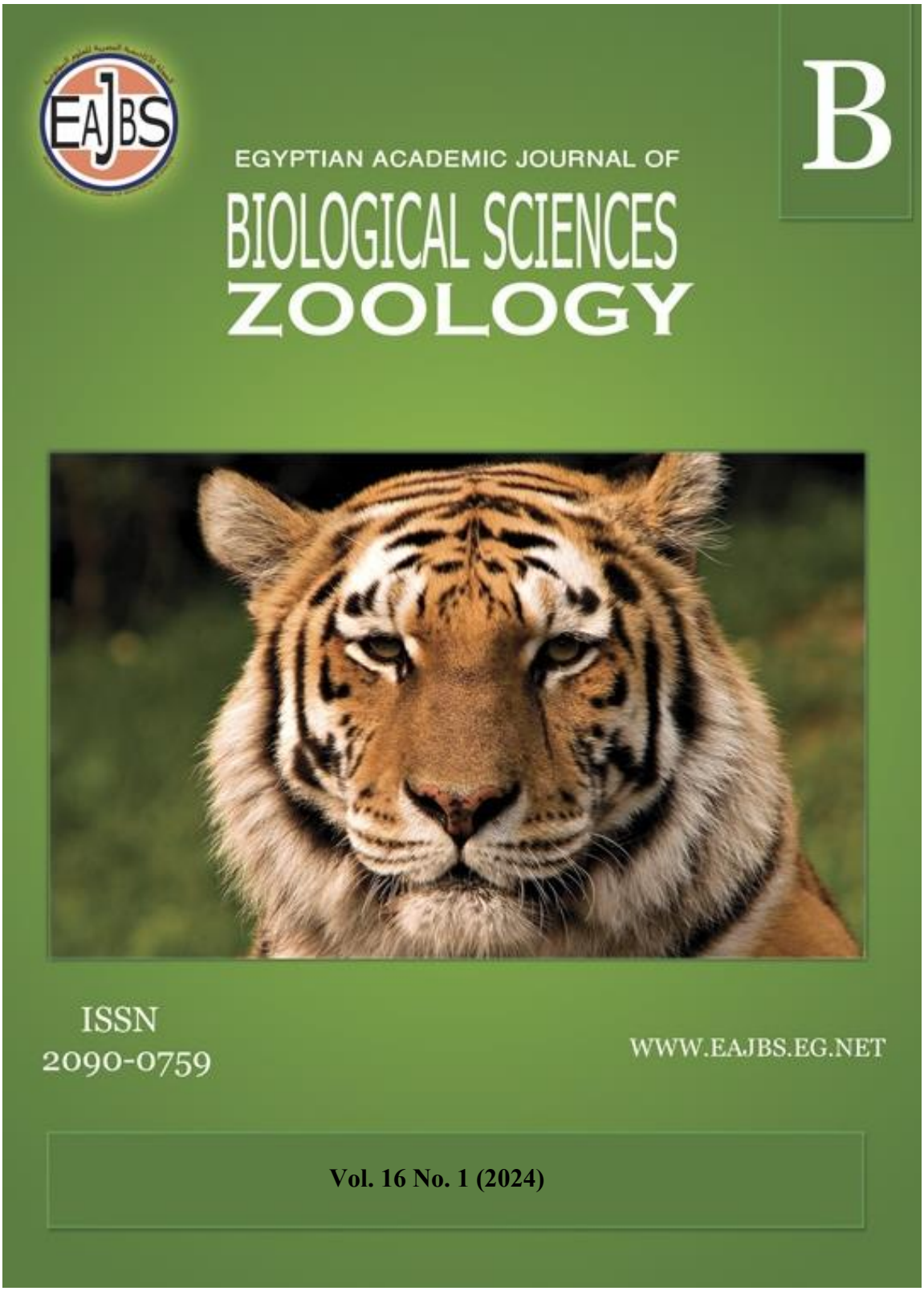

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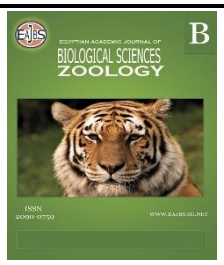

Mitigating Oxandrolone-Induced Cardiac and Hematological Changes with Bee Venom: Insights from Adult Female Rat Studies

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ABSTRACT

Anabolic androgens are used under medical supervision in treating certain diseases. Athletes up-use them to increase strength and endurance due to their lean body and muscle-building activities but this is prohibited to prevent their side effects. Nowadays females are interested in fantastic shapes affected by social media which encourages them to consume such androgens, especially oxandrolone. This research investigated possible bee venom antagonistic mechanisms to protect and cure body organs and functions against oxandrolone consumption adverse effects. Bee venom is selected due to its natural and unique components with many health-beneficial effects. Through this research oxandrolone overconsumption over time in female rats increased cardiac and ovarian tissue hypertrophy leading to their malfunction confirmed by significantly elevated ($p \leq 0.05$) cardiac serum function tests, increased plasma male sex hormone associated with decreased female sex hormones significantly ($p \leq 0.05$) in comparison with healthy group. Oxandrolone intake also increased the hematopoiesis to unpredictable levels. Oxandrolone initiated cardiac and ovarian lipolysis, oxidative and serum inflammatory status which arrested anti-oxidative and anti-inflammatory (heme oxygenase-1 and nuclear factor (erythroid-derived 2)-like 2) pathways that were confirmed by their gene expression. Contrary bee venom-treated groups in (a protective, curative, and co-administered) manner improved all biochemical and genetic deteriorations caused by oxandrolone consumption significantly ($p \leq 0.05$) via antioxidant and anti-inflammatory activities with the most significant improvement recorded in the protective group.

INTRODUCTION

Common or over-counter use of performance-enhancing drugs or supplements became a public health concern. These supplements are frequently utilized to improve muscle growth, athletic performance, and appearance in young and middle-aged individuals worldwide. Performance-enhancing supplements primarily consist of androgens, specifically androgenic anabolic steroids (Al Hashimi *et al.*, 2023).

Testosterone, the primary male sex hormone is commonly known as the progenitor of most anabolic-androgenic steroids. It results in the growth and advancement of secondary male physical characteristics. Because of its fat solubility, the substance disperses throughout the cell, penetrates the cell nucleus, interacts with it, induces biochemical alterations, and

triggers protein synthesis (Henriksen *et al.,* 2023).

Due to their protein synthesis ability; androgenic anabolic steroids were commonly used in treating hypogonadism and severe burnout but their abuse by athletes and bodybuilders is associated with many adverse effects (Abasnejad *et al.,* 2020).

Oxandrolone (Anvar) is a potent, oral anabolic androgen that is used in the treatment of several diseases including HIV-related muscle wasting, severe burn injury, trauma post-major surgery, neuromuscular diseases, and alcoholic hepatitis. It became popular among women to attain a slim body due to evolving societal pressures, such as the media, which encourage athletic beauty standards and muscular physiques for females (Piatkowski *et al.,* 2023).

Due to little or no investigation of the biochemical alterations caused by chronic consumption of anabolic steroids like oxandrolone in females; it became a necessity required with exploring possible natural treatments.Bee venom is a valuable product. It possesses a broad spectrum of biological impacts and is experiencing rapid growth in apitherapy utilization. Apitoxin, sometimes known as bee venom, is produced in the venom gland located in the abdomen of honey bees. Adult bees utilize it as their main colony defense mechanism (Bava *et al.,* 2023).

Bee venom comprises several biologically active compounds such as enzymes, peptides, amines, amino acids, phospholipids, minerals, carbohydrates, and volatile components. Key constituents of bee venom include melittin and phospholipase A2. These components have numerous healing properties. It has been utilized for millennia to combat various illnesses. Bee venom or its individual components are currently being utilized in multiple countries as a natural remedy for treating different disorders due to their minimal negative effects (Ullah *et al.,* 2023).

Consequently, this research was designed to investigate the biochemical alterations caused by oxandrolone consumption in female rats with possible protective and curative strategies of bee venom as a natural remedy.

MATERIALS AND METHODS

Rats, Diet and Chemicals:

Fifty adult female rats of (Sprague-Dawely) strain, 160–180g, purchased from The National Research Center, Giza, Egypt.

Balanced food is planned following the American Institute of Nutrition (AIN-1993 *M*) (Reeves *et al.,* 1993).

The Carniolan bee venom sample was collected from *Apis mellifera carnica* and derived fromThe Beekeeping Research Department of the Plant Protection Research Institute, Agriculture Research Centre, Giza, Egypt.

Oxandrolone (trade name Anavar) (17β-Hydroxy-17α-methyl-2-oxa-5α-androstan-3-one) in the form of tablets 10 mg each (*Meditech Human Pharmaceuticals, Ingoldheim, Germany*). Each three tablet was crushed and dissolved in 5 ml sesame oil to give each rat (30mg/kg body weight (b.wt) /day) orally on two injections (Ronchi *et al.,* 2021).

Experimental Design:

Animals were kept singular in steel crates with stable monitored temperature at 25±5°C, air humidity at 55%±10%, and light/dark cycle (12/12 hours). The overall animals were given balanced food and water *ad libitum* for 1 week (acclimatization period). Animals were separated into 5 groups, each one including ten rats -:

Group (1): Healthy control group, rats were given balanced food, orally treated with 5 ml sesame oil, and intraperitoneally injected with 1 ml saline every day for 3 months.

Group (2): Oxandrolone over supplemented group (**Oxandrolone group**), rats were given the balanced diet, orally treated with (30 mg/kg b. wt) oxandrolone dissolved in 5 ml sesame oil and intraperitoneally injected with 1ml saline every day for one month and a half.
Group (3): Protective group, rats were given the balanced diet and intraperitoneally injected with 1ml bee venom every day for one month and a half then orally treated with (30 mg/kg b.wt) oxandrolone dissolved in 5 ml sesame oil every day for one month and a half.
Group (4): Curative group, rats were given the balanced diet and orally treated with (30 mg/kg b.wt) oxandrolone dissolved in 5 ml sesame oil for one month and a half every day and then intraperitoneally injected with 1ml bee venom every day for one month and a half.
Group (5): CO-administered group, rats were given the balanced diet, orally treated with (30 mg/kg b.wt) oxandrolone dissolved in 5 ml sesame oil and intraperitoneally injected with 1ml bee venom every day for one month and a half.

Samples Collection:

After the experiment was finished, each experimental rat was weighed and sacrificed using anesthesia. Samples of blood were gathered in 3 vials during the di-estrus phase (determined by vaginal smear) (Marcondes et al., 2002). The first one contained an anticoagulant (heparin) to estimate complete blood count. The second vial was for obtaining serum and was reserved at -20°C until doing the serum analyses. The third vial is used to separate the plasma to determine sex hormones. The animal's heart and ovaries were isolated, rinsed with saline, dried on filter paper, weighed, and divided into 2 parts. The sample was homogenized in 2 mL of phosphate buffer saline (pH 7.4) using a Teflon homogenizer, followed by centrifugation at 3500 revolutions per minute for 15 minutes at 4 degrees Celsius. The supernatants were utilized for biochemical examination. The remaining portion was stored at -80°C until RNA extraction for gene analysis.

Determination of Cardiac and Ovarian Hypertrophy Indices:

Isolated hearts and ovaries from the experimental animals were weighed. The index of cardiac and ovarian hypertrophy was calculated as indicated by heart or ovary weight to body weight (Kumar *et al.*, 2012).

Hematological Measurements:

Complete blood count (CBC) and blood indices were processed with a blood counter model Kx-21 system (Beckman Coulter MAXM Analyzer, electronic kobe, Japan) (Johnson et al., 2022).

Determination of Cardiac Dysfunction:

Serum lactate dehydrogenase (LDH), creatine kinase-MB (CK-MB) activities, and troponin I (TnI) level were determined (Bergmeyer,1974; Wu and Bowers,1982 and Kiyasu,1985) using commercial kits derived from (Spinreact, Spain, Spectrum Diagnostics company, Egypt and ELISA kits derived from Eagle Biosciences company, USA) respectively.

Determination of Plasma Sex Hormones:

Plasma sex hormones such as free testosterone, progesterone, and estradiol were measured using ELISA kits obtained from Sigma Aldrich Co., USA (Catalogue # SE120089, SE120087, and SE120084, respectively), following the manufacturer's instructions. Plasma levels of luteinizing hormone (LH) and antimullerian hormone (AMH) were quantified using ELISA kits from MyBioSource, Inc., San Diego, CA, USA (Catalogue # MBS764675 and MBS264077, respectively), following the provided manual.

Estimation of Cardiac and Ovarian Tissue Oxidative Stress Parameters:

Tissues malondialdehyde (MDA), reduced glutathione (GSH), nitric oxide (NO) levels, and catalase (CAT) activity were determined using colorimetric kits manufacturer's instructions purchased from Biodiagnostic Company (Dokki, Giza, Egypt).

Serum Inflammatory Markers Determination:

Tumor necrosis factor-α (TNF- α), C-reactive protein (CRP), transforming growth factor beta1 (TGF-β1) levels and myeloperoxidase (MPO) activity were estimated using ELISA kits obtained from RayBiotech, Inc., USA (CAT.NO. ELR-TNFa-1), Thermo Fisher Scientific, Inc., Germany (CAT.NO.BMS623-3), MyBiosource company, San Diego, California, USA (CAT.NO.MBS2508830) and Abcam, USA (CAT.NO.ab105136) respectively according to the manufacturer's protocols.

Quantification of HO-1 and Nrf2 in Cardiac and Ovarian Tissues:

RNA was extracted from the cardiac ventricle and ovarian tissue samples using the Invitrogen™ TRIzol™ Plus RNA Purification Kit from Life Technologies. Total RNA was converted to cDNA using a high-capacity cDNA reverse transcription kit from Applied Biosystems. The ABI Prism 7500 Device was utilized for quantitative analysis of heme oxygenase-1 (HO-1) and nuclear factor (erythroid-derived 2)-like 2 (Nrf2) genes in 96-well optical reaction plates from Applied Biosystems. Rat primers for nuclear factor erythroid 2-related factor 2 (Nrf2) were 5′-GCTGCCATTAGTCAGTCGCTCTC -3′ and 5′-ACCGTGCCTTCAGTGTGCTTC-3′, while heme oxygenase1(HO-1) primers were 5′-TTAAGCTGGTGATGGCCTCC -3′ and 5′-GTGGGGCATAGACTGGGTTC-3′, as per manufacturer's guidelines.

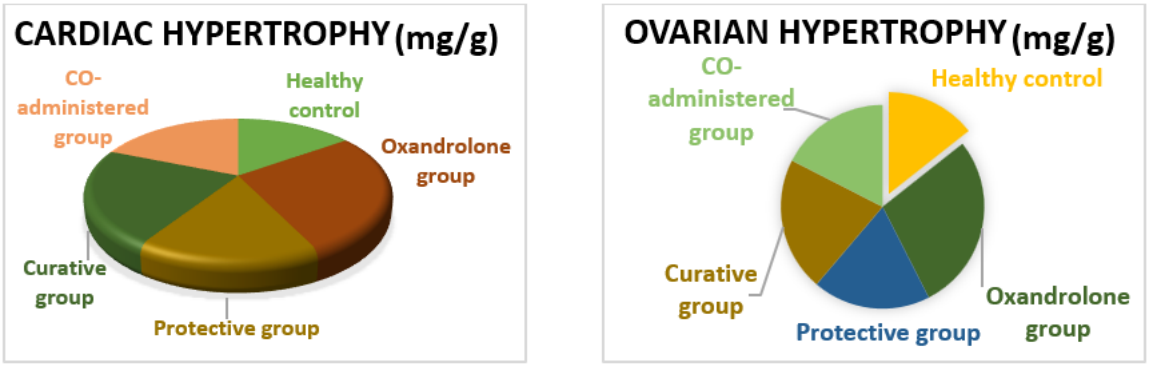
Statistical Analysis:

The data were analyzed using SPSS version 21.0. One-way analysis of variance (ANOVA) was used to conduct multiple comparisons. Statistical significance was determined based on p-values of ≤ 0.05 for differences in means among the various groups (Levesque,2007).

RESULTS

Cardiac and Ovarian Hypertrophy:

Figure 1 (a and b) illustrated that oxandrolone continued consumption resulted in cardiac and ovarian significant (p≤0.05) hypertrophy due to thickening and increased weight relative to body weight affecting their functions badly. Bee venom significantly (p≤0.05) counteracted tissue hypertrophy in supplemented groups with the best improvement (p≤0.05) recorded in the protective group.



(a) (b)

Fig. 1: Cardiac (**a**) and ovarian (**b**) hypertrophy in studied female rats.

Hematological Parameters:

One function of oxandrolone is to treat anemia and induce hematopoiesis and this significantly (p≤0.05) increased all blood components including RBC's, WBC's and PLT counts as well as Hb content in the oxandrolone group; **(Table 1).** Bee venom health-promoting functions significantly (p≤0.05) balanced hematological alterations caused by

oxandrolone over ingestion in curative, co-administered, and protective groups in an ascending ordered manner.

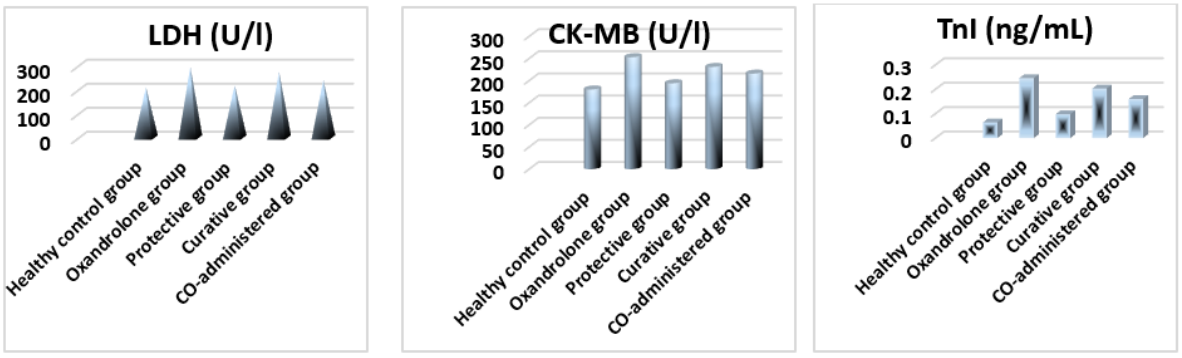
Table 1: Complete blood picture in studied female rats

Groups / Parameter	RBC's (10^6/µl)	WBC's (10^3/µl)	PLT (10^3/µl)	Hb (g/dl)
Healthy control group	10.17±0.25^{e}	11.34±0.42^{e}	186.32±4.12^{e}	14.09±0.39^{e}
Oxandrolone group	19.23±1.21^{a}	16.95±0.72^{a}	233.81±6.04^{a}	17.91±0.95^{a}
Protective Group	12.96±0.39^{d}	12.09±0.11^{d}	198.24±5.67^{d}	15.12±0.61^{d}
Curative group	17.16±0.84^{b}	15.10±0.31^{b}	221.19±3.97^{b}	17.03±0.40^{b}
CO-administered group	14.80±0.61^{c}	13.87±0.20^{c}	209.63±4.31^{c}	16.27±0.28^{c}

Values were expressed as average ±SD, n=10; with no significant differences among averages with the same letters in the same column.

Serum Cardiac Function:

On the determination of the cardiac function of tested female rats Figure 2 (a ,b and c), it became obvious that oxandrolone overconsumption for a long time significantly ($p \leq 0.05$) mangled heart functions by increasing LDH, CK-MB activities, and TnI level in comparison to the healthy control group. Bee venom consumption ameliorated cardiac dysfunction in different ingestion patterns with the most significant enhancement ($p \leq 0.05$) in the protective group.



(a) **(b)** **(c)**

Fig. 2: Cardiac function tests [LDH (a), CK-MB (b) activities, and TnI (c) level] in studied female rats.

Plasma Sex Hormones:

Oxandrolone is an anabolic steroid and its intake in female rats caused hormonal disturbances, **(**Table 2**)** by significant increment ($p \leq 0.05$) in testosterone level accompanied by decreased female sex hormone levels. On the other hand, bee venom counteracted oxandrolone-induced hormonal disturbances in supplied groups. The protective group showed the most significant ($p \leq 0.05$) hormonal regulation compared with the oxandrolone group.

Table 2: Plasma sex hormone levels in studied female rats

Groups / Parameter	Testosterone (ng/ml)	Progesterone (ng/ml)	Estradiol (pg/ml)	LH (ng /ml)	AMH (ng/ml)
Healthy control group	1.67±0.19^{e}	13.78±0.76^{a}	10.90±0.63 ^{a}	0.94±0.11 ^{a}	2.66±0.57 ^{a}
Oxandrolone group	6.93±0.34^{a}	7.82±0.31^{e}	6.41±0.25 ^{e}	0.31±0.04 ^{e}	0.98±0.09 ^{e}
Protective group	2.46±0.15^{d}	11.60±0.42^{b}	9.43±0.51 ^{b}	0.80±0.05 ^{b}	2.09±0.14 ^{b}
Curative group	5.78±0.21^{b}	9.11±0.18^{d}	7.75±0.40 ^{d}	0.49±0.03 ^{d}	1.79±0.32 ^{d}
CO-administered group	3.63±0.26^{c}	10.83±0.37^{c}	8.54±0.46 ^{c}	0.61±0.07 ^{c}	1.16±0.29 ^{c}

Values were expressed as average ±SD, n=10; with no significant differences among averages with the same letters in the same column.

Cardiac and Ovarian Oxidative Status:

Overconsumption of oxandrolone on time initiated an oxidative status in different body organs. Figure 3 (a, b, c and d) and Figure 4 (a, b, c and d) showed that oxandrolone increased lipid peroxidation and formation of reactive nitrogen species significantly ($p \leq 0.05$) in cardiac and ovarian tissues with decreased antioxidant power by decreasing GSH level and CAT activity. Antioxidant properties of bee venom voided significantly ($p \leq 0.05$) oxandrolone-induced oxidative status in female rats in all tested groups.

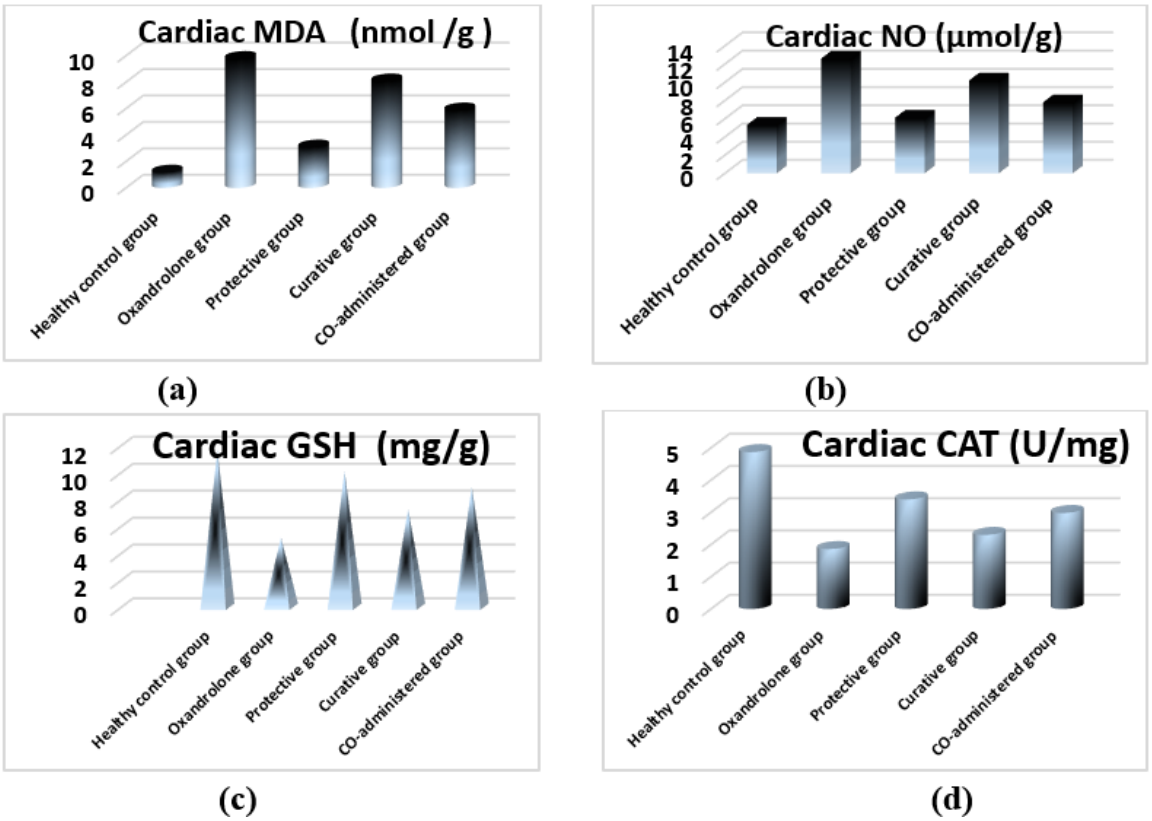


Fig. 3: Cardiac oxidative status parameters [MDA (a), NO (b), GSH(c) levels and CAT (d) activity] in studied female rats.

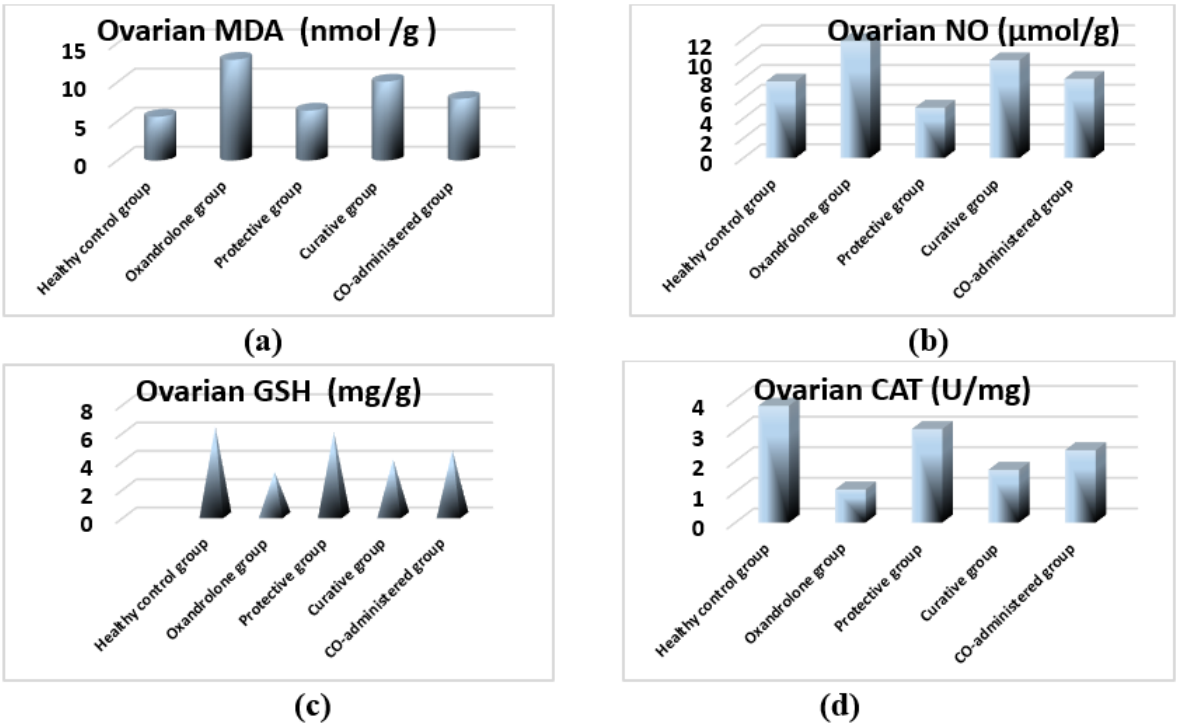


Fig. 4: Ovarian oxidative status parameters [MDA (a), NO (b), GSH(c) levels and CAT (d) activity] in studied female rats.

Serum Inflammatory Markers:

Inflammatory markers (Table 3); increased significantly ($p \leq 0.05$) in the serum of female rats who consumed oxandrolone as an indication of an inflammatory status and organ dysfunction. Inflammatory markers decreased significantly ($p \leq 0.05$) in protective, co-administered, and curative groups respectively due to bee venom biological effects.

Table (3): Serum inflammatory markers in studied female rats

Groups / Parameter	TNF- α (pg /ml)	CRP (pg/ml)	TGF-β1 (pg/ml)	MPO (U/L)
Healthy control group	26.23±1.51^{e}	16.90±0.30^{e}	20.81±0.41^{e}	11.19±0.11^{e}
Oxandrolone group	45.14±2.08^{a}	37.67±0.57^{a}	53.01±1.94^{a}	29.61±0.39^{a}
Protective group	29.12±1.69^{d}	20.96±0.43^{d}	34.93±1.36^{d}	14.28±0.17^{d}
Curative group	40.29±1.24^{b}	31.57±0.27^{b}	48.89±0.89^{b}	22.12±0.65^{b}
CO-administered group	36.51±2.15^{c}	27.14±0.16^{c}	42.36±0.50^{c}	17.91±0.20^{c}

Values were expressed as average ±SD, n=10; with no significant differences among averages with the same letters in the same column.

Cardiac and Ovarian HO-1 and Nrf2 mRNA Gene Expression:

Oxandrolone inhibited the HO-1/Nrf2 pathway significantly ($p \leq 0.05$) in Figure 5 (a and b) and Figure 6 (a and b). This explained the oxidative and inflammatory status in affected female rats. Contrary bee venom initiated and activated the HO-1/Nrf2 pathway ($p \leq 0.05$) inside affected organs as the heart and ovaries in supplemented groups to counteract oxidative stress and inflammation. Intake of bee venom before oxandrolone as a protective strategy recorded the most marked ($p \leq 0.05$) improvement.

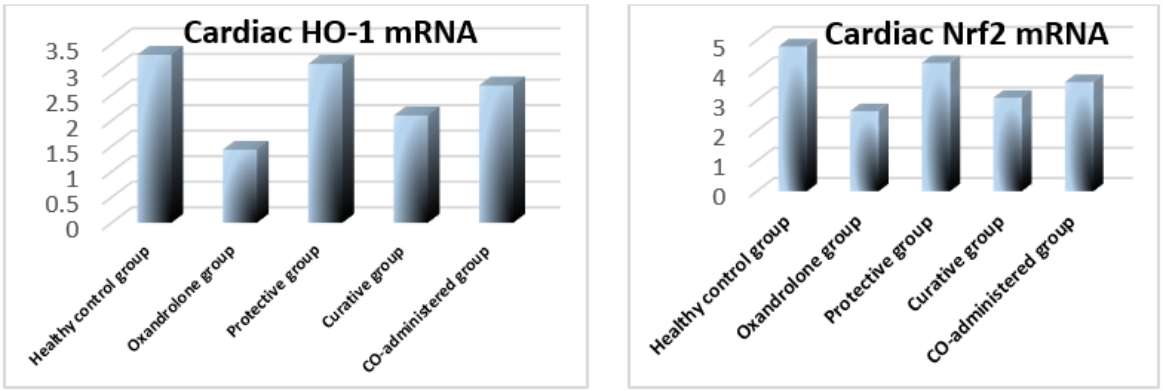


Fig. 5: Cardiac mRNA expression of [HO-1(a) and Nrf2 (b)] in studied female rats.

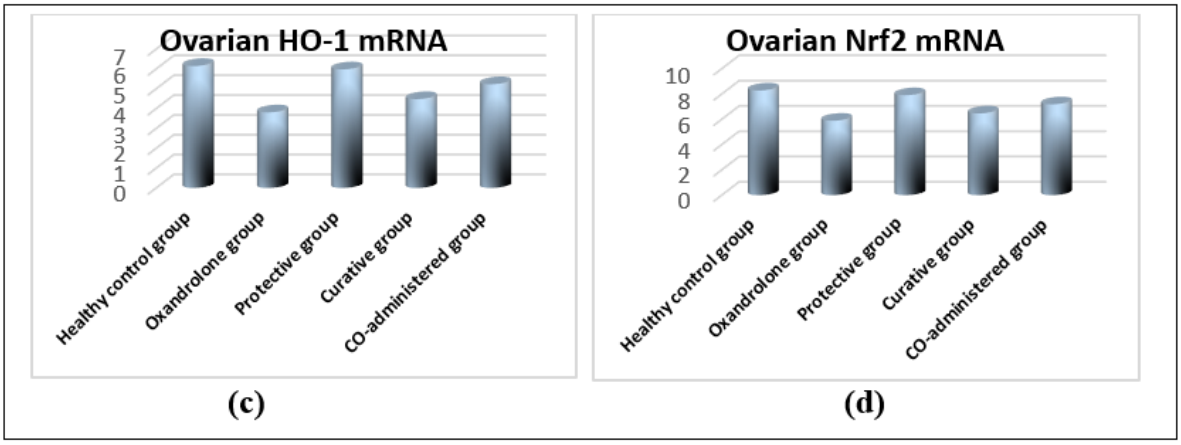


Fig.6: Ovarian mRNA expression of [HO-1(a) and Nrf2 (b)] in studied female rats.

DISCUSSION

This study aimed to provide a unique contribution to the existing literature on gender-specific information and women's utilization of anabolic steroids. There is a growing trend of women feeling increasingly empowered to engage in strength sports and develop greater muscular mass. Engaging in these sports may lead to a higher likelihood for women to subsequently misuse anabolic steroids. Women who choose to utilize anabolic steroids often prefer oral formulations, such as oxandrolone. Oxandrolone, commonly known by its brand name Anavar, is sometimes used by females to enhance muscle growth, strength, and definition. However, it is important to approach its use with caution due to potential side effects, such as the development of male secondary sexual characteristics in women.

To improve human and animal health defects, the use of natural items as substitutes for synthetic medications is becoming more and more advised as apitherapy. Bee venom therapy has been used to treat a variety of illnesses. It contains physiologically active components with medicinal qualities. It comprises tiny peptides, amines, proteins, and enzymes, including phospholipase A2, melittin, apamin, and other elements related to sugars, and minerals (Bava *et al.*, 2023).

Data illustrated that bee venom-treated groups improved significantly ($p \leq 0.05$) the thickness and weight of cardiac and ovary tissues compared with the oxandrolone group. Oxandrolone increased tissue hypertrophy due to increased testosterone production. It increases lean body mass and tissue body weight due to its anabolic activity that has been recorded previously (Grunfeld *et al.*, 2006). Oxandrolone has anabolic activity by binding to

its androgen receptor in skeletal muscle and inducing muscle protein synthesis. Oxandrolone also inhibits muscle catabolism by working as a glucocorticoid receptor antagonist (Gusti *et al.*, 2022). Anabolic androgenic steroids, when used chronically, enhance muscle growth and performance by binding to androgenic receptors, which triggers various cellular processes like gene transcription, second messenger signaling, and satellite cell activation. This leads to increased muscle protein synthesis and reduced breakdown, along with the growth of visceral adipose tissue (McCullough *et al.*, 2021).

Due to bee venom's active constituents with various healing and medicinal activities consumption corrected tissue hypertrophy. Bee venom reduced ovarian enlargement in rats by decreasing the thickness of the follicular theca layer, potentially through increased lipolysis and reduced hypertrophy of this layer (Karimzadeh et al., 2012). Shi *et al.* (2022) notified that active components of bee venom reduce fat accumulation in various body tissues and enhance heart weight in conditions like obesity and diabetes by blocking pathways such as p-adenosine monophosphate-activated protein kinase (AMPK) and p-acetyl-coenzyme A carboxylase (ACC) during the development of 3T3-L1 preadipocytes.

The hematological measures indicated a significant increase ($p \leq 0.05$) in red blood cells, hemoglobin, white blood cells, and platelet counts. This is referred to as the effect of androgens on boosting the hematopoietic system through different methods. These factors involve promoting the release of erythropoietin, enhancing bone marrow function, and facilitating the integration of iron into red blood cells (Płoszczyca *et al.*, 2024). The elevated white blood cell count is mostly due to an increase in the percentage of type B lymphocytes. The results showed a substantial rise ($p \leq 0.05$) in the percentage of lymphocytes. This could be due to oxandrolone's ability to induce inflammatory reactions that elevate lymphocyte counts. Urhausen *et al.*, (2003) noted that the androgen receptor is present in megakaryocytes and is regulated by androgens. Androgens play a role in thrombopoiesis. Reports from anabolic steroid users indicate that patients undergoing androgen therapy for aplastic anemia have seen elevated platelet counts and thrombocytosis.

Furthermore, the current study shows an increase in the PLT count overall in the oxandrolone group, these results agree with (Khetawat *et al.*,2000). Shahani *et al.*, (2009) discovered that testosterone increases the density of human platelet A2 receptors, which in turn raises human platelet aggregation responses. This finding has led to speculation that testosterone may contribute to the thrombogenicity of anabolic steroids.

Regarding the effect of bee venom on hematological parameters, it was observed that its intake in all treated groups improved hematological parameters but remained higher than the healthy control group. Bee venom can improve blood circulation in micro-blood capillaries, raise coronary and peripheral circulations, and stimulate the production of new erythrocytes (Shi *et al.*, 2022 and Abo-Zaid *et al.*, 2023). These results are in agreement with the results of Mohammed and Hassan, (2019) who recorded a significant increase in the values of Hb with a decrease in the values of WBC's and platelets in the bee venom-treated arthritis groups.

Results illustrated a significant elevation ($p \leq 0.05$) in the activities of LDH, CK-MB, and TnI levels of the oxandrolone group when compared to the control group. Organ health is negatively impacted by anabolic androgenic steroids. When anabolic androgenic drugs are utilized, oxidative stress and apoptosis are two causes of harm (Ayubi *et al.*, 2023). The most common organ affected by anabolic androgenic steroid use is the heart; prolonged high-dose administration of these drugs causes cardiac dysfunction due to increased bad lipid fraction as low-density lipoprotein, insulin resistance, and increased visceral fat deposition linked with hypertension (McCullough *et al.*, 2021).

Also, these results are in accordance with Shaaban et al., (2019) as the maximum serum troponin I level was recorded in the oxandrolone group. In contrast, the protective group had the lowest amount. The antioxidant properties of bee venom, which inhibit the

production of free radicals, protect the heart restricting the release of such tested enzymes by scavenging free radicals, stopping lipid peroxidation of membranes, and preserving membrane integrity. According to reports, bee venom could improve heart function by reducing oxidative stress and inhibiting the NF-kβ pathway (Shi *et al.,* 2022 and Bava *et al.,* 2023).

Testosterone level was elevated significantly ($p \leq 0.05$) accompanied by decreased female sex hormone levels in the oxandrolone group on continuous intake (Jeschke *et al.,* 2007) which may cause de-menstruation. High concentrations of anabolic androgens exhibit an antiestrogenic impact because of a decreased activity of androgen receptors and competing with estrogens for their receptors (Arazi *et al.,* 2017).

Conversely, a significant ($p \leq 0.05$) decreased testosterone and increased female sex hormones, respectively in all the bee venom-treated groups (protective, curative, and CO-administered groups) as compared to the oxandrolone group. Bee venom has the potential to enhance the reproductive characteristics of both male and female rabbits. This suggests that bee venom could serve as a sustainable synthetic chemical medication (sex stimulants) in rabbit breeding, leading to enhancements in reproductive traits, immune system response, and overall health (El-Hanoun *et al.,* 2020 and Elkomy *et al.,* 2021).

Oxandrolone intake initiated oxidative stress by increasing lipolysis, lipid peroxidation and reactive oxygen species (ROS) levels while arresting antioxidant activities and levels. Oxandrolone abuse can promote changes in redox balance and induce oxidative stress (Ronchi *et al.,* 2021). Cytochrome P450 mono-oxygenases metabolize anabolic steroids at high concentrations (Pey *et al.,* 2003 and Arazi *et al.,* 2017). Reactive oxygen species are produced as a result, which causes an up-regulation eventually leading to the antioxidant enzymes' activity running out. Additionally, anabolic steroids boost the lipase enzyme's activity, which raises the rate of lipolysis, this consequently raises the availability of long-chain fatty acids for ATP synthesis and mitochondrial oxidation, which develops lipid peroxidation and produces ROS (Langfort *et al.,* 2010).

On the other hand, bee venom groups improved these results due to its strong antioxidant effect (Bava et al., 2023) which impeded lipid peroxidation, reduced MDA and ROS levels with increased antioxidant enzyme activities and non-enzymatic antioxidant levels as GSH in different body tissues (Shi *et al.,* 2022 and Abo-Zaid *et al.,* 2023).

On consumption of anabolic androgens as a treatment for burns or wasting diseases; they are anti-inflammatory agents. Contrary chronic consumptions of these androgens initiate an inflammatory process. Results reported that the levels of serum inflammatory markers in the oxandrolone group were significantly ($p \leq 0.05$) elevated compared to the healthy control group. Chronic administration of large amounts of anabolic androgens can cause inflammation that might contribute to triggering cardiac injury (Arazi *et al.,* 2017 and Albano *et al.,* 2021). Bee venom has an anti-inflammatory effect by suppressing the activity of pro-inflammatory cytokines. These findings were consistent with the study by Lee *et al.,* (2020) who demonstrated that treatments with apamin and bee venom were notably successful in reducing the production of key inflammatory cytokines. Bee venom and its primary constituents suppress the body's redox equilibrium, inhibit the production of inflammatory cytokines and chemokines, and modify the expression of proteins associated with cell apoptosis (Abo-Zaid *et al.,* 2023 and Bava *et al.,* 2023).

The transcription factor Nrf2 is crucial in regulating the expression of antioxidant genes that have anti-inflammatory effects. Nrf2 and Keap1 are key players in maintaining redox balance within cells and controlling inflammation. Nrf2 has been shown to play a role in regulating the heme oxygenase-1 (HO-1) axis, which is a powerful target for reducing inflammation. Recent research demonstrated a link between the Nrf2/antioxidant response element system and the regulation of inflammatory mediators, the NF-κB pathway, and macrophage metabolism. As a result of inflammation associated with increased lymphocyte

count leading to inflammatory cytokines production the Nrf2 and HO-1 gene expression in ovaries and cardiac tissues were arrested in the oxandrolone group on the other hand bee venom antioxidant and anti-inflammatory components linked activities improved gene expression of both Nrf2 and HO-1 in affected tissues counteracting oxidative stress and inflammation (Saha *et al.,* 2020 and Nguyen *et al.,* 2022).

CONCLUSION

Anabolic androgen misuse became a public health concern due to different associated adverse effects. Oxandrolone female overconsumption progressively increased to achieve body shaping and building. Oxandrolone caused biochemical and genetic alterations with many deleterious health sequences. Finding natural remedies is essential to compete with such stresses. Bee venom contains various active constituents with many health-beneficial activities that can counteract oxandrolone side effects. Anabolic androgens especially oxandrolone must be included in prescribed drugs and not be used except under medical supervision. Bee products especially bee venom extraction, production, and validation for human consumption to improve general health and antagonize possible biological alterations must be promoted.

Declarations:

Ethical Approval: The research ethics committee of the Faculty of Women for Arts, Science, and Education maintains records pertaining to animal laboratory regulations and treatment (sci1432305003) that were followed during the animal experiments.

Conflicts of Interest: The authors claim that there are no conflicts of interest.

Authors Contributions:I hereby verify that all authors mentioned on the title page have made substantial contributions to the conception and design of the study, have thoroughly reviewed the manuscript, confirm the accuracy and authenticity of the data and its interpretation, and consent to its submission.

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REFERENCES

Abasnejad , M.; Mohammadi A.; and Khajehlandi, A.(2020) . Investigation of the chronic effects of winstrol and oxandrolone anabolic steroids on liver enzymes in male bodybuilding athletes. *Jundishapur Journal of Chronic Disease Care,* 9(3): e106517. https://doi.org/10.5812/jjcdc-141089.

Abo-Zaid, M. A.; Yatimi, K. A.; and Ismail, A. H. (2023). The role of bee venom on immunological and hematological parameters in albino rats. *The Egyptian Journal of Immunology,* 30(2), 11–25.

Al Hashimi, M.; Farahat, Y.; Kandil, H.; and Al Khalidi, I. (2023). Androgenic-anabolic steroid abuse trend and management: A prospective, cross-sectional, questionnaire-based survey. *Health Science Reports,* 6(1): e1032. https:// doi. org/10. 1002/ hsr2. 1032.

Albano, G. D.; Amico, F.; Cocimano, G.; Liberto, A.; Maglietta, F.; Esposito, M.; Rosi, G. L.; Di Nunno, N.; Salerno, M.; and Montana, A. (2021). Adverse effects of anabolic-androgenic steroids: A literature review. *Healthcare (Basel, Switzerland),* 9(1):97. https://doi.org/10.3390/healthcare9010097.

Arazi, H.; Mohammadjafari, H.;and Asadi, A.(2017). Use of anabolic androgenic steroids produces greater oxidative stress responses to resistance exercise in strength-trained men. *Toxicology Reports*, 4: 282–286. https://doi.org/ 10.1016/j. toxrep. 2017.05.005.

Ayubi, N.; Kusnanik, N. W.; Herawati, L.; Komaini, A.; Mutohir, T. C.; Callixte, C.; Aljunaid, M.; Nurhasan, N.; Muhammad, H. N.; Purwanto, B.; Rifki, M. S.; Putri,

D. R. S.; and Syafawi, A. (2023). Abuse of anabolic-androgenic steroids and adverse effects on human organ health: A Review. *Biointerface Research in Applied Chemistry, 13*(3): 281. https://doi.org/10.33263/BRIAC133.281

Bava, R.; Castagna, F.; Musella, V.; Lupia, C.;Palma, E.; and Britti, D. (2023). Therapeutic use of bee venom and potential applications in veterinary medicine. *Veterinary Sciences,* 10(2): 119. https://doi.org/10.3390/vetsci10020119.

Bergmeyer, HU.(1974). Methods of enzymatic analysis I. 2nd Edition. New York: Academic Press, pp.321–7.

El-Hanoun, A.; El-Komy, A.; El-Sabrout, K.; and Abdella, M. (2020). Effect of bee venom on reproductive performance and immune response of male rabbits. *Physiology and Behaviour,* 223: 112987. https://doi.org/10.1016/j.physbeh.2020.112987.

Elkomy, A.; El-Hanoun, A.; Abdella, M.; and El-Sabrout, K. (2021). Improving the reproductive, immunity and health status of rabbit does using honey bee venom. *Journal of Animal Physiology and Animal Nutrition,* 105 (5): 975–983. https://doi.org/10.1111/jpn.13552.

Grunfeld, C.; Kotler, D.P.; Dobs, A.; Glesby, M.; and Bhasin ,S. (2006). Oxandrolone in the treatment of HIV-associated weight loss in men: a randomized, double-blind, placebo-controlled study. *Journal of Acquired Immune Deficiency Syndromes,* (41): 304-311. https://doi .10.1097/01.qai.0000197546.56131.40.

Gusti, N. R. L.; Saputro, I. D.; Rizaliyana, S.; and Putra, O. N. (2022). Effects of oxandrolone on lean body mass (LBM) in severe burn patients: A randomized, double-blind, placebo-controlled trial. *Annals of Burns and Fire Disasters,*35(1): 55–61.

Henriksen, H. C. B.; Havnes, I. A.; Jørstad, M. L.; and Bjørnebekk, A. (2023). Health service engagement, side effects and concerns among men with anabolic-androgenic steroid use: a cross-sectional Norwegian study. *Substance Abuse Treatment, Prevention, and Policy,* 18(1): 19. https://doi.org/10.1186/s13011-023-00528-z.

Jeschke, M. G.; Finnerty, C. C.; Suman, O. E.; Kulp, G.; Mlcak, R. P.; and Herndon, D. N. (2007).The effect of oxandrolone on the endocrinologic, inflammatory, and hypermetabolic responses during the acute phase postburn. *Annals of Surgery,* 246(3), 351–362. https://doi.org/10.1097/SLA.0b013e318146980e.

Johnson, A.; Huang, Y. C.; Mao, C. F.; Chen, C. K.; Thomas, S., Kuo, H. P.; and Kong, Z. L. (2022). Protective effect of ethanolic extract of *Echinacea purpurea* contained nanoparticles on meniscal/ligamentous injury induced osteoarthritis in obese male rats. *Scientific Reports,* 12(1):5354. https://doi.org/ 10.1038/s41598-022-09380-w.

Karimzadeh, L.; Nabiuni, M.; Sheikholeslami, A.; and Irian, S. (2012). Bee venom treatment reduced C-reactive protein and improved follicle quality in a rat model of estradiol valerate-induced polycystic ovarian syndrome. *Journal of Venomous Animals and Toxins including Tropical Diseases,* 18(4), 384–392. https://doi.org/ 10.1590/ S1678-91992012000400006.

Khetawat, G.; Faraday, N.; Michele, L.; Nealen, K.; Vijayan, V.; Bolton, E.; Stephen, J.; Paul, N.; and Bray, F.(2000). Human megakaryocytes and platelets contain the estrogen receptor β and androgen receptor (AR): Testosterone regulates AR expression. *Blood,* 95(7): 2289–2296. https://doi.org/ 10.1182/BLOOD. V95. 7.2289.

Kiyasu, Y. (1985). Current status of detecting Troponin-I for patient management. *American Clinical Production Reviews,*4:29–31.

Kumar, S.; Singh, R.; Vasudeva, N.; and Sharma, S. (2012). Acute and chronic animal models for the evaluation of anti-diabetic agents. *Cardiovascular Diabetology,*(11): 9. https://doi.org/10.1186/1475-2840-11-9.

Langfort, J.; Jagsz, S.; Brzezinska, Z.; Klapcinska, B.; Galbo, H.; Gorski, J.; and Dobrzyn, P. (2010). Testosterone affects hormone-sensitive lipase (HSL) activity and lipid

metabolism in the left ventricle. *Biochemical and Biophysical Research Communications. 4*: 670-676. https://doi.org/ 10.1016/j.bbrc.2010.07.140

Lee, Y. M.; Cho, S. N.; Son, E.; Song, C. H.; and Kim, D. S. (2020). Apamin from bee venom suppresses inflammation in a murine model of gouty arthritis. *Journal of Ethnopharmacology,* 257: 112860. https://doi.org/ 10.1016/J.JEP.2020.112860.

Levesque, R. (2007). SPSS programming and data management: a guide for SPSS and SAS users. Spss.3 rd edition, USA.

Marcondes, F. K.; Bianchi, F. J.; and Tanno, A. P. (2002). Determination of the estrous cycle phases of rats: some helpful considerations. *Brazilian journal of biology = Revista brasleira de biologia,* 62(4A): 609–614. https://doi.org/10.1590/s1519-69842002000400008.

McCullough, D.; Webb, R.; Enright, K. J.; Lane, K. E.; McVeigh, J.; Stewart, C. E.; and Davies, I. G. (2021). How the love of muscle can break a heart: Impact of anabolic androgenic steroids on skeletal muscle hypertrophy, metabolic and cardiovascular health. *Reviews in Endocrine and Metabolic Disorders,* 22(2):389–405. https:// doi.org/10.1007/s11154-020-09616-y.

Mohammed, Z. I.; and Hassan, A. J. (2019). Effect of bee venom on some blood and biochemical parameters in formaldehyde induced arthritis male rats in comparison with prednisolone drug. *Journal of Physics: Conference Scientific,* 1234(1), 1-11. https://doi.org/ 10.1088/1742-6596/1234/1/012066.

Nguyen, C. D.;Yoo, J.; Hwang, S. Y.; Cho, S. Y.; Kim, M.; Jang, H.; No, K. O.; Shin, J. C.; Kim, J. H.; and Lee, G. (2022). Bee venom activates the nrf2/ho-1 and trkb/creb/bdnf pathways in neuronal cell responses against oxidative stress induced by $a\beta_{1-42}$. *International journal of Molecular Sciences,* 23(3):1193.https://doi. org/ 10.3390/ijms23031193.

Pey, A.; Saborido, I.; Blázquez, J.; Delgado, A.; and Megías, A. (2003) .Effects of prolonged stanozolol treatment on antioxidant enzyme activities, oxidative stress markers, and heat shock protein HSP72 levels in rat liver. *Journal of Steroid Biochemistry and Molecular Biology,* 87(4–5): 269–277. https://doi. org/ 10. 1016/j.jsbmb.2003.09.001.

Piatkowski, T.; Robertson, J.; Lamon, S.;and Dunn, M. (2023). Gendered perspectives on women's anabolic-androgenic steroid (AAS) usage practices. *Harm Reduction Journal,* 20(1): 56. https://doi.org/10.1186/s12954-023-00786-x.

Płoszczyca, K. ; Czuba, M.; Zakrzeska, A.; and Gajda, R.(2024). The Effects of six-gram d-aspartic acid supplementation on the testosterone, cortisol, and hematological responses of male boxers subjected to 11 days of nocturnal exposure to normobaric hypoxia. *Nutrients,* 16(1):76. https://doi.org/10.3390/nu16010076.

Reeves, P.; Nielsen, F.; Fahe, G; and Jr, M. (1993) AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the formulation of the AIN-76A rodent diet. *The Journal of Nutrition,* 123(11):1939–1951. https://doi.org/10.1016/j.tjnut.2023.12.011.

Ronchi, S. N.; Mass, E. M. S. W.; Bernardina, N. R. D.; de Melo Júnior, A. F.; Dos Santos, W. C.; de Andrade, T. U.; Brasil, G. A.; and Bissoli, N. S. (2021). Low and high doses of oxandrolone promote pathological cardiac remodeling in young male rats. *Steroids,* (170): 108814. https://doi.org/10.1016/j.steroids.2021.108814.

Saha, S.; Buttari, B.; Panieri, E.; Profumo, E.; and Saso, L.(2020). An Overview of Nrf2 signaling pathway and its role in inflammation. *Molecules. 25*:5474. https:// doi. org/10.3390/molecules25225474

Shaaban, M. M.; Hamza, R. G.; and El shahat, A. N. (2019). Studying the ameliorative effect of bee venom against damage and inflammation induced in gamma-irradiated rats.

Arab Journal of Nuclear Sciences and Applications, 52(1), 178–184. https://doi.org/10.21608/AJNSA.2018.3107.1071.

Shahani, S. ; Braga-Basaria, M. ; Maggio, M.; and Basaria, S. (2009). Androgens and erythropoiesis: past and present. *Journal of Endocrinological Investigation*, 32(8):704–716. https://doi.org/ 10.1007/BF03345745.

Shi, P.; Xie, S.; Yang, J.; Zhang, Y.; Han, S.; Su, S.; and Yao, H. (2022). Pharmacological effects and mechanisms of bee venom and its main components: Recent progress and perspective. *Frontiers in Pharmacology*, 13:1001553. https://doi.org/10. 3389/ fphar.2022.1001553.

Ullah, A.; Aldakheel, F. M.; Anjum, S. I.; Raza, G.; Khan, S. A.; and Tlak Gajger, I. (2023). Pharmacological properties and therapeutic potential of honey bee venom. *Saudi Pharmaceutical Journal (SPJ) the official publication of the Saudi Pharmaceutical Society*, 31(1): 96–109. https://doi.org/10.1016/j.jsps.2022.11.008.

Urhausen, A.; Torsten, A.; and Wilfried, K. (2003). Reversibility of the effects on blood cells, lipids, liver function and hormones in former anabolic-androgenic steroid abusers. *Journal of Steroid Biochemistry and Molecular Biology*, 84 (2–3): 369–375. https://doi.org/10.1016/S0960-0760(03)00105-5.

Wu, A.H.; and Bowers, G. N., Jr (1982). Evaluation and comparison of immunoinhibition and immunoprecipitation methods for differentiating MB and BB from macro forms of creatine kinase isoenzymes in patients and healthy individuals. *Clinical Chemistry*, 28:2017–2021. https://doi.org/10.1093/clinchem/hvad145.