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Effect of the 5-alpha-reductase inhibitor finasteride on experimentally-induced pain, inflammation, gastric lesions and liver injury

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ABSTRACT

Finasteride, a 5-alpha-reductase inhibitor, used in treatment of benign prostatic hypertrophy was evaluated in acute nociceptive pain models of thermal, chemogenic and visceral inflammatory pain, carrageenan-induced paw oedema, Porsolt's forced swimming test and Morri's water maze test in mice. Finasteride was also tested in the indomethacin-induced gastric mucosal damage and in acute liver injury caused by carbon tetrachloride (CCl_4) in the rat. Finasteride given intraperitoneally (i.p.) 1h beforehand at doses of 0.25-2 mg/kg significantly increased the response latency in the mouse hot plate test. The drug given orally (0.5-2 mg/kg) or intraperitoneally (i.p.) (0.25-1 mg/kg) caused significant dose-dependent antinociceptive effect in the mouse acetic-acid-induced writhing assay. The co-administration of finastertide (2 mg/kg, i.p.) with either ketoprofen or celecoxib resulted in increased analgesic effect. The drug given at 0.5-1 mg/kg, i.p. reduced the duration of paw licking induced by intradermal capsaicin injection. Finasteride (0.5- 1 mg/kg, i.p.) inhibited the paw oedema response to subplanter carrageenan injection in a dose-dependent manner. Finasteride (0.125-1 mg/kg, i.p.) did not alter immobility time in the Porsolt's forced swimming test. The drug (1 or 2 mg/kg, i.p.) had no significant effect on the antidepressant activity of fluoxetine but increased that of imipramine. The latency to locate the submerged platform in the MWM test was not affected by the administration of finasteride. The drug (2 mg/kg, i.p.) increased gastric mucosal lesions caused by indomethacin. Finasteride at 1 or 2 mg/kg showed no significant effect on CCl_4-induced increase in serum liver enzymes but the drug at 2 mg/kg reduced the silymarin-induced liver protection. In conclusion, finasteride displayed antinociceptive and anti-inflammatory activities. It does not appear to have depressant-like properties or impair short-term working memory at the doses employed in the present study. The drug may increase gastric lesions due to non-steroidal anti-inflammatory agents and cause liver damage.

Keywords: finasteride, visceral pain, thermal pain, antidepressant activity, gastric ulcer

1. INTRODUCTION

Finasteride is type II 5α-reductase inhibitor in use for the treatment of benign enlargement of prostate causing prostatic shrinkage and relief of symptoms of bladder obstruction (Rittmaster, 2008). The drug is also approved for the pharmacologic management of androgenic alopecia or male pattern loss of hair (De Nunzio et al., 2008). The action of the enzyme 5α-reductase is to convert testosterone into its main active form dihydrotestosterone. The latter is considered responsible for prostate growth Marks, (2004) and the decrease in absorption of nutrients needed for hair growth (Libecco and Bergfeld, 2004). Finasteride appears to possess other important pharmacological actions for it has been shown to be an efficient chemo preventive agent that reduces the risk of not only prostatic cancer Reed and Parekh, (2009) but also oesophageal carcinoma (Rabbani et al., 2022).

Finasteride therapy, however, has been complicated by a number of important adverse effects which may persist after discontinuing the drug, the so called "post-finasteride syndrome" (Ganzer et al., 2015). These include chronic fatigue, gynaecomastia, decreased libido and cognitive effects eg. Slow cognition, difficulty to maintain attention, depressed affect, nervousness and depressive symptoms (Ganzer et al., 2015; Irwig, 2014; Diviccaro et al., 2020). Besides inhibiting the conversion of testosterone to dihydrotestosterone, the 5 alpha-reductase enzyme catalyzes the conversion of progesterone to dihydroprogesterone and deoxycorticosterone to dihydrodeoxycorticosterone. The subsequent 3alpha-reduction results in steroid derivatives termed neuroactive steroids Paul and Purdy, (1992) that have rapid non-genomic effects on brain functioning and behavior, mainly by increasing the action of gamma-amino butyric acid on GABA receptors (Finn et al., 2006).

In view of the above, the present study was designed to investigate the effects of finasteride on acute nociceptive pain, inflammation, cognition and memory performance in mice. The study was also extended to investigate the effect of the drug on acute gastric injury caused by the non-steroidal anti-inflammatory drug indomethacin and on acute liver injury evoked by the hepatotoxic agent carbon tetrachloride in the rat.

2. MATERIALS AND METHODS

Animals

Male Swiss albino mice (23-25 g) and male Sprague–Dawley rats (150–160 g) were used. Animals were housed under standardized conditions with free access to food and water. Experimental procedures were performed in accordance with the Ethics Committee of the National Research Centre and the Guide for Care and Use of Laboratory Animals by the U.S. National Institutes of Health (Publication No. 85-23, revised 1996). Equal groups of 6 mice or rats/group were used in all experiments. The doses of finasteride used in the study were based upon the human dose after conversion to that of mice and rat according to Paget and Barnes conversion tables (Paget and Barnes, 1964).

Drugs and Chemicals

Finasteride (Bristol Global Napi Pharmaceutical Co. Cairo, A.R.E.), carrageenan, capsaicin (Sigma, USA), indomethacin (Kahira Pharm & Chem. IND Co., Cairo, A.R.E.), imipramine hydrochloride (Novartis Pharma, Cairo, A.R.E.), fluoxetine hydrochloride (Amon Co., Cairo, A.R.E.) were used. Stock solutions of capsaicin (10 mg/ml) contained 10% ethanol, 10% Tween 80, 80% saline solution. Analytical-grade glacial acetic acid (Sigma, USA) was diluted with pyrogen-free saline to provide a 0.6% solution for i.p. injection. All drugs were dissolved in isotonic (0.9% NaCl) saline solution immediately before use, except indomethacin which was dissolved in a 5% solution of sodium bicarbonate.

Experimental Methods

Tests of nociception

Hot-plate assay

An electronically controlled hotplate (Ugo Basile, Italy) heated to 53 °C (± 0.1 °C) was used. Each mouse was placed unrestrained on the hot plate for the baseline measurement just prior to saline or drug administration. Different groups of mice (n = 6/group) were given either finasteride (0.125, 0.25, 0.5 or 1 mg/kg, i.p.) or saline (control). Measurements were then taken 30, 60, 90 and 120 min after drug administration. Latency to lick a hind paw or jump out of the apparatus was recorded for the control and drug-treated groups. The cut-off time was 30s (Le Bars et al., 2001).

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Acetic acid-induced writhing

Separate groups of 6 mice each were administered either saline (control) or finasteride at doses of 0.125, 0.25, 0.5 or 1 mg/kg; 0.2 ml, i.p. Other groups were given finasteride at doses of 0.5, 0.1 or 2 mg/kg; 0.2 ml orally. After 1h of saline or drug administration, an intraperitoneal (i.p.) injection of 0.6% acetic acid (0.2 ml) was given (Koster et al., 1959). In other experiments, the effect of finasteride (1 or 2 mg/kg, orally) on the antinociceptive effect of the non-steroidal anti-inflammatory drugs celecoxib and ketoprofen was examined. Drugs were co-administered 1h prior to the abdominal constriction assay.

Capsaicin-induced hind paw licking

Finasteride (0.25, 1 or 2 mg/kg, i.p.) or saline was administered 30 min before the injection of the noxious capsaicin at the dose of 1.6 µg/paw (25 µl) beneath the dorsum of the right hind paw skin of mice. Observation was done after the injection of capsaicin and for 5 min duration. The time spent by each mouse licking the injected paw was calculated with the use of a stopwatch (Sakurada et al., 1992).

Carageenan-induced paw oedema

Paw swelling was induced by subplantar injection of 50 µl of 1% sterile lambda carrageenan suspension in saline given into the mouse right hind paw (Winter et al., 1962). An equal volume of saline was injected into the left hind paw. Quantification of the inflammatory oedema was performed by the measurement of the increase in hind footpad thickness using a micrometer caliper just before injection of the inflammogen and at 4h thereafter (Abdel-Salam et al., 2003). Oedema was expressed as the percentage of the basal (pre-saline or pre-drug) values.

Behavioral tests

Porsolt's forced-swimming test

In this test, the mouse was placed in a glass cylinder measured diameter 12 cm and 24 cm in height filled up with water to 12 cm height. The temperature of water was kept at 25 °C. The mouse was forced to swim for a period of 6 min and the time it spent immobile, remained floating and stopped struggling is calculated. The floating time, considered as a measure of despair Porsolt et al., (1977), was determined for finasteride (0.125, 0.25, 0.5 or 1 mg/kg, i.p.), saline or imipramine, a tricyclic antidepressant (15 mg/kg, i.p.) treated groups. In other experiments, the effect of finasteride (0.5 or 1 mg/kg, i.p.) on the antidepressant-like effect of the serotonin reuptake inhibitor fluoxetine (10 or 20 mg/kg, i.p.) or imipramine (15 or 30 mg/kg, i.p.) was examined. Drugs were co-administered 60 min prior to experiment.

Cognitive testing

The Morris water maze (MWM) test was performed to study spatial learning and memory. The maze consisted of a glass tank which measured 20 cm wide, 40 cm height and 70 cm in length. The maze was filled with water to a depth of 21 cm and the temperature of the water was kept at 25 °C. The escape glass platform was hidden from sight, submerged 1 cm under the water surface at the end of the tank (Dunnett et al., 2003). Mice rapidly learn to swim directly to the escape platform and climb out. Once the mice reached the platform, it remained there for 15 s. After each trial, the mouse was towel dried, returned to its home cage where a heat lamp was available and 3 min were allowed to elapse before the next trial which used the same platform location and start position as in trial 1. The latency to find the platform (s) is determined using a stopwatch. The effect of finasteride on working memory was studied in mice treated with scopolamine (1 mg/kg, i.p.) to induce cognitive impairment (Smith et al., 2002). Mice were pre-treated with scopolamine alone (2 mg/kg, i.p.) or in combination with finasteride (0.5, 1 or 2 mg/kg, i.p.) (n=6/group) 60 min prior to testing.

Gastric ulcer studies

Gastric mucosal injury was induced in rats by i.p. indomethacin at a dose of 20 mg/kg. Finasteride was i.p. given at doses of 0.5, 1 or 2 mg/kg at the same time of indomethacin injection. Rats were allowed access to food and water and euthanized 24 h later, their stomachs excised and opened along the greater curvature, washed with saline and examined for the presence of gastric lesions. The number of mucosal lesions was noted and severity calculated as previously described (Abdel-Salam et al., 1997).

Carbon tetrachloride-induced liver injury

Liver injury was induced by treating rats by gavage with CCl_4–olive oil (1:1, v/v) at a dose of 2.8 ml/kg through an orogastric tube (Abdel-Salam et al., 2014). The industrial solvent CCl_4 is a hepatotoxic agent which is widely used in experimental animals to

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induce liver injury (Muriel et al., 2001). Rats were randomly allocated into seven equal groups (6 rats each). Group 1 received the vehicle (olive oil) orally. Groups 2, 3, 4, 5, 6 & 7 were orally treated with CCl_4–olive oil, either alone (group 2: CCl_4 control) or together with orally administered silymarin at a dose of 10 mg/kg (group 3), finasteride at doses of 1 or 2 mg/kg (groups 4 & 5) or silymarin (10 mg/kg) combined with finasteride at doses of 1 or 2 mg/kg (groups 6 & 7). Drugs were administered for 2 successive days. Rats were allowed food and drinking water freely during the study. Thereafter, blood samples collection from the retro-orbital vein plexuses was done under light ether anaesthesia. Rats were then euthanized by cervical dislocation. The liver of each rat was then fixed in freshly prepared 10% neutral buffered formalin, processed routinely and embedded in paraffin.

Determination of liver enzymes

Serum activities of aspartate aminotransferase (ALT) and alanine aminotransferase (AST) were measured according to Reitman-Frankel colorimetric transaminase procedure Crowley, (1967) whereas alkalinephosphatase (ALP) activity was measured by the method of Belfield and Goldberg, (1971) using commercially available kits (Bio diagnostic, Egypt).

Liver Histopathology

Five μm thick paraffin sections were prepared and stained with hematoxylin and eosin for histopathological examination (Drury and Walligton, 1980). Sections were examined using a light microscope. Olympus Cx 41 with DP12 Olympous digital camera (Olympous optical Co. Ltd, Tokyo, Japan) was used.

Statistical Analysis

Statistical analysis was carried out with the use of one-way analysis of variance (ANOVA) with Tukey's multiple comparisons test for group comparisons. Kruskal-Wallis test followed by Dunn's multiple comparisons test was used for the severity of gastric lesions. Graph Pad Prism version 6 for Windows (Graph Pad Software, San Diego, CA, USA) was used. A probability value less than 0.05 was considered to be statistically significant.

3. RESULTS

Effect of finasteride on thermal pain

Finasteride given i.p. at doses of 0.25-2 mg/kg significantly increased hot-plate latency in a dose-dependent manner. The anti-nociceptive effect of the drug was produced with a dose of 0.25 mg/kg 1h after injection and with 193.1% and 194.4% maximal increase in latency by 1 or 2 mg/kg finasteride 1 h after drug administration (Figure 1).

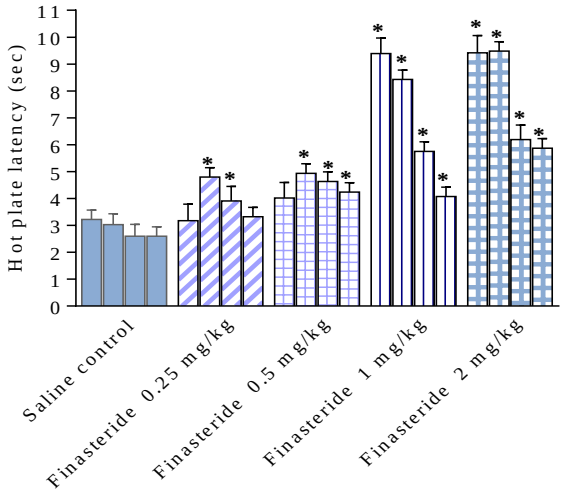

Figure 1 Hot plate latencies (mean ± SEM) after saline or different doses of finasteride. Saline or finasteride was i.p. given 30 min before the test. Measurements were taken 30, 60, 90 or 120 min after injections. *p<0.05 vs. saline control value at the corresponding time point

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Effect of finasteride on visceral nociception

Finasteride administered *via* i.p. or oral route produced a significant and a dose- decrease in acetic acid-induced writhing in mice. The effect of celecoxib or ketoprofen was increased by the addition of 2 mg/kg finasteride but not with the dose of 1 mg/kg (Figure 2).

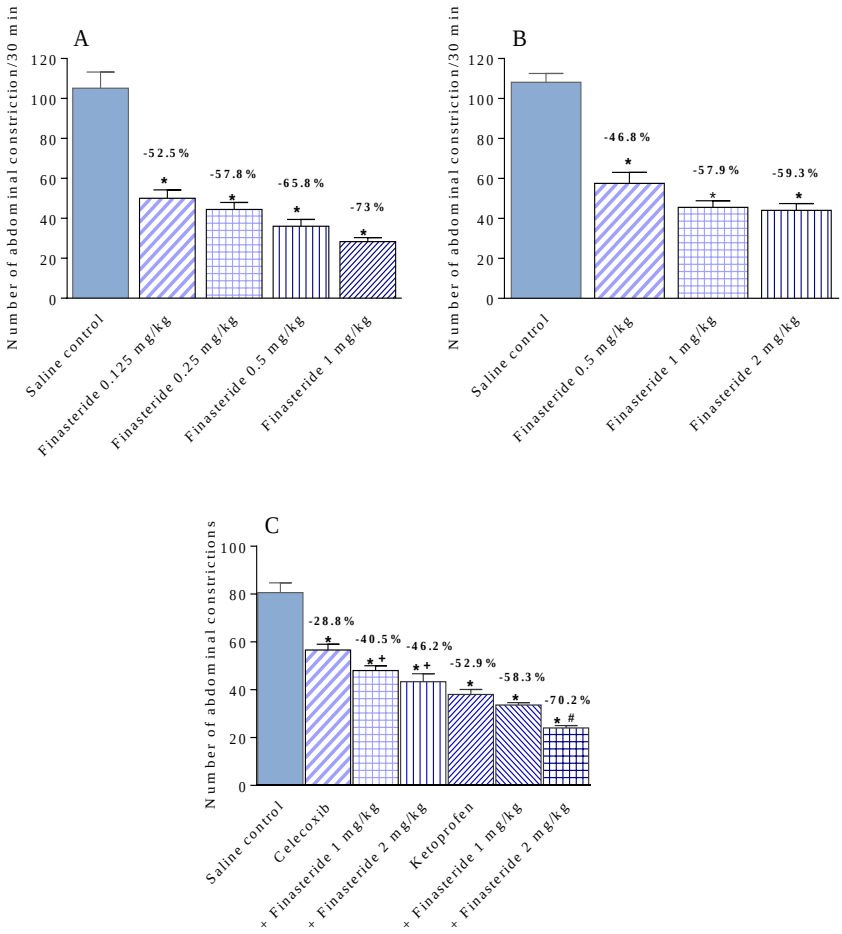

Figure 2 Effect of finasteride on the number of abdominal constrictions in the mouse acetic-acid writhing assay. Finasteride or saline was given via i.p. (A & C) or oral (B) routes 1h prior to i.p. injection of acetic acid. Data expressed as mean ± SEM. *p<0.05 vs. saline control value and between different groups as shown in the graph. +p<0.05 vs. celecoxib control, #p<0.05 vs. ketoprofen control, the percent (%) inhibition as compared with saline control is shown

Effect of finasteride on capsaicin-induced paw licking

The duration of the paw-licking after the injection of capsaicin was significantly decreased by finasteride at 0.5 or 1 mg/kg. Values were 39.69 ± 1.13 for the saline control and 33.56 ± 1.32 and 32.15 ± 1.61 for finasteride at 1 or 2 mg/kg, respectively (Figure 3).

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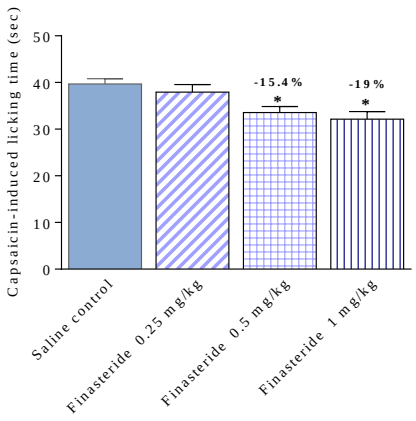

Figure 3 Effect of different doses of finasteride on paw- licking duration after capsaicin injection. Mice received i.p. saline or finasteride, 30 min prior to capsaicin injection. Data expressed as mean ± SEM and percent (%) inhibition compared with saline. *p<0.05 vs. saline control

Effect of finasteride on paw oedema caused by carrageenan

Compared to saline contol value, paw odema significantly increased by 94.1 ± 3.2% at 4h time point after the injection of carrageenan. Paw oedema was significantly decreased by 23%, 38.9% and 56.7% by finasteride at doses of 0.5, 1 and 2 mg/kg, respectively (Figure 4).

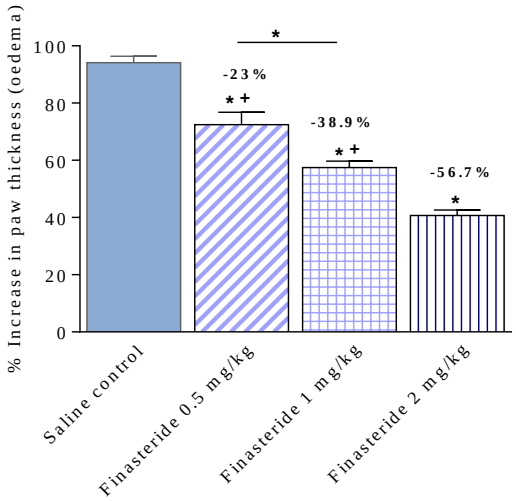

Figure 4 The anti-oedema effect of finasteride in the carrageenan-induced paw oedema assay in mice. Results are presented as mean ± SEM and percent (%) inhibition compared to saline. *p<0.05 vs. saline control and between different groups as shown in the figure, +p<0.05 vs. finasteride 2 mg/kg treated group

Effect of finasteride in Porsolt's forced-swimming test

Finasteride administered at doses of 0.125, 0.25, or 0.5 mg/kg had no significant effect on the immobility time. Meanwhile, the antidepressant imipramine caused significant decrease of the immobility time by 27.1% (Figure 5A). The administration of finasteride did not significantly alter the antidepressant effect of fluoxetine but increased that of impiramine (Figure 5B).

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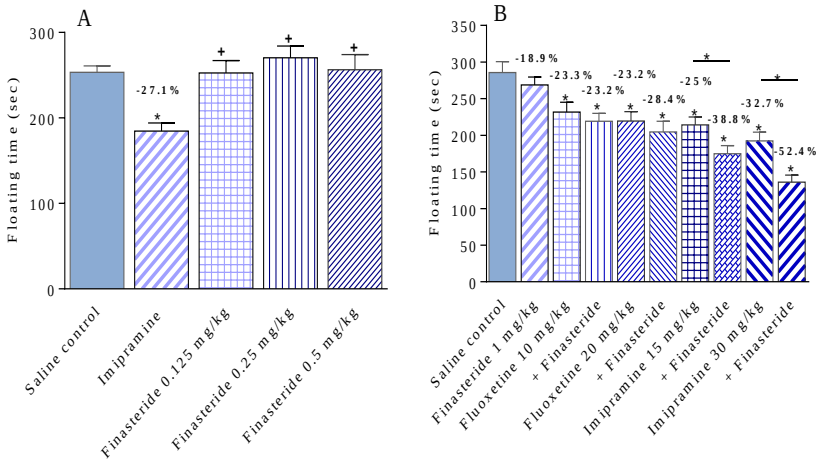

Figure 5 Effect of finasteride on the immobility time. Results shown are mean ± SEM and percent (%) inhibition compared to saline. *p<0.05 vs. saline control and between different groups as shown in the figure, +p<0.05 vs. imipramine

Effect of frinasteride in Morris water maze test

We investigated whether finasteride would increase the memory impairment in scopolamine-treated mice. Scopolamine (1 mg/kg, i.p.) impaired memory performance leading to higher latencies to locate the plateform than that of the saline control group. Finasteride given at 1 or 2 mg/kg showed non-significant effect on the latency to find a hidden platform in mice treated with scopolamine (Figure 6A & B).

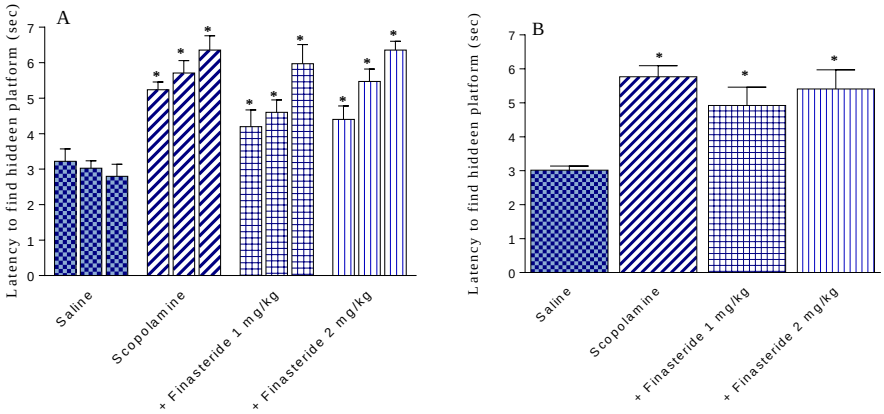

Figure 6 Effect of scopolamine given alone or with finasteride in the Morris water Maze test. Mice received i.p. injections of scopolamine alone (1 mg/kg, i.p.) or in combination with finasteride (1 or 2 mg/kg), 60 min prior to testing. Data are mean ± SEM. (A) The columns represent the first, second and third trail, respectively for each treatment group. Asterisks indicate significant change from trial 1. (B) The average mean latency ± SEM of three trials to locate a submerged plate in the MWM test. Mice received i.p. injections of scopolamine alone (2 mg/kg, i.p.) or in combination with finasteride (1 or 2 mg/kg) 60 min prior to testing. *p< 0.05 *vs.* the saline control group

Effect of finasteride on gastric mucosal damage

Finasteride administered i.p. at doses of 0.5 or 1 at time of indomethacin (IND) injection had no significant effect on gastric lesions. The higher dose of 2 mg/kg resulted in significant increase in number and severity of IND-induced gastric mucosal lesions. Values were 3.57 ± 0.3 and 4.0 ± 0.25 for finasteride 2 mg/kg + IND vs. IND control values of 1.83 ± 0.31 and 2.0 ± 0.36, respectively (Figure 7).

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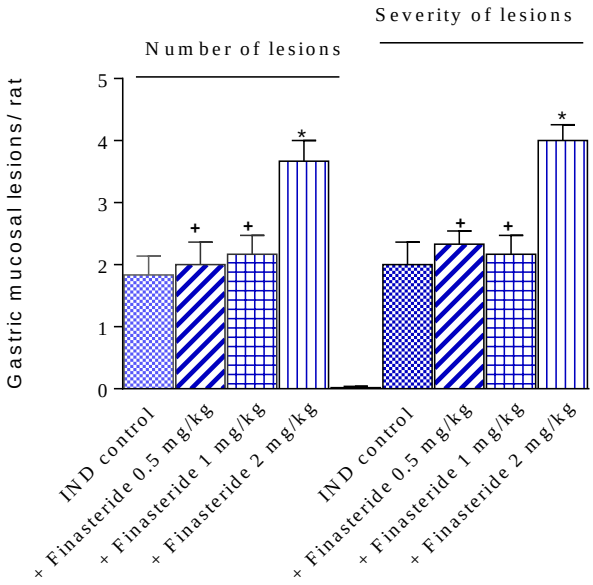

Figure 7 The effect of finasteride on gastric mucosal injury induced by indomethacin (IND) in rats. Indomethacin (20 mg/kg, i.p.) was given alone or with different doses of finasteride (0.5, 1 or 2 mg/kg, i.p.) and rats euthanized 24 h later. Results are expressed as mean ± SEM. *p<0.05 vs. IND control. +p<0.05 vs. IND + finasteride 2 mg/kg group

Effect of finasteride on liver injury

Serum liver enzymes

In CCl_4-treated rats, the activities of ALT, AST and ALP in serum were significantly increased by 146.8% (203.1 ± 6.1 vs. 82.3 ± 3.4 U/l), 93.0% (181.0 ± 7.0 vs. 93.8 ± 2.8 U/l) and 96% (127.2 ± 4.8 vs. 64.9 ± 3.3), respectively, compared to their respective vehicle control values. The treatment of rats with finasteride at 1 or 2 mg/kg had no significant effects on serum enzyme activities. Moreover, after treatment with silymarin/finasteride, serum enzyme activities were significantly lower compared with the CCl_4 control group, indicating that finasteride did not prevent the hepatic protective effect of silymarin. Significant differences in AST and ALP activities, however, were present between silymarin-treated rats given either 1 or 2 mg/kg finasteride, which suggest that the finasteride at 2 mg/kg may have reduced the silymarin effect (Figure 8).

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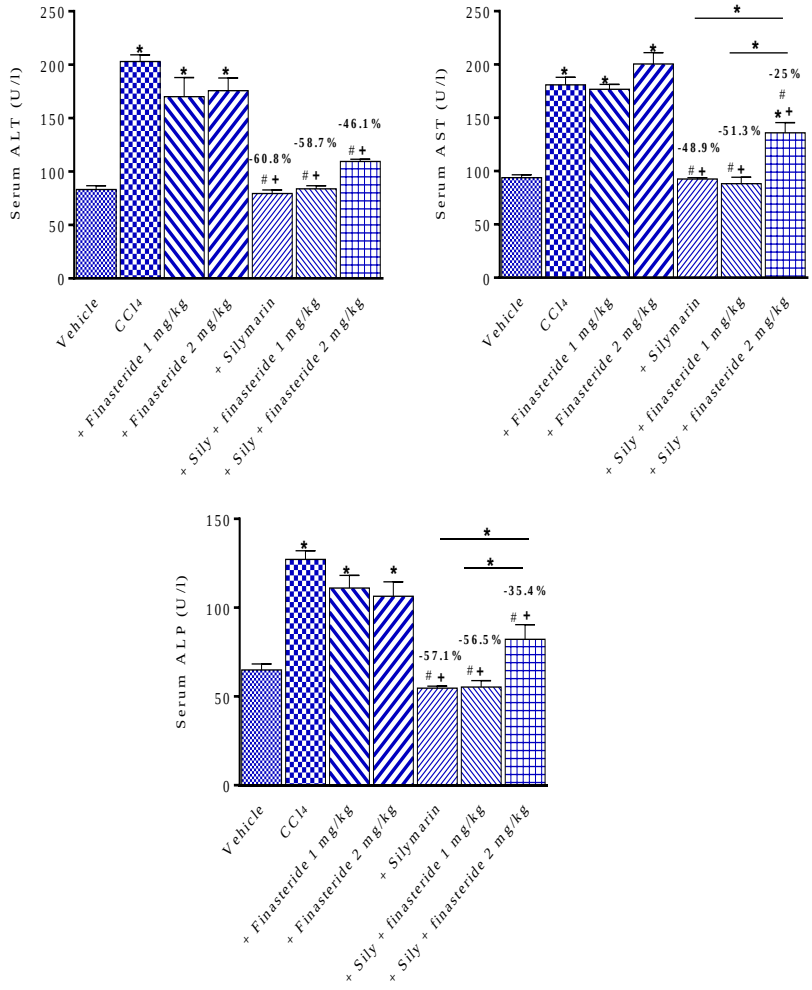

Figure 8 Serum ALT, AST and ALP activities in CCl_4-treated rats given finasteride and/or silymarin (Sily). Results are mean ± SEM and percent (%) inhibition compared to CCl_4 control. *p<0.05 vs. vehicle control and between different groups as shown in the graph, +p<0.05 vs. CCl_4 control, #p<0.05 vs. CCl_4 + finasteride-treated groups

Liver histopathology

The histological study showed that whilst silymarin protected against liver injury, rats treated silymarin and finasteride (2 mg) showed degeneration of the hepatocytes, vacuolation and hydropic degeneration of the hepatocytes (Figure 9).

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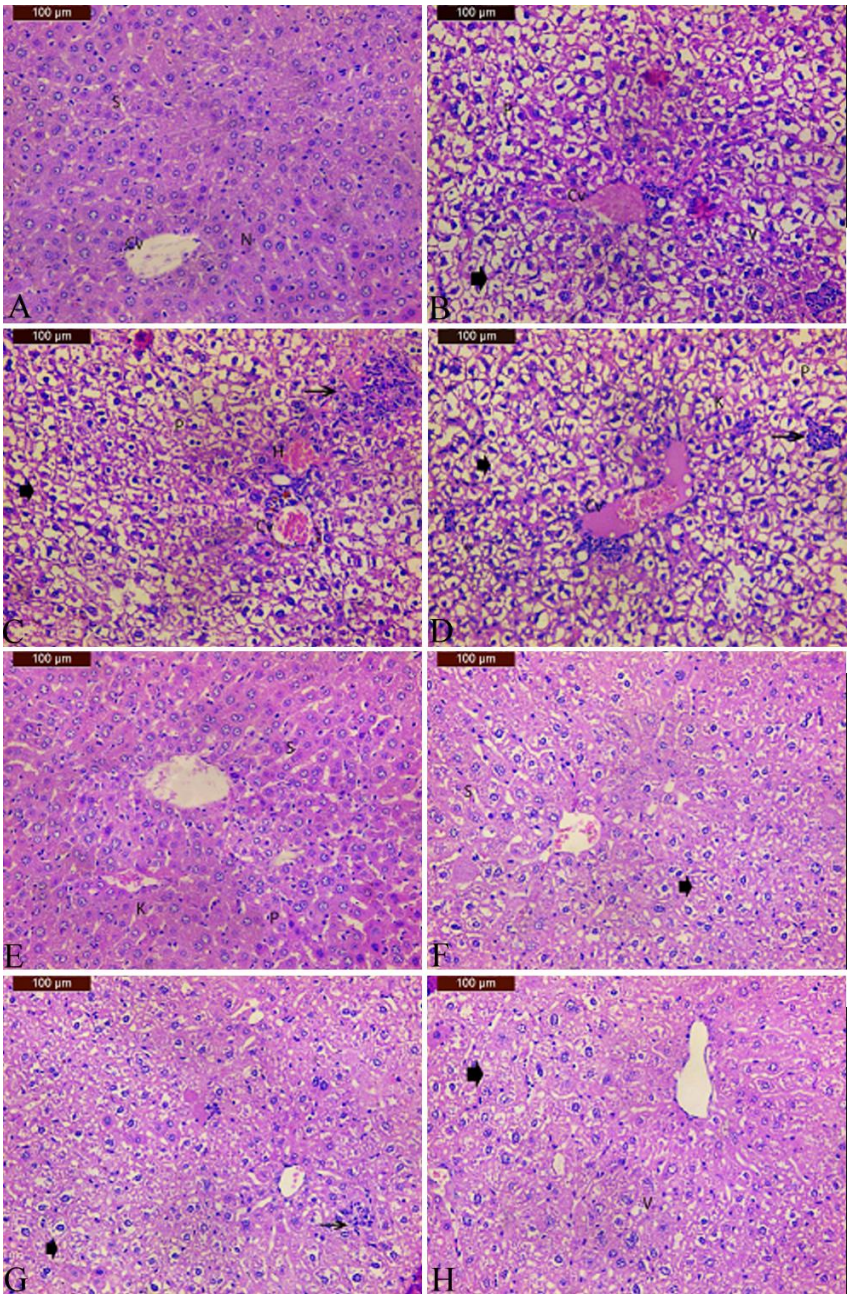

Figure 9 Representative photomicrographs of rat liver sections after treatment with: (A) Vehicle: Normal hepatic architecture. central vein (CV), hepatocytes (H), blood sinusoids (S) and nucleus (S); (B) CCl_4 control: Loss of the normal architecture of hepatic lobules, central vein congestion (CV) with few inflammatory cells particularly around the central vein and pre-necrotic changes (arrowhead), hydropic degeneration and vacuolation (V) in the hepatocytes and pyknotic nuclei (P); (C) CCl_4 + finasteride 2 mg/kg: Disturbance of the hepatic lobules architecture. Notice the congested central vein (CV), hydropic degeneration (arrowhead), few inflammatory cells (arrow), pyknotic (P) and karyolysic (K) nuclei; (D) CCl_4 + finasteride 2 mg/kg: Focal necrosis associated with few inflammatory cell infiltration (arrow), congestion central vein (CV), hydropic degeneration (arrowhead), haemorrhage (H) and pyknotic nuclei (P); (E) CCl_4 + silymarin: Almost nearly normal structure with few pyknotic nuclei (P), slight activated Kupfer cells (K) and slight dilated blood sinusoid (S); (F) CCl_4+ silymarin + finasteride 1 mg/kg: The hepatic lobule architecture appears more or less like normal. Notice thehydropic degeneration (arrowhead) and dilatation of the sinusoids (arrows); (G) CCl_4+ silymarin + finasteride 2 mg/kg: Degeneration of the hepatocytes that surrounded the central vein (arrowhead) and vacuolation (V) in the hepatocytes; (H) CCl_4+ silymarin + finasteride 2 mg/kg: Degeneration of the hepatocytes (arrowhead) and few inflammatory cells (arrow).

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4. DISCUSSION

The present study showed that finasteride, a 5-α reductase inhibitor, exerted antinociceptive effects in thermal, visceral and chemogenic pain models. Finasteride attenuated the nociceptive response to thermal stimulation and reduced the pain behavior in the acetic acid-induced abdominal constriction assay. It also reduced chemogenic pain induced by subplantar injection of capsaicin which is caused by C fiber excitation.

Heat stimulates cutaneous nociceptors, the heat-sensitive transient receptor potential cation channel vanilloid subfamily member 1 (TRPV1) on nerve terminals of sensory neurons with thin fiber afferents (C-and A-delta) (Szolcsányi, 2014). These nociceptive neurones release glutamate as a central neurotransmitter in the spinal cord as well as release neuropeptides eg. substance P, neurokinin A, calcitonin gene-related peptide and somatostatin during intense stimulation (Dray, 1992; Winter et al., 1995). Our results showed that finasteride produced antinociceptive effect against thermally-induced pain (i.e., hot plate test). The anti-nociceptive effect was produced with a dose of 0.25 mg/kg and reached 193.1% and 194.4% of the basal value after i.p. finasteride at 1 or 2 mg/kg, respectively. Finasteride given *via* oral or i.p. routes was effective in inhibiting the behavioral pain response induced by i.p. injection of acetic acid with the drug given i.p. at 0.125 mg/kg producing 52.5% inhibition of abdominal constrictions. After i.p. administration, finasteride was more effective in inhibition of the writhing response compared to the oral route. This difference in action is most likely is due to more rapid and better absorption of the drug after systemic administration. The writhing response is caused by the release of prostacyclin in the abdominal cavity (Berkenkopf and Weichman, 1988). It is effectively inhibited by non-steroidal anti-inflammatory drugs (NSAIDs) Santos et al., (1998), morphine Baamonde et al., (1989), antidepressant drugs eg., fluoxetine Singh et al., (2001) and *Hypericum perforatum* Abdel-Salam, (2005) as well as the dopamine blocking drug cinnarizine Abdel-Salam, (2007). Our results showed that the behavioral response to i.p. injection of acetic acid was also inhibited by ketoprofen, a non-selective cyclooxygenase inhibitor and to lesser extent by the selective cyclooxygenase-2 (COX-2 isoenzyme) inhibitor celecoxib. The analgesic effects of the NSAIDs were increased by the co-administration of finasteride at 2 mg/kg but not with the lower dose of the drug.

We in addition showed that finasteride reduced chemonociception caused by subplanter capsaicin injection. Capsaicin evokes pain by selectively stimulating TRPV1 (the capsaicin receptor) on the peripheral nerve endings of the capsaicin-sensitive polymodal nociceptive neurons (Szolcsányi, 2014). These fibers have sensory-efferent functions in that they convey the nociceptive information from the skin and viscera to the spinal cord and then to the central nervous system (CNS) and releases neuropeptides from sensory nerve terminals resulting in the neurogenic component of inflammation (Szolcsányi, 1984; Maggi and Meli, 1988). In visceral inflammation, local tissue injury results in the release of a variety of chemical and inflammatory mediators such as prostaglandins, bradykinin, adenosine triphosphate from inflammatory cells and these stimulate vagal, spinal or pelvic afferents to signal to the CNS (Bueno and Fioramonti, 2002). Other researchers using the tail-flick assay reported mild, but non-significant analgesic effect for orally given finasteride at 0.5 mg/kg for 30 days. The drug, however, increased morphine antinociception (Duborija-Kovacevic et al., 2008). There is evidence that changes in the local levels of neurosteroids at the spinal cord and dorsal root ganglia may modulate pain transmission (González and Ferreyra, 2022). This could be one mechanism by which finasteride modulates the pain response. The drug may also act through neurosteroids increasing γ-aminobutyric acid inhibitory neurotransmission Finn et al., (2006) or by modulating other brain neurotransmitters eg. dopamine (Li et al., 2018).

We also found that finasteride exerted marked anti-oedema effect in the carrageenan-induced paw oedema assay. The effect of the drug was dose-related with a 56.7% reduction of oedema after 1 mg/kg finasteride, suggesting an anti-inflammatory effect for the drug. In their study, Duborija-Kovacevic et al., (2008) reported 19.9% decrease in formalin-induced paw oedema by orally administered finasteride at 0.5 mg/kg given 4h prior to testing. Finasteride therapy has been associated with depressive symptoms (Altomare and Capella, 2002). These persist after discontinuation of the drug and probably result from alterations in levels of brain neurosteroids (Traish et al., 2001; Melcangi et al., 2016). A study by Beckley and Finn, (2007) reported increased immobility time in the forced swimming test in mice after the administration of a high dose of finasteride (100 mg/kg). Other researchers showed that finasteride prevented the antidepressant like action of fluoxetine (Molina-Hernández et al., 2009). In the present study, the potential for finasteride to exert depressive-like effects was assessed in Porsolt's forced swimming test. Mice treated with finasteride showed no significant effect on the floating time (a measure of despair) compared with the saline control. Finasteride did not alter the antidepressant effect of the SSRI fluoxetine. The drug, however, increased the antidepressant action of the tricyclic agent impiramine. The mechanism that underlies this effect of finasteride is not clear.

Memory problems, recall impairment, slowing of mental processes and cloudiness of mind which persist after stopping the medication have been reported by subjects treated with finasteride (Ganzer et al., 2015). In the present work, the ability of finasteride to cause memory impairment was examined in the Morris water maze test. The treatment of mice with the anti

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cholinergic drug scopolamine resulted in increased latency to find the submerged platform, indicative of impaired working memory. Our results, however, showed no significant effect for finasteride administration on the memory impairment caused by scopolamine. It is still possible that long-term treatment with finasteride rather than single dosing is required for affecting memory processing.

Our results in addition showed that finasteride given at 2 mg/kg caused a significant increase in gastric mucosal lesions evoked by the NSAID indomethacin. This effect of finasteride is not due to local effect since the drug was given via systemic route. The effect of finasteride on liver injury is not clear. A study by Hazlehurst et al., (2016) in healthy male volunteers, found increased hepatic insulin resistance and intra-hepatic lipid accumulation after three weeks of administering dutasteride, a competitive inhibitor of both type-1 and type-2 isoenzymes of 5-α-reductase, but not after finasteride therapy. In the present work we investigated the effect of finasteride in the presence of acute liver injury due to CCl_4. We found no significant effects for the drug on serum enzyme activities in CCl_4-treated rats. However, an increase in histologic injury by 2 mg/kg finasteride was observed. Silymarin, a standardized plant extract, derived from the milk thistle plant is a hepatic protective agent used for the treatment of various liver disorders in view of its antioxidant effects and membrane stabilizing properties (Flora et al., 1998; Saller et al., 2001). In animal studies of liver injury, the herb was shown to markedly alleviate the increase in serum liver enzymes and provide histologic protection against liver toxicants (Abdel-Salam et al., 2014; Muriel et al., 1992). In the present study, silymarin and finasteride were co-administered to CCl_4-treated rats so as to investigate a possible modulatory action for finasteride on the liver protection by silymarin. Our results suggest that the higher dose of 2 mg/kg reduced the hepatic protection by the antioxidant silymarin indicated by the increase in AST and ALP activities and the extent of histologic damage.

5. CONCLUSIONS

The present study indicates that the 5-alpha-reductase inhibitor finasteride possesses antinociceptive and anti-inflammatory properties. The drug does not appear to have depressant-like effects or impair short-term working memory at the doses used in the study. Finasteride, however, may increase gastric damage due to NSAIDs and reduce the protective effect of silymarin in the liver.

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Ethical approval

Experimental procedures were performed in accordance with the Ethics Committee of the National Research Centre and the Guide for Care and Use of Laboratory Animals by the U.S. National Institutes of Health (Publication No. 85-23, revised 1996).

Author contribution

O.M.E.A.S. and A.A.S. conducted the research and biochemical studies, E.A.O. performed the histopathology and its interpretation, O.M.E.A.S. wrote and prepared the manuscript, O.M.E.A.S. and A.A.S. and E.A.O. approved the final version of the manuscript.

Conflict of Interest:

The authors declare that there are no conflicts of interests.

Data and materials availability:

All data associated with this study are present in the paper.

REFERENCES

1. Abdel-Salam OME, Baiuomy AR, El-Shenawy SM, Arbid MS. The anti-inflammatory effects of the phosphodiesterase inhibitor pentoxifylline in the rat. Pharmacol Res 2003; 47:3 31–340.
2. Abdel-Salam OME, Sleem AA, Shafee N. Hepatoprotective effects of *Cynara extract* and silymarin on carbon tetrachloride-induced hepatic damage in rats. Comp Clin Pathol 2014; 23(3):709-716.
3. Abdel-Salam OME, Szolcsányi J, Mózsik GY. The indomethacin-induced gastric mucosal damage in rats. Effect of gastric acid, acid inhibition, capsaicin-type agents and prostacyclin. J Physiol 1997; 91:7-19.
4. Abdel-Salam OME. Anti-inflammatory, anti-nociceptive and gastric effects of *Hypericum Perforatum* in rats. Sci World J 2005; 5:585–596.

5. Abdel-Salam OME. Modulation of visceral nociception, inflammation and gastric mucosal injury by cinnarizine. Drug Target Insights 2007; 2:29–38.
6. Altomare G, Capella GL. Depression circumstantially related to the administration of finasteride for androgenetic alopecia. J Dermatol 2002; 29(10):665-9.
7. Baamonde A, Hidalgo A, Andres-Trelles F. Sex-related differences in the effects of morphine and stress on visceral pain. Neuropharmacology 1989; 28:967–970.
8. Beckley EH, Finn DA. Inhibition of progesterone metabolism mimics the effect of progesterone withdrawal on forced swim test immobility. Pharmacol Biochem Behav 2007; 87(4):412-9.
9. Belfield A, Goldberg DM. Human serum glucose-6 phosphatase activity: Confirmation of its presence and lack of diagnostic value. Clin Chim Acta 1971; 31:81-85.
10. Berkenkopf JW, Weichman BM. Production of prostacyclin in mice following intraperitoneal injection of acetic acid, phenylbenzoquinone and zymosan: Its role in the writhing response. Prostaglandins 1988; 36:693–709.
11. Bueno L, Fioramonti J. Visceral perception: Inflammatory and non-inflammatory mediators. Gut 2002; 51(1):i19–i23.
12. Crowley LV. The Reitman-Frankel colorimetric transominase procedure in suspected myocardial infarction. Clin Chem 1967; 13(6):482–7.
13. De Nunzio C, Miano R, Trucchi A, Finazzi Agrò E, Tubaro A. Finasteride for prostatic disease: An updated and comprehensive review. Expert Opin Drug Metab Toxicol 2008; 4(12):1561-8.
14. Diviccaro S, Melcangi RC, Giatti S. Post-finasteride syndrome: An emerging clinical problem. Neurobiol Stress 2020; 12:100209.
15. Dray A. Mechanism of action of capsaicin-like molecules on sensory neurons. Life Sci 1992; 51:1759-1765.
16. Drury RVA, Walligton EA. Carleton's histological technique, 5th edition, Oxford University Press, New York 1980; 206.
17. Duborija-Kovacevic N, Jakovljevic V, Sabo A, Tomic Z. Anti-nociceptive and anti-inflammatory properties of 5alpha-reductase inhibitor finasteride in experimental animals. Eur J Drug Metab Pharmacokinet 2008; 33(3):181-6.
18. Dunnett SB, Bensadoun JC, Pask T, Brooks S. Assessment of motor impairments in transgenic mice. In: Crawley JN (ed). Mouse behavioral phenotyping. Washington DC: Society for Neuroscience 2003; 1–13.
19. Finn DA, Beadles-Bohling AS, Beckley EH, Ford MM, Gililland KR, Gorin-Meyer RE, Wiren KM. A new look at the 5alpha-reductase inhibitor finasteride. CNS Drug Rev 2006; 12(1):53-76.
20. Flora K, Hahn M, Rosen H, Benner K. Milk thistle (Silybum marianum) for the therapy of liver disease. Am J Gastroente rol 1998; 3:139–143.
21. Ganzer CA, Jacobs AR, Iqbal F. Persistent sexual, emotional and cognitive impairment post-finasteride: A survey of men reporting symptoms. Am J Men's Health 2015; 9(3):222–228.
22. González S, Ferreyra S. Neurosteroids and neuropathic pain: An up-to-date perspective. Curr Opin Endocr Metab Res 2022; 22:100314.
23. Hazlehurst JM, Oprescu AI, Nikolaou N, Di Guida R, Grinbergs AEK, Davies NP, Flintham RB, Armstrong MJ, Taylor AE, Hughes BA, Yu J, Hodson L, Dunn WB, Tomlinson JW. Dual-5α-reductase inhibition promotes hepatic lipid accumulation in man. J Clin Endocrinol Metab 2016; 101:103–113.
24. Irwig MS. Persistent sexual and nonsexual adverse effects of finasteride in younger men. Sex Med Rev 2014; 2:24-35.
25. Koster R, Anderson M, De Beer EJ. Acetic acid for analgesic screening. Fed Proc 1959; 18:412.
26. Le Bars D, Gozariu M, Cadden SM. Animal models of nociception. Pharmacol Rev 2001; 53:597–652.
27. Li L, Kang YX, Ji XM, Li YK, Li SC, Zhang XJ, Cui HX, Shi GM. Finasteride inhibited brain dopaminergic system and open-field behaviors in adolescent male rats. CNS Neurosci Ther 2018; 24:115–125.
28. Libecco JF, Bergfeld WF. Finasteride in the treatment of alopecia. Expert Opin Pharmacother 2004; 5(4):933-40.
29. Maggi CA, Meli A. The sensory-efferent function of capsaicin-sensitive sensory neurons. Gen Pharmac 1988; 19 (1):1-43.
30. Marks LS. 5 alpha-reductase: History and clinical importan ce. Rev Urol 2004; 6(9):S11-21.
31. Melcangi RC, Giatti S, Garcia-Segura LM. Levels and actions of neuroactive steroids in the nervous system under physiological and pathological conditions: Sex specific features. Neurosci Biobehav Rev 2016; 67:25–40.
32. Molina-Hernández M, Téllez-Alcántara NP, Olivera-Lopez JI, Jaramillo MT. Olanzapine plus 17-beta estradiol produce antidepressant-like actions in rats forced to swim. Pharmacol Biochem Behav 2009; 93(4):491-7.
33. Muriel P, Alba N, Perez-Alvarez VM, Shibayama M, Tsutsumi VK. Kupffer cells inhibition prevents hepatic lipid peroxidation and damage induced by carbon tetrachloride. Comp Biochem Physiol C Toxicol Pharmacol 2001; 130(2):21 9–26.
34. Muriel P, Garciapina T, Perez-Alvarez V, Mourelle M. Silymarin protects against paracetamol induced lipid peroxidation and liver damage. J Appl Toxicol 1992; 12:439–442.

35. Paget GE, Barnes JM. Toxicity tests. In Evaluation of Drug Activities Pharmacometics. Eds. Laurence DR, Bacharach AL. London and NewYork: Academic Press 1964; 1–135.
36. Paul SM, Purdy RH. Neuroactive steroids. FASEB J 1992; 6: 2311–2322.
37. Porsolt RD, Le Pichon M, Jalfre M. Depression: A new animal model sensitive to antidepressant treatments. Nature 1977; 266:730–732.
38. Rabbani S, Santoni G, Lagergren J, Xie SH. Use of anti-androgenic 5α-reductase inhibitors and risk of oesophageal and gastric cancer by histological type and anatomical sub-site. Br J Cancer 2022; 127:892–897.
39. Reed AB, Parekh DJ. The utility of 5-alpha reductase inhibitors in the prevention and diagnosis of prostate cancer. Curr Opin Urol 2009; 19(3):238-42.
40. Rittmaster RS. 5alpha-reductase inhibitors in benign prostatic hyperplasia and prostate cancer risk reduction. Best Pract Res Clin Endocrinol Metab 2008; 22(2):389-402.
41. Sakurada T, Katsumata K, Tan-No K, Sakurada S, Kisara K. The capsaicin test in mice for evaluating tachykinin antagonists in the spinal cord. Neuropharmacology 1992; 31: 1279-1285.
42. Saller R, Meier R, Brignoli R. The use of silymarin in the treatment of liver diseases. Drugs 2001; 61:2035–2063.
43. Santos AR, Vedana EM, De-Freitas GA. Antinociceptive effect of meloxicam, in neurogenic and inflammatory nociceptive models in mice. Inflamm Res 1998; 47:302–307.
44. Singh VP, Jain NK, Kulkarni SK. On the antinociceptive effect of fluoxetine, a selective serotonin reuptake inhibitor. Brain Res 2001; 915:218–226.
45. Smith JW, Evans T, Costall B, Smythe JW. Thyroid hormones, brain function and cognition. A brief review. Neurosci Biobehav Rev 2002; 26:45–60.
46. Szolcsányi J. Capsaicin and sensory neurones: A historical perspective. In: Capsaicin as a Therapeutic Molecule (Progress in Drug Research 68) (Abdel-Salam OME, Ed.). Springer, Basel, Switzerland 2014; 1–37.
47. Szolcsányi J. Capsaicin-sensitive chemoceptive neural system with dual sensory-efferent function. In Antidromic Vasodilatation and Neurogenic Inflammation (Chahl LAJ, Szolcsányi J, Lembeck F. Eds.). Akademiai Kiado, Budapest 1984; 27–56.
48. Traish AM, Hassani J, Guay AT, Zitzmann M, Hansen ML. Adverse side effects of 5-α reductase inhibitors therapy: Persistent diminished libido and erectile dysfunction and depression in a subset of patients. J Sex Med 2001; 8:872-884.
49. Winter CA, Risley EA, Nuss GW. Carrageenan induced edema in hind paw of the rat as an assay for antiinflammatory drugs. Proc Soc Exp Biol Med 1962; 111: 5 44-552.
50. Winter J, Bevan S, Campbell EA. Capsaicin and pain mechanisms. Br J Anaesth 1995; 75:157-168.

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