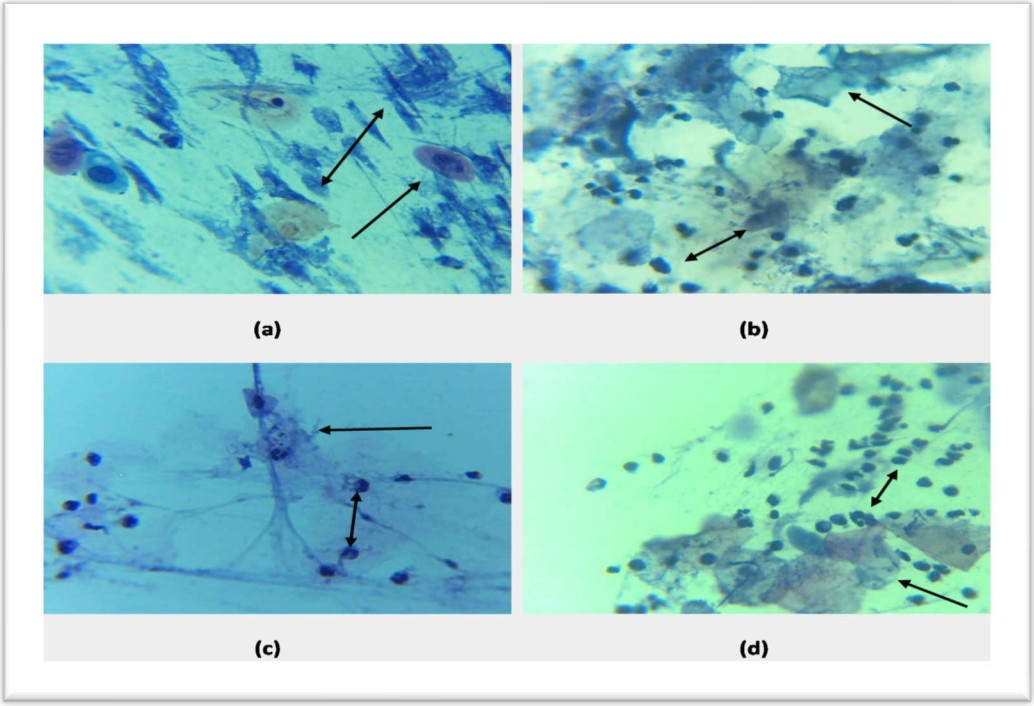

Fig. 6: Effect of MC fruit & seed extracts on regular estrous cycle of female albino Wistar rats

Fig. 6 illustrates cytological analysis of female adult rats' estrous cycle. Proestrus phase (a) displays cohesive clusters, sheets, strands (double arrowhead), and primarily round nucleated epithelial cells (arrow). Estrous phase (b) shows a section of the tissue having Anucleated keratinized epithelial cells (arrow) and sporadic nucleated epithelial cells (double arrowhead). Metestrus Phase (c) demonstrating the co-existence of dispersed neutrophils (double arrowhead) and Anucleated keratinized epithelial cells (arrow). Diestrus phase (d) indicates that there are more neutrophils (double arrowhead) than Anucleated epithelial cells (arrow). Ovaries from mature female rats can be seen that are photographed using a light microscope (HE×40).

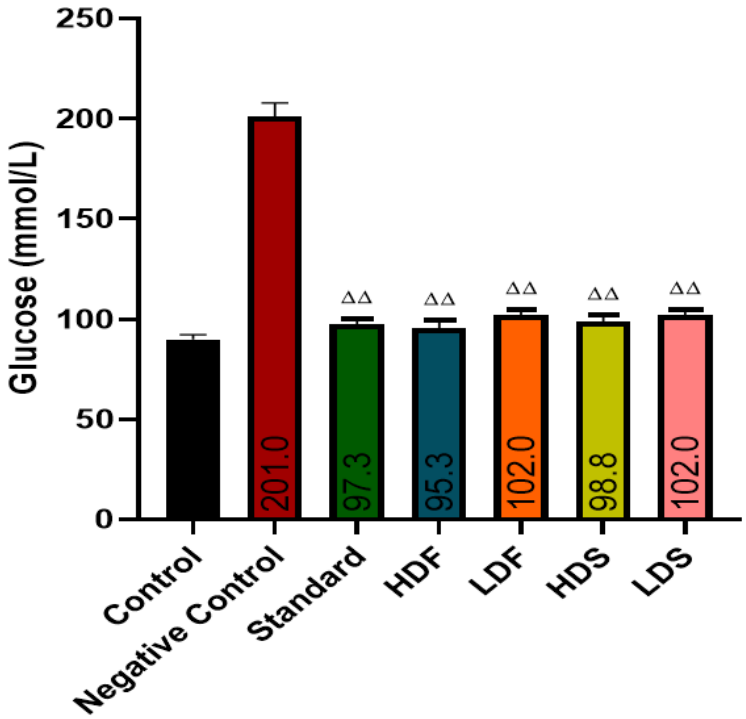

Fig. 7: Effect of MC fruit and seed extracts on glucose following 60 days of dosing.

The results are presented as the mean ± SD determinations for each group (n = 6).

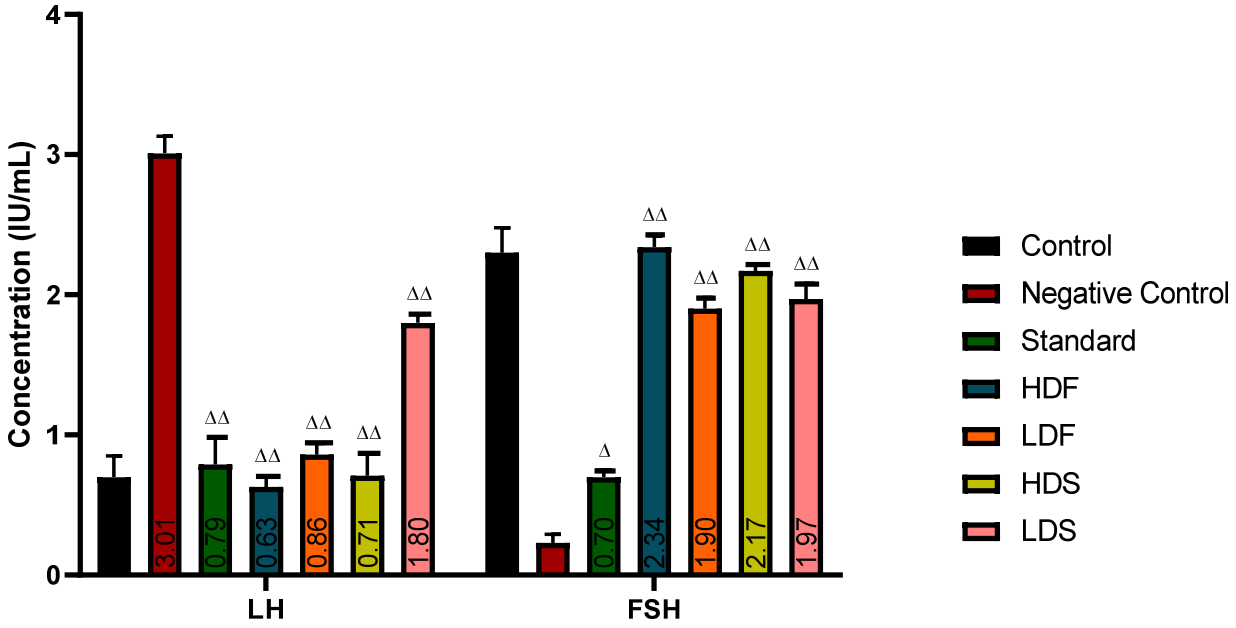

Fig. 8 (a): Effect of MC fruit and seed extracts on LH & FSH following 60 days of dosing.
The results are presented as the mean ± SD determinations for each group (n = 6)

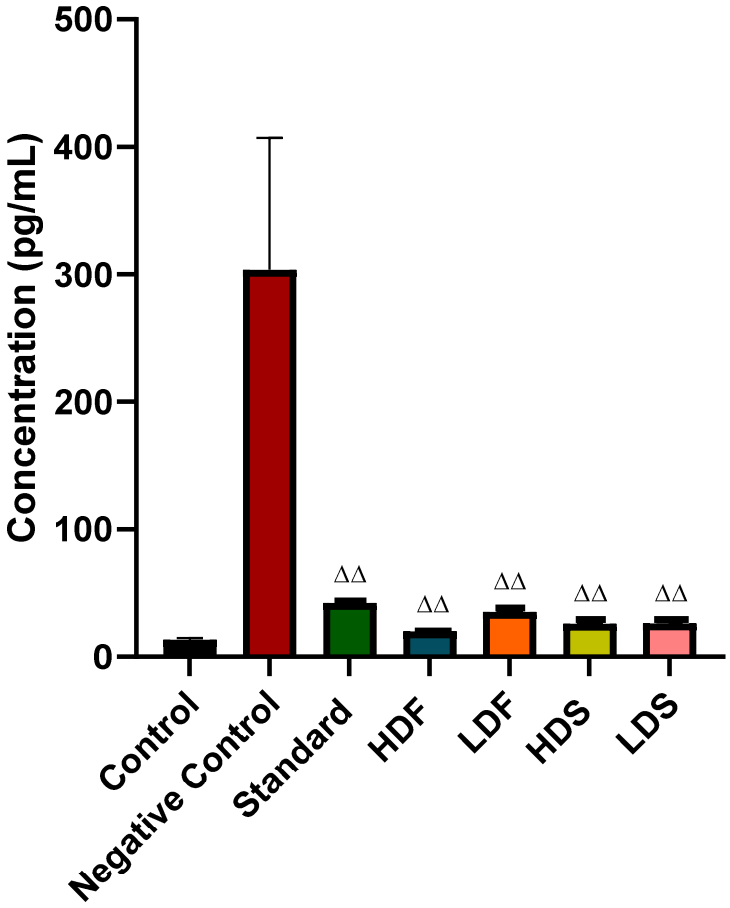

Fig. 8 (b): Effect of MC fruit and seed extracts on estradiol following 60 days of dosing. The results are presented as the mean ± SD determinations for each group (n = 6)

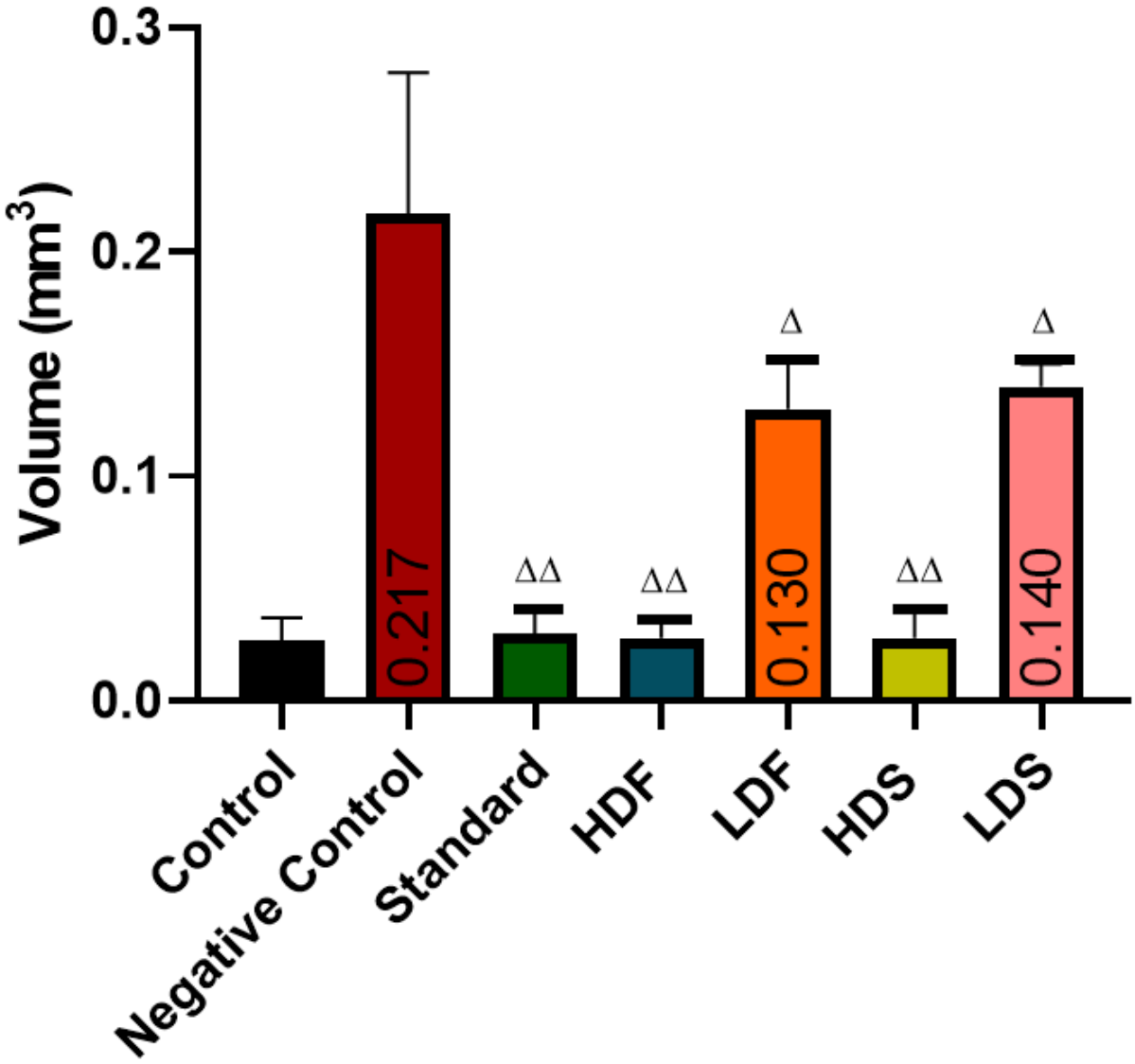

Fig. 9: Effect of MC fruit and seed extracts on ovarian volume following 60 days of dosing.
The results are presented as the mean ± SD determinations for each group (n = 6)

Table 4: Effect of MC fruit and seed extracts on superoxide dismutase & glutathione levels

	GROUPS								
	Control	NC (4.5mg/kg)	Standard (350mg/kg)	HDF (1000mg/kg)	LDF (500mg/kg)	HDS (1000mg/kg)	LDS (500mg/kg)	F Value	P Value
SOD	161.56±10.89	160.5±2.22	158.1±8.25	165.5±9.27	155.2±8.53	162.7±8.95	158.7±8.77	1.12	>0.05
GPx	45.26±2.21	28.83±1.52	38.99±2.43 $^{\Delta\Delta}$	45.3±2.72 $^{\Delta\Delta}$	32.59±2.01 $^{\Delta}$	43.6±1.90 $^{\Delta\Delta}$	22.81±2.5 $^{\Delta}$	15.15	<0.01

DISCUSSION

This study explored the impact of *Momordica charantia* L. fruit and seed extracts on a rat model with estradiol valerate-induced PCOS. The findings confirmed that both extracts effectively mitigated PCOS-related changes. Induction of PCOS altered body weight, estrous cycle, glucose, insulin levels, ovarian morphology, lipid profiles and hormonal status. However, treatment with *Momordica charantia* L. fruit and seed extracts improved these disturbances.

We successfully developed the PCOS rat model via estradiol valerate that is the pro-ester of estradiol. It induces hyperandrogenemia in addition to an elevated sympathetic activity and also raises blood glucose levels with subsequent development of hyperinsulinemia, both of which are involved in the pathogenesis of polycystic ovary syndrome.

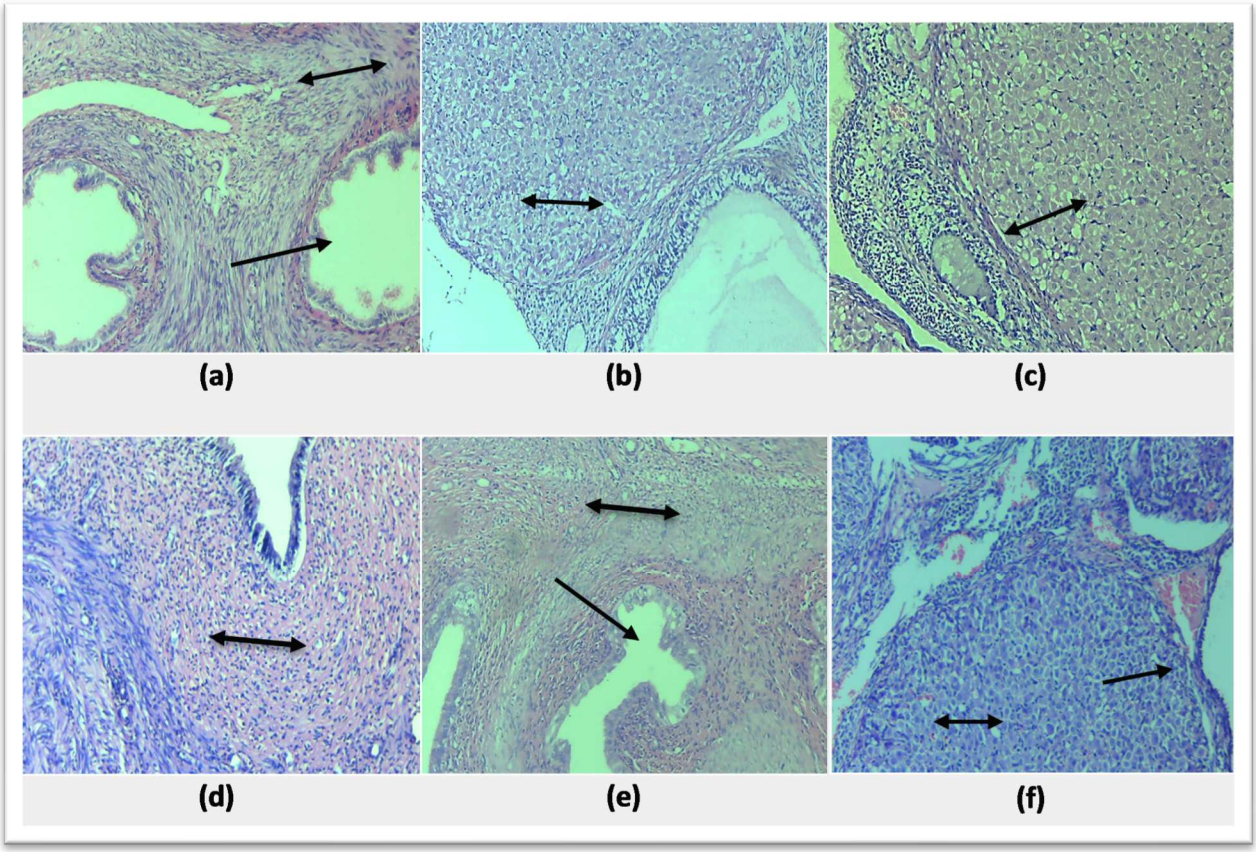

Fig. 10: Effect of *Momordica charantia* L. fruit & seed extracts on ovarian histopathology of female albino wistar rats
Figure 10 presents light microscopic images of rat ovaries stained with hematoxylin and eosin (HE × 40). Image descriptions: (a) Polycystic ovary with multiple cysts and collagenized stroma (Arrow: cyst; Double arrow: collagenized stroma). (b) Metformin-treated ovary showing luteinized stroma (Double arrow). (c-d) HDF and HDS treatments for 60 days, both displaying luteinized stroma without cysts (Double arrow). (e-f) LDF and LDS treatments for 60 days, both showing luteinized stroma with few cystic follicles, but no large cysts (Arrow: cyst; Double arrow: luteinized stroma). Scale bar: 200µm.

Our findings confirmed previous studies that reported the necessity of using estradiol valerate to prepare a PCOS model (Ajayi and Akhigbe, 2020). These results were demonstrated for the first time when rat models were administered *Momordica charantia* L. fruit and seed extracts, which significantly altered the levels of estradiol, LH and FSH due to various active phytochemical constituents, including high carbohydrates, alkaloids, steroids, saponins, tannins and flavonoids content, with proven anti-diabetic, hypolipidemic, anti-obesity, anti-inflammatory and anti-estrogenic effects (Dandawate *et al*., 2016).

Our study assessed the acute toxicity profile of *Momordica charantia* L. using a standard MTT assay, which revealed that the aqueous fruit extract was non-toxic and maintained HeLa cell viability. This finding is in accordance with previous research (Kumar and Bhowmik, 2010). The antioxidant capacity of *Momordica charantia* L. fruit and seed extracts was evaluated by DPPH, displaying concentration-dependent radical scavenging activity. The antioxidant ability of the extracts was further substantiated by estimates of the total phenolic content and *in-vivo* tests. Hazra *et al*., (Hazra *et al*., 2022) in 2022 reported that MC fruit had been recognized for its anti-oxidant potential due to its higher contents of phenolic compounds. Superoxide dismutase (SOD) and glutathione have significant roles in protecting reproductive health. For example, superoxide dismutase is closely associated with oocyte maturation. As mentioned earlier about the study by Jain *et al*. (2007), who investigated the protective role of *Momordica charantia* L. against lipid peroxidation and oxidative stress. The study, titled 'Antioxidant Capacity of *Momordica charantia* L. Extract and its Protective Effect on Testicular Damage in Valproic Acid-Induced Rats' published in the International Journal of Morphology illustrates that bitter melon extract significantly enhances antioxidant enzyme activities, including superoxide dismutase (SOD) and catalase (CAT) and decreases malondialdehyde (MDA) levels in liver tissues. This research underscores the efficacy of *Momordica charantia* L. as a potent antioxidant agent, supporting our rationale for its inclusion in our study to assess impacts on oxidative stress markers. Our current study on *Momordica charantia* L. (MC) substantiates high antioxidant capability of MC that exhibited dose-dependent scavenging activity by DPPH method with

simultaneous estimation of total phenolic content and *in-vivo* testing. Investigation of fruit and seed extracts of MC discovered no significant change in the SOD content ($p>0.05$) and a significant ($p<0.01$) increase in glutathione level especially at treatments of 1000 mg/kg with over-expression compared with negative control and also notable increases with 500mg/kg vs. negative control. This pattern has also been observed in standard drug, metformin HCl, which did not affect SOD content but significantly increased glutathione level ($p<0.05$), as did the MC treatment groups.

Further anti-inflammatory in-vitro assays and carrageenan-induced inflammatory animal model were conducted to assess the anti-inflammatory role of MC fruit and seed extracts. The carrageenan-induced inflammation model, though not PCOS-specific, reflects MC's systemic anti-inflammatory effects, which are relevant given the role of NF-κB and cytokines in PCOS (Arigela *et al*., 2021). Recently, numerous inflammatory markers have been observed in PCO women. Raised levels of white blood cell count, C-reactive protein and some cytokines, including interleukin and tumor necrosis factor-α (TNF-α), have been found in PCO patients. Our results indicated robust anti-inflammatory effects of MC fruit and seed extracts that align with Arigela *et al*., (2021) who reported that bitter gourd and its constituents have anti-inflammatory properties by modulating NF-κB and pro-inflammatory cytokines. Moreover, Lii *et al*., researched bitter melon's anti-inflammatory effects on macrophages stimulated by lipopolysaccharides (LPS). Their findings revealed that bitter melon extracts could inhibit the activation of NF-κB (Nuclear Factor- Kappa B), a transcription factor that plays a critical role in the inflammatory response (Lii et al., 2009).

Hyperlipidemia is another frequently identified comorbidity in women suffering from PCOS. The condition might be diagnosed in up to 70% of cases. Consequently, our experiment confirmed the high degree of improvement ($p<0.01$) in the total lipid profile due to the treatment with MC fruit and seed extract in the experimental model of PCO. The significant further improvement was observed after 60 days of treatment reflected by increased HDL and decreased TC, TG and LDL. Due to the presence of widely known hypolipidemic components, including saponins, phenolic compounds, triterpenes, alkaloids and unsaturated fatty acids, bitter melon appears to be a highly effective hypolipidemic agent (Fan *et al*., 2019). Other researchers have also precisely demonstrated a similar effect significantly reducing body weight, visceral fat and high-fat diet accumulation (Saeed *et al*., 2017).

Elevated adiposity and increased fat cells may cause hyperinsulinemia and insulin resistance. This is due to disruptions in the production of reproductive hormones by adipose tissues, including a decrease in the secretion of adiponectin, a cytokine that helps increase insulin sensitivity (Jaclyn Carr *et al*., 2019). The current study showed that MC fruit and seed extract (500mg/kg and 1000mg/kg per day dosing) successfully reduced fasting blood sugar, cholesterol, low-density lipoprotein (LDL) and triglycerides (TGs) compared to the negative control group. MC fruit, which is rich in oleanolic acid 3-O-glucuronide, oleanolic acid 3-O-monodesmoside, charantin polypeptide-P and momordicin has been found to possess hypoglycemic activity (Saeed *et al*., 2018). The pharmacological action was also demonstrated in various studies. Fan *et al*., (2019) showed that bitter melon extracts resulted in decreased blood sugar levels might be attributed to the stimulation of the expression of Peroxisome proliferator-activated receptor gamma (PPARγ). It is a receptor that regulates lipid and glucose homeostasis by coordinating the expression of genes that encode enzymes involved in these processes. Noruddin *et al*., (2021) demonstrated that MC fruit exerts a hypoglycemic effect by acting as PPARγ ligand with inducing muscle glucose uptake activity. In addition to insulin resistance, PCOS patients experience obesity resulting as a consequence of leptin resistance (Jaclyn Carr *et al*., 2019).

Higher insulin and leptin concentrations in the body lead to an enhanced craving for food, the accumulation of adipose tissue and an ultimate increase in body weight. The present study showed MC fruit and seed extracts significantly reduced body weight after 60 days of treatment; this reduction can be attributed to the anti-obesity properties of bitter melon, which contains saponins, alkaloids, flavonoids, triterpenoids and unsaturated fatty acids. These active constituents have been shown to impact reducing body weight.

Patients with polycystic ovary syndrome have dysregulated neuroendocrine system, which leads to the dysfunctioning of the hypothalamic-pituitary-ovarian axis. This dysregulation leads to an excessive release of gonadotropins. The elevated hypothalamic gonadotropin-releasing hormone (GnRH) axis stimulates the production of the β-subunit of luteinizing hormone more than the follicle-stimulating hormone. As a result, there is an increase in luteinizing hormone (LH) formation compared to follicle stimulating hormone (FSH), which results in an increased luteinizing hormone to follicle stimulating hormone ratio (Ashraf *et al*., 2019). This situation leads to hyperandrogenism because of the larger follicles and the upregulated androgen synthesis enzymes. Our recent investigation on the extracts of *Momordica charantia* L. fruit and seeds demonstrated significantly decreased LH concentrations after 60 days of treatment. Additionally, we found highly significantly raised levels of FSH for both fruit and seed extracts after 60 days of dosing.

In assessing the anti-estrogenic effect of bitter melon against PCOS, we found significant results in relation to the negative control group. Both MC seed and fruit extracts

successfully reduced estradiol levels. Bitter melon fruit was found to reduce serum estradiol and increase ESR-1 and ESR-2 levels (Akpınar, 2013).

Since increased ovarian volume is the characteristic feature of PCOS after 60 days of dosing, bitter melon produced highly significant results ($p < 0.01$). The outputs of our ovarian histological investigation side by side affirmed the hormonal & biochemical findings, demonstrating favourable results in terms of the kind and morphology of the luteinized and collagenized stroma and the existence of cysts in the estradiol-induced PCOS group.

These research findings are in line with previous research that has identified a connection between hypercholesterolemia, hyperinsulinemia and leptin resistance, all of which can further disrupt the release of hormones such as FSH, LH and androgens. The study results indicate that the fruit and seed extracts of bitter melon contain ingredients that can manage the symptoms of PCOS. Both the fruit and seed extracts were each effective, but the researchers noted that the extract of the bitter melon seed has a slight advantage over that of the fruit, emphasizing the potential of the bitter melon seed extract as a slightly better alternative for managing PCOS, underlining the therapeutic value of bitter melon to manage the condition.

CONCLUSION

Our study demonstrates that the fruit and seed extracts of *Momordica charantia* L. possess significant therapeutic potential for managing the symptoms of PCOS. Both extracts were effective, with the seed extract showing a slight advantage over the fruit extract. This underscores the therapeutic value of bitter melon in addressing the complex metabolic and hormonal dysregulations associated with PCOS, providing a promising avenue for future research and clinical applications.

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Authors' contributions
1. Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work- Saira Saeed Khan, Anosh Tahir, Sadaf Naeem, Nazish Jaffar, Sadia Sardar Sheikh
2. Drafting the work or revising it critically for important intellectual content- Anosh Tahir, Saira Saeed Khan, Nuzhat Sultana, Adnan Iqbal
3. Final approval of the version to be published- Anosh Tahir, Saira Saeed Khan, Sadaf Naeem
4. Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved-Anosh Tahir, Saira Saeed Khan, Sadaf Naeem, Adnan Iqbal, Nazish Jaffar, Sadia Sardar Sheikh, Nuzhat Sultana

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Data availability statement
Data are available upon reasonable request.

Ethical approval
The research protocols were approved by the Institutional Bioethical Committee of the University of Karachi (IBC KU 123(A)/2020).

Conflict of interest
The authors declare no conflict of interest in this study.

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