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Ameliorative effect of pomegranate peel extract on the testicular dysfunction in obese rats

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ABSTRACT

Infertility and sexual incapacity worsened due to the pathophysiological circumstances in obese individuals. The impact of pomegranate peel extract (PPE) treatment on testicular dysfunction and its effect on testicular histological changes in obese rats was examined. Four groups of forty mature male Sprague Dawley rats (n = 10) were used. The 1st group (Gp1) was used as a negative control and fed a typical baseline diet. For twelve weeks, Gp2, Gp3, and Gp4 were fed a high-fat diet to cause obesity. Then, Gp2 was left as obese rats, while Gp3 and Gp4 were administered Konjac (50 mg/kg b.wt) and PPE (100 mg/kg b.wt) every day for eight weeks. Lipid profile and body weight changes were measured. Several biochemical parameters, including superoxide dismutase, catalase activities, and malondialdehyde levels, as well as the male reproductive hormones, were measured. In testicular tissues, the immunohistochemical changes in caspase-3 and Bcl-2 expression, as well as the histological changes, were examined. Testis tissues of obese rats had various pathophysiological and histological modifications. However, treating obese rats with Konjac or PPE reduced these changes, as shown by improving the lipid profile, increasing antioxidant enzyme activity, increasing the hormonal shift, and improving the testicular histological abnormalities.

Keywords: Antioxidants, Bcl-2, Hormonal dysfunction, Obese, Pomegranate peel extract, p53, Testicular

1. Introduction

Obesity is a serious global health issue that affects both adults and children at a high rate (WHO, 2021). Excessive body fat buildup is the hallmark of this complicated, multifactorial illness, which is frequently measured using the body mass index (BMI) (Mayoral et al., 2020). Obesity is generally defined as having a BMI of 30 kg/m² or greater. Over the past few decades, the rate has dramatically increased on a global scale. By 2025, 16% of the world's population may be obese, accounting for 45% (> 4 billion) (Ahmed and Mohammed, 2025). Numerous health issues are linked to obesity (Bodirsky et al., 2020). In fact, obesity increases the incidence of coronary heart disease, type 2 diabetes (T2-DM), and secondary hypogonadism in males (Sarma et al., 2021). It is exclusively associated with increased leptin levels, pro-inflammatory markers (Obradovic et al., 2021). Infertility and decreased muscle mass are all worsened by pathophysiological changes in obese individuals (De Lorenzo et al., 2018).

Male reproductive hormones are altered by obesity, which has an impact on lifestyle. For instance, it has been found that obese people have significantly altered the male reproductive hormones such as luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone (TST) levels. The enlargement of adipose tissue and the enzyme aromatase, which transforms TST into estrogen, are both increased.

Hypoestrogenism lowers LH secretion, which prevents Leydig cells from producing TST (De Lorenzo et al., 2018). The condition known as obesity-associated hypotestosteronemia is reversible, and low TST levels increase the development of adipocytes into pluripotent stem cells. This promotes the aromatization of TST to oestradiol, which suppresses the generation of TST (Kelly and Jones, 2013). It has been observed to deteriorate sperm parameters, producing a decrease of count, motility, and morphological deformations, and enhanced reactive oxygen species (ROS) production and deoxyribonucleic acid damage of sperm (Billah et al., 2022).

The primary strategies for losing weight include changing one's lifestyle to include more exercise, eating a balanced diet, and avoiding foods high in processed foods (Lingvay et al., 2022). Blood pressure, triglycerides (TG), fatty acids, and blood glucose are all significantly impacted by this approach (Hall et al., 2021). Losing weight lowers the risk of sleep apnea, non-alcoholic fatty liver disease, dyslipidemia, cardiovascular disease, and T2-DM (García and Jiohanna, 2019). Currently, the Food and Drug Administration has approved anti-obesity medications such as Bupropion-naltrexone, Liraglutide, Orlistat, Phentermine-topiramate, and Konjac for short-term or long-term therapies (Powell, 2020). Most of the anti-obesity medications reduce food intake and suppress appetite by acting on the central nervous system (Müller et al., 2022).

Physicians suggested Orlistat as a treatment for obesity (Bansal et al., 2022). It operates in the lumen of the stomach and small intestine as a reversible inhibitor of pancreatic and gastric lipases, greatly reducing the absorption of fat (Bray et al., 2016). Diarrhea, dyspepsia, and flatulence are among the gastrointestinal side effects of Orlistat that are frequently observed (Othman et al., 2021). Also, it was reported that there is a higher chance of acute pancreatitis, gallstone disease, and breast cancer (Patel et al., 2018).

According to Hassan et al. (2022), medicinal plants are used for treating obesity and are thought to be safer when used over an extended period. Compared to synthetic medications for weight loss, natural products are more affordable, more widely available, and have fewer adverse effects (Hasani-Ranjbar et al., 2013). A well-known polysaccharide, Konjac glucomannan (KGM), was extracted from *Amorphophallus konjac* (Devaraj et al., 2019). It is made up of a straight chain of 1:1.6 M ratios of $\beta-1,4$-linked d-glucose and D mannose, with 8% branching via β-1,6 glucosyl links (Li et al., 2017). Konjac helps with a number of conditions, including constipation, elevated blood fat/glucose levels, and obesity, by reducing nutritional imbalance through increased dietary fiber consumption (Gómez et al., 2019). It exhibits numerous health-promoting properties, such as anti-inflammatory and anti-diabetic (Longchen et al., 2021).

Punica granatum L. (pomegranate) belongs to the Family Lythraceae. Since the 1990s, pomegranate peel fruit has been widely marketed to consumers in the US as a medical food (Jurenka, 2008). Pomegranate extracts as anti-obesity agents have been studied due to their antioxidant properties (Essam et al., 2019). These extracts have been studied for a variety of pharmacologic properties (Kang et al., 2016). The current study aims to assess the impact of pomegranate peel extract (PPE) treatment on testicular dysfunctions and histological changes in obese rats.

2. Materials and methods

Konjac capsules were obtained from October Pharmaceutical Co. in Egypt. The Bio Diagnostic Company (Egypt) supplied the kits for total cholesterol (TC), TG, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), malondialdehyde (MDA), catalase (CAT), and superoxide dismutase (SOD). The Sigma Aldrich Company (Berlin, Germany) supplied the phosphate buffer saline (PBS), bovine serum albumin (BSA), caspase-3, and B-cell lymphoma 2 (Bcl-2) antibodies. The testosterone (TST) MAIA® kit was bought from Biochem Immuno System. The follicle-stimulating hormone (FSH) ELISA kit was acquired from MYBIOSOURCE Company (MBS2021901). Rat LH, ELISA Kit (E-EL-R0026) was obtained from Elabscience Company.

Preparation of pomegranate peel extract

Pomegranate fruits were purchased from the Tanta City, Egypt, local market. Pomegranate peel was chopped into little pieces and left to dry in the shade. After crushing these compartments, 50 g of each was added to 500 ml of 70% ethanol, and the mixture was left for three days. In order to obtain the hydro-alcohol PPE, the supernatants were filtered and allowed to dry in an air-conditioned environment.

Determination of the phytochemical analysis

According to Miliauskas et al. (2004), the total phenolic content (TPC) was calculated. Zhishen et al. (1999) method was used to measure the total flavonoid concentration (TFC). Saponin content was determined according to the method of Ebrahimzadeh and Niknam (1998). Total antioxidant capacity (TAC) was evaluated using the phospho-molybdenum procedure, according to Prieto et al. (1999). DPPH scavenging activity was measured using the Blois (1958) method.

Gas chromatograph–mass spectrometry analysis

The Trace GC 1310-ISQ mass spectrometer (Thermo Scientific, Austin, TX, USA) was used to analyze the phytochemical contents of PPE. The temperature of the column oven was raised by 30 °C per minute to 300 °C, held for two minutes. The temperatures of the injector and MS transfer line were maintained at 270 °C and 260 °C, respectively. As a carrier gas, helium was employed at a steady flow rate of one meter per minute. Diluted samples of 1µl were automatically injected using an autosampler AS1300 coUSLed with GC in split mode after a 3-minute solvent delay. EL mass spectra were obtained in full scan mode across the m/z 45-600 range at 70 eV ionization voltages.

Composition of normal basil diet and high-fat diet

The normal basil diet (NBD) for control rats was made according to the method of Pugh et al. (1999). Rats were fed a high-fat diet (HFD) that included 44.6% fat, 40.6% carbohydrates, and 14.8% protein according to the method of Boyce et al. (2020).

Animals

Sprague Dawley male rats (150 ± 5 g) were purchased from the Faculty of Agriculture, Alexandria University (Alexandria, Egypt). The rats were housed under a 12:12 h light–dark cycle for a 1-week acclimatization. The experiments were carried out under the ethical number MU-ACUC (SC.R.25.09.33).

Experimental design

Thirty obese and ten normal rats were distributed into four groups (n = 10). Gp1 was fed NBD and used as a negative control. Gp2 to Gp4 were fed on an HFD for 12 weeks. Then, Gp3 received administration of Konjac (50 mg/kg), and Gp4 was given oral PPE (100 mg/kg) daily for two months. Each rat's weight growth was tracked on a weekly basis. Rats were sacrificed under anesthesia (Isofluran). Blood samples were collected in clean tubes for biochemical analysis. Testicular tissues were collected for biochemical, histopathological, and immunohistochemical investigations.

Determination of the biochemical parameters

Serum TC, TG, HDL-C, and LDL-C were determined according to the manufacturer's kits. After homogenizing testis tissue pieces, the homogenates were centrifuged at 13,000 rpm. The supernatant was used to measure the antioxidants and oxidant parameters. The methods of Minami and Yoshikawa (1979), Aebi (1984), and Esterbauer and Cheeseman (1990) were used to determine the SOD, CAT activity, and the MDA level, respectively. Following the manufacturer's instructions, TST, LH, and FSH hormones were measured in serum samples.

Histopathological and immunohistochemistry investigations

Testicular tissues were removed and preserved for a full day in 10% formalin. Then, sectioned at 5 µm and embedded in paraffin wax. Hematoxylin and eosin (H&E) staining was applied to the sections in order to perform histopathological analyses. p53 and Bcl-2 were identified for immunohistochemistry. In brief, paraffin sections with a thickness of 5 µm were deparaffinized in xylene, rehydrated with alcohol of decreasing grades, and then cleaned in PBS. Sections were submerged in 3% H_2O_2 and then

rinsed with PBS to deactivate endogenous peroxidase. Slides were then boiled for 20 minutes in 10 mM citrate buffer (pH 6.0) and allowed to cool for an additional 20 minutes at room temperature. 5% BSA was added to Tris-buffered saline to prevent nonspecific binding. After applying primary antibodies at a 1:100 dilution for 60 minutes at room temperature, PBS was used for washing. The secondary antibodies were then incubated on the slides. After that, sections were cleaned, visualized, and examined. The streptavidin–biotin peroxidase staining approach was used to identify Bcl-2-positive cells and p53 (Bancroft and Gamble, 2008).

Statistical analysis

The significant differences between treatment groups were evaluated using one-way analysis of variance (ANOVA). To demonstrate the treatment's considerable impact, the Dunnett test was performed to compare each group to the control group. The statistical significance threshold was established at $p<0.05$. Every data point is displayed as mean ± SD.

3. Results

Phytochemical content of pomegranate peel extract

To assess the phytochemical composition of PPE, the TPC, TFC, saponin, TAC, and DPPH were determined. The results showed that the TPC content was 18.91mg /g. Ex, The TFC content and the saponin content were 268.21 µg QE/mg EX and 217.61mg/g EX ,respectively. The TAC and DPPH scavenging activity were 3.05 mg AE/g Ex and 56.69%, respectively (Table 1).

GC-MS analysis of pomegranate peel extract

As shown in Table 2, the GC-MS analysis of PPE showed that there was a high component of 5-hydroxymethylfurfural at peak area (P.A.) 47.37% at R.T. 10.60 min. the 5-aminolevulinic acid and 9-Octadecenoic acid (Z)-, methyl ester compounds were recorded with P. A 4.23 and 4.91%, at R.Ts, 4.40 and 30.56, respectively (Fig. 1 and Table 2).

Table 1: Phytochemical analysis of pomegranate peel extract

Phytochemical parameters	PPE
Total phenolic content (mg GAE/g EX)	18.91 ±1.28
Total flavonoid contents (µg QE/mg EX)	268.21 ± 2.39
Saponin (mg/g EX)	217.61 ±1.97
TAC (mg AE/g EX)	3.05 ± 0.5
DPPH scavenging activity (%)	56.69 ± 2.23

The values represented mean ± SD; EX: Extract; DW: Dry weight; PPE: Pomegranate peel extract; GAE: Gallic acid equivalent; QE: Quercetin equivalent; TAC: Total antioxidant capacity; AE: Ascorbic acid equivalent; DPPH: 2,2-Diphenyl-1-picrylhydrazyl.

Table 2: GC-MS analysis of pomegranate peel extract.

No.	R.T (min)	Name	M.Wt	M.F	P.A (%)
1.	4.40	5-Aminolevulinic acid	130	C5H9NO3	4.23
2.	8.32	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	144	C6H8O4	5.91
3.	10.60	5-Hydroxymethylfurfural	126	C19H32	47.37
4.	13.58	PHENOL, 2-METHOXY-4-(2-PROPENYL)	164	C10H12O2	10.96
5.	17.59	Phenol, 2-methoxy-4-(2-propenyl)-, acetate	206	C12H14O3	2.94
6.	20.74	Cyclopentaneacetic acid, 3-oxo-2-pentyl-, methyl ester	226	C13H22O3	2.56
7.	21.29	1-(4-Isopropylphenyl)-2-Met Hylpropyl Acetate	234	C15H22O2	1.34
8.	27.23	Hexadecanoic Acid, Methyl Ester	270	C17H34O2	1.03
9.	30.38	9,12-Octadecadienoic Acid, Methyl Ester, (E,E)	294	C19H34O2	2.72
10.	30.56	9-Octadecenoic acid (Z)-, methyl ester	296	C19H36O2	4.91

RT: Retention time; M.Wt: Molecular weight; M.F: Molecular formula; P.A%: Peak area percentage.

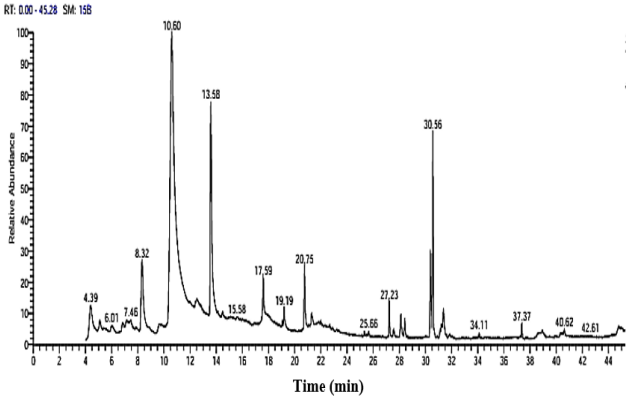


Fig.1: GC-MS chromatogram of PPE and the fragmentation pattern of its constituents.

The treatment with PPE or Konjac decreased the % B.wt of obese rats.

As shown in Fig. 2, Table 3, compared to the group of obese rats, the treatment with konjac or PPE led to a significant decrease in the percentage (%) of B.wt changes by -14.62 and -16.33%, respectively ($p \leq 0.05$).

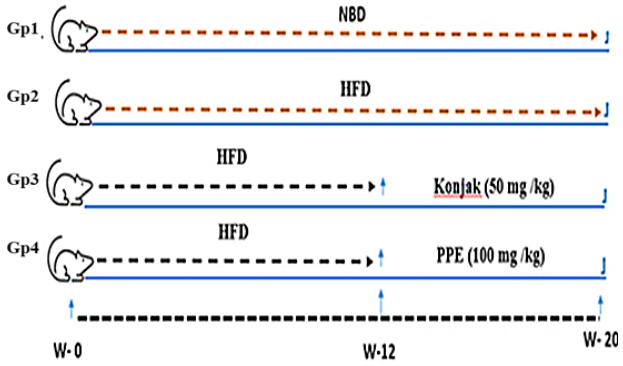


Fig. 2. Timeline of the experimental plan

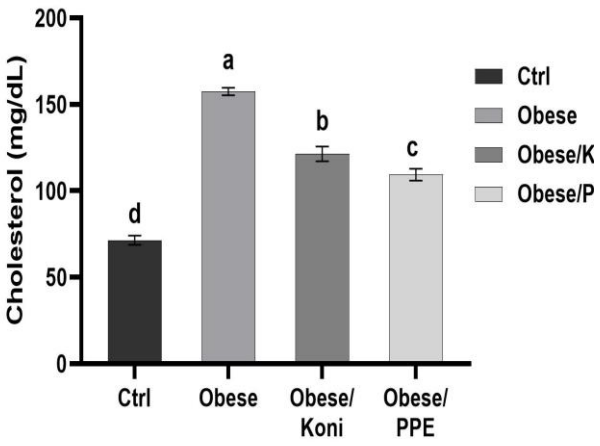
Table 3. Initial, final body weight and the % of body weight change in different groups.

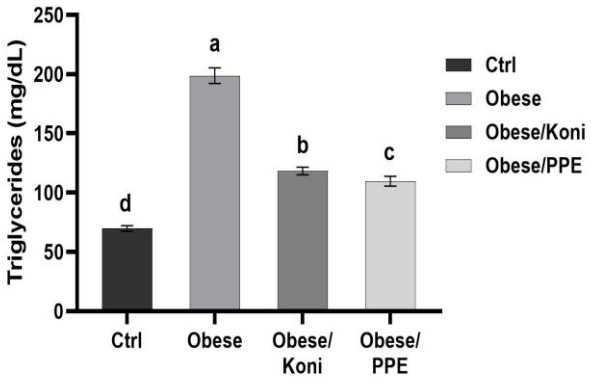
Groups	I.B.Wt (g)	F.B.Wt (g)	% b. wt change
Ctrl.	177 ± 8.76 [b]	208 ± 9.38[b]	17.51%
Obese.	246 ± 8.52 [a]	354 ± 9.09 [a]	43.91%
Obese/Konj.	253 ± 10.13 [a]	216 ± 7.12 [b]	-14.62%
Obese/PPE.	251± 9.47 [a]	210 ± 9.06 [b]	-16.33%
F-value	78.5	113.4	
p-value	< 0.001	< 0.001	

The values represented mean ± SD; I.B.Wt: Initial body weight; F.B.Wt: Final body weigh; Ctrl: Control; Konj: Konjac; PPE: Pomegranate peel extracts. P value ≤ 0.05 was considered to be statistically significant. Means that do not share a letter are significantly different.

Treatment with PPE augmented the lipid profile changes in obese rats

Rats were fed on HFD for 12 weeks and had significant changes in lipid profile when compared to normal rats ($p \leq 0.05$). Significant increases in the levels of TC, TG, in obese rats when compared to normal rats ($p \leq 0.05$). The levels of HDL-C and LDL-C in obese rats were 19.49 and 40.01 mg/dl, respectively. In the control group, these values were 39.02 and 18.43 mg/dL. The treatment with konjac, however, augmented those changes in the lipid profile in obese rats. Additionally, treatment with PPE showed a significant improvement in these parameters compared to those in obese rats ($p \leq 0.05$) (Fig. 3).





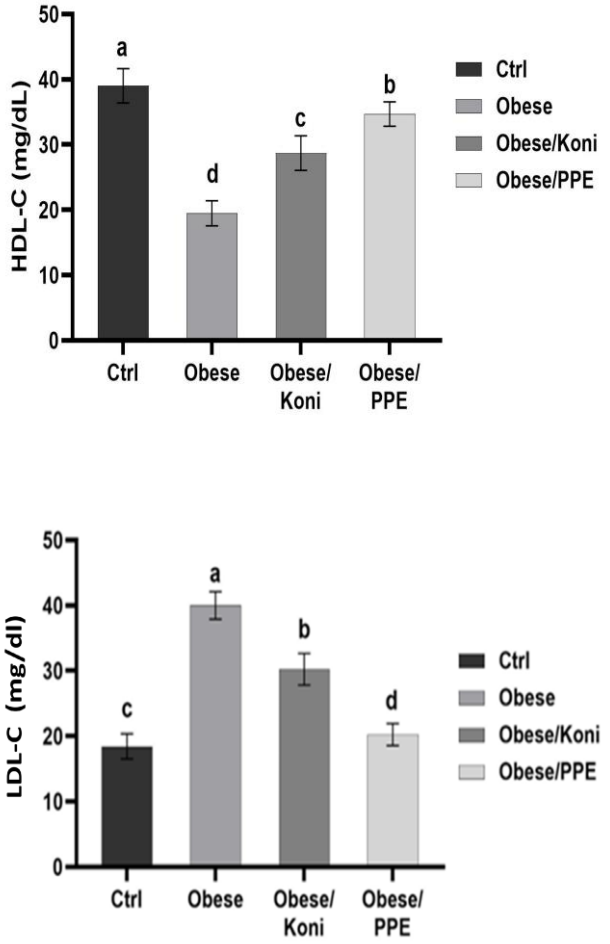


Fig. 3: The TC, TG, HDL-C, and LDL-C levels in the different groups.

Treatment of obese rats with PPE ameliorated the antioxidant status

Compared to the control group, the SOD and CAT activities decreased significantly in obese rats ($p \leq 0.05$). At the same time, the level of MDA was significantly increased ($p \leq 0.05$). The treatment with konjac or PPE significantly mitigated the antioxidant/oxidant changes in obese rats. Interestingly, the effect of treatment with PPE was superior to that of Konjac in ameliorating these effects (Table 4).

Table 4. Hepatic SOD, CAT activities, and MDA levels in the different groups

Groups	SOD (U/ g tissue)	CAT (U/g tissue)	MDA (nmol/ g tissue)
Ctrl.	8.31 ± 0.52 [a]	77.83 ± 3.01 [a]	38.57 ± 1.3 [d]
Obese.	4.34 ± 0.21 [c]	49.47 ± 2.34 [c]	65. 75 ± 3.9 [a]
Obese/Konj.	3.23 ± 0.31 [d]	38.18 ± 1.98 [d]	56.31 ± 2.15 [b]
Obese/PPE.	5.13 ± 0.43 [b]	54.36 ± 2.97 [b]	45.64 ± 1.94 [c]
F-value	159.9	204.6	106.1
p-value	< 0.001	< 0.001	< 0.001

The values represented mean ± SD; SOD: Superoxide dismutase; CAT: Catalase; MDA: Malondialdehyde; Ctrl: Control; Konj: Konjac; PPE: Pomegranate peel extracts. P value ≤ 0.05 was considered to be statistically significant. Means that do not share a letter are significantly different.

Effect of treatment of obese rats with PPE and Konjac on hormones

The figure shows the effects of PPE and Konjac treatments on the reproductive hormones included TST, LH, and FSH of obese rats compared with a control group (Gp1). Gp1 had normal hormone levels of TST (2.65 ± 0.31 ng/ml), LH (3.09 ± 0.86 ng/ml) and FSH (6.15 ± 1.45 ng/ml). Obese group (Gp2) showed a significant reduction in all the above-mentioned hormones. TST level decreased to 1.09 ± 0.11 ng/ml. LH reduced to 2.18 ± 1.09 ng/ml. FSH decreased to 3.65 ± 1.07 ng/ml. The group of obese rats which are treated with PPE improved hormone levels compared to obese rats alone. TST was partially restored to 2.15 ± 0.25 ng/ml, the level of LH was 2.64 ± 0.74 ng/ml, while the FSH level improved to 4.72 ± 0.87 ng/ml. Obese rats treated with Konjac also improved the levels of the reproductive hormones. In this group, the level of TST was 1.94 ± 0.33 ng/ml, the level of LH was 2.33 ± 0.89 ng/ml, while the levels of FSH were 4.25 ± 0.98 ng/ml (Table 5).

Table 5. Levels of TST, LH, and FSH in the sera of the experimental groups

Groups	TST (ng/ml)	LH (ng/ml)	FSH (ng/ml)
Ctrl.	2.65 ± 0.31[a]	3.09 ± 0.86	6.15 ± 1.45[a]
Obese.	1.09 ± 0.11[c]	2.18 ± 1.09	3.65 ± 1.07[b]
Obese/Konj.	1.94 ± 0.33[b]	2.33 ± 0.89	4.25 ± 0.98[b]
Obese/PPE.	2.15 ± 0.25[a]	2.64 ± 0.74	4.72 ± 0.87[b]
F-value	30.27	0.99	4.58
p-value	< 0.001	0.423 n.s.	0.017

The data were displayed as the mean ± SD. Means that do not share a letter showed a significant difference, $p \leq 0.05$.

Treatment with PPE decreased the histological alterations in testes tissues in obese rats

Control slices of testes tissues displayed normal testicular structure. Seminiferous tubules are normally spherical and have a well-organized seminiferous epithelium, indicating active and full spermatogenesis. Sertoli cells and germinal epithelium, which are made up of multiple layers

of spermatogenic cells, line seminiferous tubules. In the basal region of the tubules, spermatogonia were small, rounded cells. Primary spermatocytes were larger in size than the spermatogonia with large spherical nuclei. Early spermatids had smaller, rounded cells with paler nuclei. The long tails of sperm were visible in the tubule lumen. The connective tissue and groups of Leydig cells with vesicular nuclei and acidophilic cytoplasm populated the small interstitial spaces between the tubules. There are several completely grown spermatozoa in the lumen (middle) of the tubules with the higher magnification (right).

In the obese group (Gp2), sections showed symptoms of testicular injury that are frequently linked to obesity. The form of the seminiferous tubules may seem erratic. The seminiferous epithelium frequently exhibits disarray, including a decrease in the amount of mature spermatozoa in the lumen, vacuolization (clear voids) inside the germ cells, and possibly fewer layers of growing germ cells. In comparison to Gp1, the overall cell density may be lower. Compared to the obese group (b), the obese/Konjac group showed some improvement, the seminiferous tubules' structure seems to be better structured and retained. The epithelium may have more germ cells and less vacuolization.

In comparison to the obese group (b), the obese/PPE group showed an improvement, comparable to or possibly superior to the obese/Konjac group. Similar to the control group (a), the spermatogenic epithelium seems thick and well-organized, and the seminiferous tubules are well-formed. Spermatozoa are probably rather prevalent in the lumen (Fig. 4).

Immunohistochemical investigations

In cells lining the seminiferous tubules, GpI displayed strong positive Bcl-2 cytoplasmic immunoreaction (Fig. 5A), whereas Gp2 showed weak Bcl-2 immunoreaction in seminiferous tubule cells (Fig. 5B), but strong Bcl-2 positivity in seminiferous tubule cells of Gp3 and Gp4 (Fig. 5C, D). In comparison to the control group (Fig. 5E), immunohistochemical analysis of P_{53} revealed high expression in Gp2 (Fig. 5F), decreases in seminiferous tubular diameter, epithelial height, and an increase in germ cell loss that may be linked to germ cell death. The germ cells in the seminiferous tubules, which were weakly stained, significantly decreased in Gp3 (Obese/Konjac) (Fig. 5G) and Gp4 (Obese/PPE) (Fig. 5H) due to P53 immunoreactivity expression.

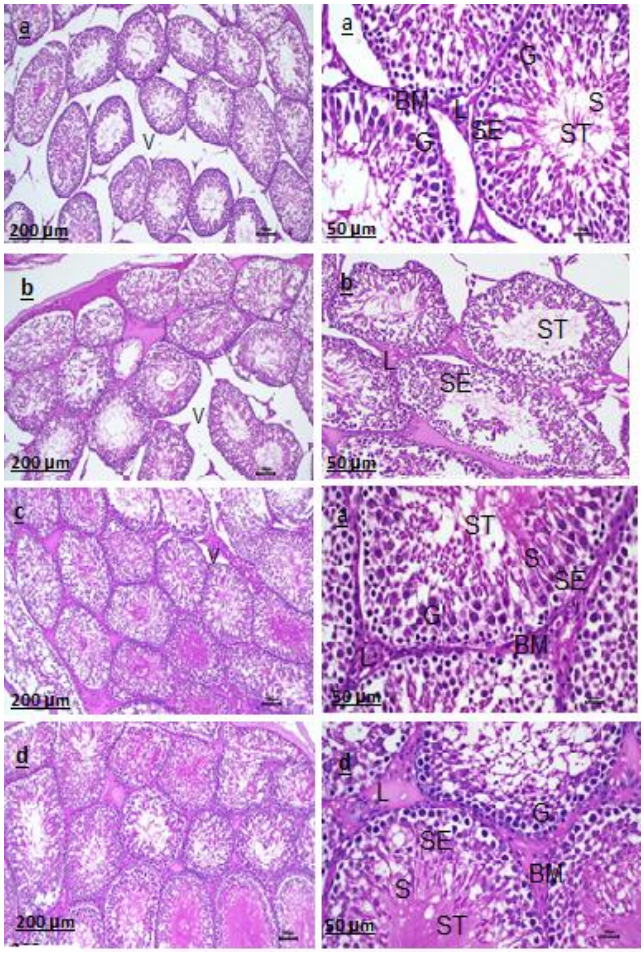


Fig. 4: H&E-stained testis photomicrographs in (a) normal control, (b) obese, (c) obese/Konjac, and (d) obese/PPE. NC stands for control; group I (a) has typical seminiferous tubules (ST) with Sertoli cells (SE) lying on the basement membrane (BM) and germinal epithelium (G). The lumen contains sperm (S). The interstitial cells of Leydig (L) are found in the narrow normal interstitial spaces (IS) between the tubules. The histology of the testis in the obese group II (b) demonstrates a significant decrease in the number of germ cells (G); shedding and degenerating cells have dark-stained nuclei with large gaps between them. A few Leydig cells (L) and vacuoles (V) may be seen in the broad interstitial tissue. Obese/PPE-treated animals (c and d) showed extensive areas with high germ cell and sperm populations in comparison to NC and Obese/Konjac; lower panel magnification: ×400, scale bar = 50 µm).

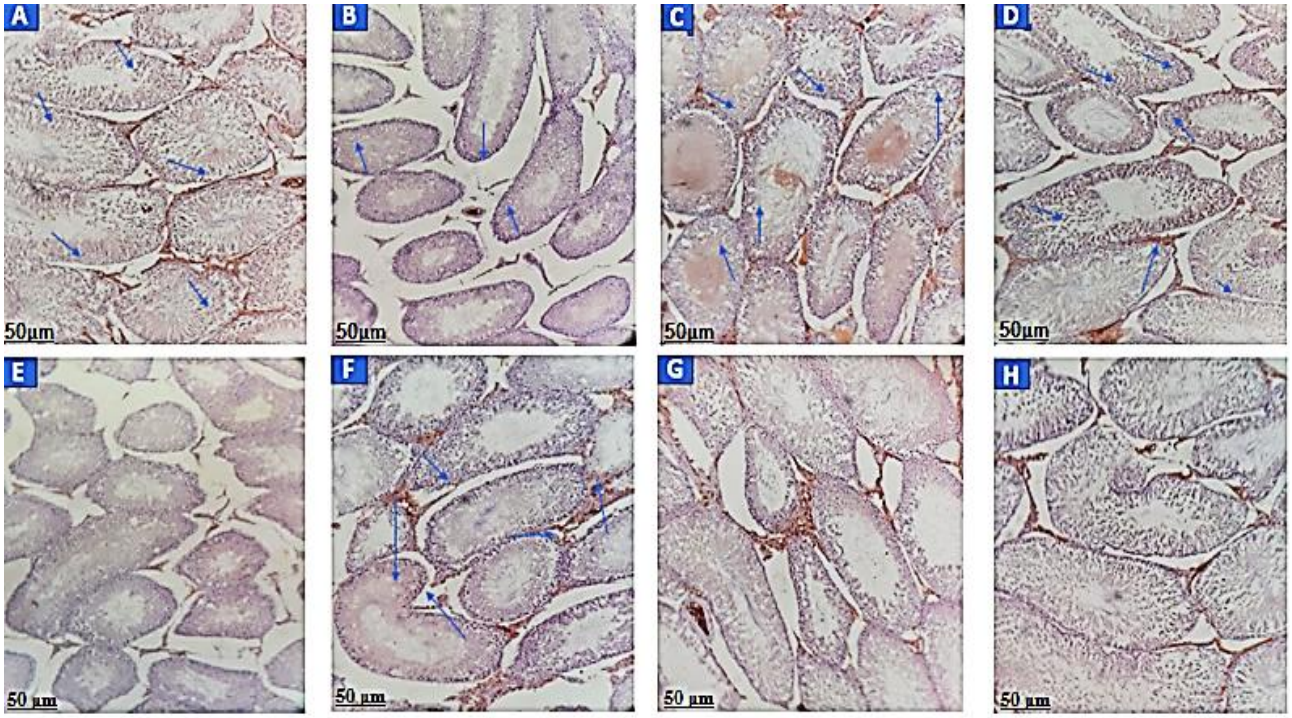


Fig. 5. Immunohistochemical localization of Bcl-2 and p53 biomarkers in testicular tissues of groups I (A,E) Control, II (B,F) Obese, III (C,G) Obese/Konjac, and IV (D,H) Obese/PPE. (A,C,D) shows strong positive Bcl-2 immunoreaction in the seminiferous tubule cells (arrow) in contrast to (B), which shows minimal Bcl-2 immunoreaction in the seminiferous tubule cells (arrow). Conversely, the germ cells in the seminiferous tubules in (E, G, H) displayed a weak p53, whereas in (F) (arrow) (×400, scale bar=50 µm), it appeared significantly positive

4. Discussion

The phytochemical investigation found that PPE had a considerable quantity of bioactive compounds. Natural herbs play a number of roles in removing free radicals (Enas and Omnia, 2024). The data showed that the TFC of the PPE was 18.91 ± 1.28 mg GAE/g EX and 268.21 ± 2.39 g QE/mg EX (Table 1). A previous study demonstrates that pomegranate peel is one of the best sources of flavonoids and phenolics among fruit by-products (Elfalleh et al., 2012). Given that flavonoids are known to scavenge radicals, the high TFC content in particular points to considerable antioxidant potential (Panche et al., 2016). Additionally, PPE has a high saponin content, which is consistent with saponins' capacity to control lipid metabolism and its anti-obesity properties (Shi et al., 2014). DPPH scavenging activity and antioxidant capacity provide additional evidence of its noteworthy potential to neutralize free radicals. This finding suggests that PPE may be a good natural source of bioactive compounds for use in functional food and medical applications.

The GC-MS investigation of PPE revealed several bioactive compounds with potential therapeutic use. 5-hydroxymethylfurfural (47.37%) has been demonstrated to have anti-inflammatory and antioxidant qualities (Nasseri et al., 2022). It may have a significant part in the biological activities of the PPE due to its significant content. Among other phenolic compounds, phenol, 2-methoxy-4-(2-propenyl) (10.96%), and its acetate derivative (2.94%) were also discovered. Maltol derivative 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl (5.91%) has been associated with antioxidant and flavor-enhancing properties (Zhang et al., 2019). Similarly, 5-aminolevulinic acid (4.23%) has use in photodynamic treatment and has been linked to anticancer activities (Khalid et al., 2021). Hexadecanoic acid, methyl ester (1.03%), 9,12-octadecadienoic acid, methyl ester (2.72%), and 9-octadecenoic acid (Z)-, methyl ester (4.91%) were also identified as fatty acid methyl esters.

These compounds have antioxidants, hypocholesterolemic, and anti-inflammatory qualities (Rashid et al., 2021). The GC-MS profile demonstrates that PPE is a rich source of several bioactive compounds of pharmacological importance. These findings are consistent with previous studies that showed PPE has therapeutic

advantages as a helpful ingredient in nutraceuticals (Singh et al., 2022). The body weight of the control group increased by a modest 17.51 %, which is consistent with normal physiological growth. Conversely, the obese group gained the most weight (43. 1%), indicating that food manipulation was effective in producing obesity. This result is in line with previous studies that demonstrate significant weight gain and adiposity are caused by diets high in fat or calories (Wang et al., 2020). Interestingly, both treatment groups (Obese/Konjac and Obese/PPE) saw a considerable decrease in body weight, with declines of –14.62% and –16.33%, respectively. Additionally, the highly significant p-values (<0.001) confirm that the observed effects are closely linked to the interventions and not the product of chance. These results offer strong proof that natural dietary therapies like PPE and Konjac may be useful therapeutic agents for the treatment of obesity.

This study revealed significant variations in lipid profiles, particularly in relation to obesity-induced dyslipidemia. Figure 3 shows that the obese group's TC and TG were considerably higher than the control group. Furthermore, obese mice exhibited considerably reduced HDL-C and greater LDL-C. These findings are consistent with the known association between obesity and dyslipidemia, which is characterized by low HDL-C, and hypertriglyceridemia (Grundy, 2016). Following therapy with Konjac extract (Obese/Konj.), TC and TG were considerably lower than in the obese group. Furthermore, HDL-C levels slightly increased while LDL-C dropped. According to previous studies, glucomannan, a soluble fiber derived from Konjac root, can lower serum TG and TC levels by modifying bile acid metabolism and lipid absorption. These results suggest that Konjac may have a lipid-lowering effect (Zhang and Hu, 2020). It's interesting to note that the PPE-supplemented group's lipid profiles improved noticeably more (Obese/PPE). TG and TC levels decreased to 109.67 ± 4.15 mg/dL and 109.34 ± 3.42 mg/dL, respectively, whereas HDL-C sharply increased to 34.68 ± 1.87 mg/dL.

Accordingly, LDL-C was significantly lower (20.25 ± 1.68 mg/dL), closely matching the levels in the control group. These findings suggest that PPE supplementation restores lipid homeostasis more successfully than Konjac. These results imply that taking Konjac and PPE supplements together can assist with treating dyslipidemia caused by obesity, with PPE being more successful in restoring lipid balance.

The SOD and CAT activities were significantly lower in obese rats compared to the control group, while MDA levels were significantly greater. These findings imply a connection between obesity and oxidative stress, which is characterized by weakened antioxidant defenses and increased lipid peroxidation. This corroborates recent research showing that obesity increases reactive oxygen species (ROS) generation, overtaxing the antioxidant system and promoting oxidative damage (Furukawa et al., 2017). Compared to the obese group, PPE-treatment significantly boosted SOD and CAT activity and decreased hepatic MDA levels.

This suggests that in order to offer protection, PPE lowers lipid peroxidation and boosts antioxidant enzyme activity. PPE phytochemicals, particularly polyphenols such flavonoids and ellagitannins, have antioxidant qualities that reduce oxidative stress in obesity (Banihani, 2021). PPE supplementation could strengthen the body's natural antioxidant defenses and scavenge free radicals to lessen oxidative damage to liver cells (Abdel Moneim, 2012). Obese rats' serum TST, LH, and FSH levels differed significantly from those of the control group. Obesity was linked to a significant decrease in testosterone in comparison to controls, suggesting hypogonadism in obese individuals. This finding is consistent with previous studies demonstrating that obesity induces hormonal dysregulation by raising aromatase activity, which enhances the conversion of testosterone to estradiol and ultimately reduces blood levels of testosterone (Pasquali, 2021). Interestingly, there was no significant difference in LH levels across the groups ($p = 0.423$), even though TST significantly decreased in obese rats. A disruption of the hypothalamic-pituitary-gonadal axis is indicated by the lack of the expected compensatory increase in LH production in response to decreasing testosterone. Similar

findings have been documented, suggesting a condition of functional hypogonadism in obesity (Corona et al., 2019). Additionally, FSH levels were considerably lower in the obese group than in the control group confirming the idea that obesity inhibits the release of gonadotropin (Katib, 2015).

Reduced FSH may result in poor spermatogenesis, which is consistent with research linking obesity to male infertility (Sermondade et al., 2013). Following treatment with Konjac extract (Obese/Konj.) and PPE (Obese/PPE), testosterone levels were partially restored and were like the control group. These improvements suggest that both extracts include bioactive compounds that may stimulate androgen production or reduce oxidative stress in testicular tissue. PPE, on the other hand, contains a wealth of polyphenols with anti-inflammatory and antioxidant qualities that protect Leydig cells and increase testosterone release (Sayed, et al., 2025). According to the H &E staining and immunohistochemical analysis of testis sections from Gp2 showed substantial degenerative alterations in the seminiferous tubules, including a noticeable decrease in the quantity, disarray, and vacuolation of the majority of germinal epithelial cells. There were few Leydig cells and vacuoles in the interstitial tissue. Additionally, Bcl-2 immunostaining in seminiferous tubule cells was significantly higher in the obese group than in the control group. Many disorders, including obesity, are typically linked to apoptosis. Seminiferous tube structure and seminiferous luminal spermatozoa content significantly improved in Konjac and PPE-treated mice.

According to these findings, Konjac and PPE may enhance spermatogenesis in obese individuals. According to this study, testicular oxidative stress, inflammation, and apoptosis were brought on by HFD-induced obesity. In addition to controlling body weight, treatment with Konjac, or PPE, improved these symptoms.

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