THE PROTECTIVE ROLE OF PHITOFERT AGAINST NAPROXEN-INDUCED TOXICITY IN THE PROSTATE GLAND OF RATS

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ABSTRACT

The current study was designed to investigate the effects of the treatment with naproxen drug on the physiological functions "Zinc, prostatic and serum PSA concentration", some biochemical markers "Total protein and GSH, CAT and MDA levels", as well as histometrical and the histopathological aspects of prostate ventral lobe. Also this study examined the phitofert product and selenium in treating naproxen's effect. Therefore, twenty four adult male rats were divided into 3 groups (8 rats each) as follows Groups 1; rats were administrated with 1 ml of d.w. for 28 successive days. Group 2; rats were administrated with 21 mg.Kg⁻¹ of naproxen for 28 successive days. Groups 3; rats were administrated with 21 mg.Kg⁻¹ b.w., and at 5-6 h later they were administrated with 7 mg.Kg⁻¹ b.w of phitofert for 28 successive days. Naproxen-treated rats “Group” exhibited a significant (P≤0.05) declining in the zinc and PSA concentration, PVL total protein, GSH concentration, CAT activity and a high increment in the MDA level that indicated to appearance of oxidative stress status. The naproxen had passive impact on the weight, histometrical and histopathological of glandular "acini" and excretory "ducts" portions. While, the administration of phitofert abrogated all above naproxen induced passive effects.

Keywords : MDA, prostrate gland, naproxen, phitofret, rats

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INTRODUCTION

The prostate gland was considered one of the major accessory glands "prostate and seminal vesicle" of the male reproductive system. This gland was composed of ventral lobe, dorsal lobe and pairs of latteral lobes in rats, and it was able to produce and releasing several important substances "Citrate, zinc, prostate-specific antigen, amines, metal ions and enzymes” , that essential for normal function of sperms(1). Additionally, the prostate secreted plenty amount of fluids that allow, the transport and supply nutrition to sperms(2).

It was well known that, its development, growth and function control by androgens(3). On the other hand, several factors adversely affected the prostate gland such as testosterone replacement therapy, heavy metal and some drugs(3).

Meanwhile the phitofert for men i.e, phitofert sperm plus product was a food supplement containing several components such as some plant extracts "Maca, Ginseng and Withania” as adaptogenic and tonic. Ditto, this drug includes some of the vitamins i.e., C and B₁₂ that had a role in the cell divission process. The other
ingredients was cellulose, coenzyme Q_{10}, niacin and glutathion as bulking agents. Meanwhile, it contained the anti-caking agents such as magnesium salts of fatty acid, silicon dioxide, and riboflavin. This phitofert sperm plus was manufactured in UE for promo pharma s.p.a (www.promopharma.it) knowing, maca that one of its component was utilized to remedy the infertility in both sex via increased fertility in several species\cite{4}. Also maca was characterized by possessing of antioxidant role\cite{5}.

Naproxen drug belongs to nonsteroidal anti-inflammatory drugs "NSAIDs", it was widely used to treat the inflammation chronically disorder and to relieve magrain, fever and subacute disease, also it was considered as prostaglandin inhibiting drug\cite{6}.

Little studies have been focused on the influence of long-term treatment with naproxen and the other members of NSAIDs in the male reproductive functions especially of the prostate gland. Therewith these studies showed that, the NSAIDs had several detrimental impacts on male reproductive physiology and they associated with declining in fertility\cite{7}. Also some studies suggested that the NSAIDs usage may caused increased the benign prostatic hyperplasia\cite{8}.

Given, there was very few studies about of the effect of naproxen on the prostate gland, there was no study about the effect of phitofert on this gland. The aim of the current study was to know body and organ weight, pathophysiological, histopathological, total protein of tissue, hormonal profile, and oxidative markers of ventral prostate posttreatment with naproxen and to elucidate the capacity of phitofert against naproxen-induced adverse changes in the above mentioned parameters compared to the capacity of selenium using.

The goal of this study was to evaluate the histopathological and biochemical markers such as total protein tissue and oxidative stress in the prostate ventral lobe in addition to study its histostucture and histopathological changes that induced by a naproxen, and to investigate whether these alternations could be attenuated by treatment with phitofert.

**MATERIAL AND METHODS**

Thirty two of male Sprague-Dawley rats were kept at controlled conditions of temperature (25±9°C) and 14h-10h light dark cycle. The animal were provided with pellet and water *ad libitum*. The current study was conducted in the reproductory physiology lab at the College of Education for Pure Science Ibn Al-Hitham, University of Baghdad. The rats were randomly divided into 3 groups of eight rats for each as follows: Group1: rats were orally administrated by 1 ml of d.w for 28 days (control). Group2: rats orally administrated by 1 ml of 21 mg.Kg\textsuperscript{-1} b.w. naproxen for 28 days. Group3: rats were orally administrated by 1 ml of 21 mg.Kg\textsuperscript{-1} b.w. of naproxen and post 5 to 6 h they treated with 1 ml of 7 mg.Kg\textsuperscript{-1} b.w of phitofert for 28 days.

After 24h of the last treatment, the rats of each groups were weighed and the blood was collected by heart puncture. Then, collected blood samples were left to clot for 2 h. The sera were extracted post centrifugation for 10 min at 3000 rpm and kept at -20°C until used.

Thereafter, the prostatic lobes of all experimental groups were divided into equal two parts. The first parts of each group were immediately proceeded for histometrical and histopathological examination. While, the second parts were frozen until used for various assays.
Exp.1: preprocessing of ventral prostate homogenate. The second parts of ventral prostate lobes of each group was individually homogenized in sterilized phospho buffer salaine (PBS) by using a homogenizer at 4°C. The homogenates were centrifuged at 3000 g for 15 min at 4°C. The supernatant fractions were used for the various assays as follows:

Assessment of physiological parameters

Assay of prostatic tissue zinc level.

By using the zinc colorimetric assay kit (Cochesion Biosciences, UK/China), the prostatic zinc level was estimated. Wherein, the zinc in alkaline medium reacted with nitro-PAPs to form a colored complex. The intensity of color was directly proportional to the amount of zinc present in sample. The measurement was done by smart spectrophotometer Biorad (USA) at 578 nm wavelength.

Assay of serum and prostatic tissue prostatespecific antigen (PSA) levels.

An enzyme immuno assay ELISA "Sandwich" method was followed to evaluate the serum and prostatic tissue PAS levels, by using the PSA kits (Elabscience®) and ELISA human reader Hs (Germany) at 450 nm wavelength.

Biochemical markers assay

Assay of prostatic tissue total protein concentration

The total protein in the supernatant fraction was evaluated by the colorimetric assay that, based on the principle of Biuret reaction. The formed color intensity was relative to the total protein concentration in sample. The measurement was done by smart spectrophotometer Biorad USA at 450 nm wavelength.

Assay of the oxidative status in the ventral prostate tissue (OS)

The activity of oxidant enzyme i.e., lipid peroxidation (MDA) was estimated according to the method of [10]. Whereas, the levels and activity of nonenzymatic and enzymatic antioxidants i.e., glutathion (GSH) and catalase (CAT) were respectively measured according to [11,12].

Measurements of the serum T hormone

Enzyme immuno assay (EIA) method was followed to determine the serum T hormone concentration for all experimental groups by using specific kits and ELISA Human reader Hs (Germany).

Histometrical and histopathological examination

The one parts of prostate ventral lobes were fixed by using 10% formaline. The paraffin sections of these parts lobes were cut transverse at 5 μm by using routine histological technique and stained with hematoxylin and eosin, to study the histometrical and histopathological aspects.

RESULTS AND DISCUSSION

Obviously, the treatment with naproxen induced perturbation influences in the prostatic functions via assay some of markers such as zinc concentration and prostatic-specific antigen in PVL tissue and serum.

PVL zinc concentration

Our findings revealed that, the naproxen "groups" induced a significan (P≤0.05) decrement in the prostatic ventral lobe zinc concentration compared to values in control "group1" (Figure1). Many previous studies proved

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that, efficacy of prostate gland to concentrate a high amounts of trace element zinc in their acini epithelial cells was more than doing by other cells of soft tissues\(^{13, 14}\). Additionally, it was known that, zinc played a vital role in the prostate function and it was considered as a marker of prostatic function\(^{15}\). The accumulation of zinc at a high levels in mitochondria of the prostate epithelial cells was essentially for inhibition the aconitase enzyme "that involving the citrate oxidation" activity hence, the citrate was accumulated and secreted into the seminal fluid, also the secretion of citrate was considered as a major physiological function of prostate\(^{13}\).

In this study, the reduction in the PVL zinc concentration of rats with naproxen "group2" accompanied with declining in the serum T levels. This result supported the previous study by\(^{16}\) who demonstrated that, testosterone hormone caused a significant decrease in the zinc level of ventral prostatic cells, whereas it caused a significant increase in the zinc levels in the lateral prostatic cells \textit{in vivo} or \textit{in vitro}. Therefore, the decrement of prostatic ventral lobe zinc concentration maybe attributed to either a high sensitive of their acini epithelial cells to the naproxen-induced oxidative stress resulting to zinc transporter protein damage and reduction in their numbers, or it could attribute to incidence a reduction in the zinc transporter expression and in affinity between the zinc transporter and zinc. Also, the declining in zinc concentration was confirmed by the histopathological alternation in the PVL that considered as intoxication influences of naproxen.

\textbf{PSA concentration}

Meanwhile, the prostatic specific antigen (PSA) concentration was significantly (\(P\leq 0.05\)) elevated in PSA of ventral lobe tissue and serum of rats treating with naproxen; group 2 compared to values of control; group1 (Figures 2 and 3). It was known that, the main function of prostate gland was to produce a milky fluid by prostate ductal and acini epithelium, and this fluid contained an androgen-regulated protein knwon as PSA\(^{17}\). The PSA was a glycoprotein produced in low quantities by cells of prostate gland, in healthy males it was released into the blood at low concentration too, whereas a high serum PSA levels had been correlated with prostate cancer\(^{18}\). In addition, it was used as a marker for prostatic volume in benign prostatic hyperplasia (BPH) and prostatic cancer\(^{19}\). Also, it was observed the elevation of plasma PSA and testosterone in BPH-induced male rats\(^{20}\). Besides, it was reported that, the level of free testosterone in the blood was considered to be pivotal in BPH progression\(^{21}\).

Interestingly, a significant increment in the PSA concentrations were accompained with the reduction in the serum testosterone concentration therefore, this elevation could not be due to one of both prostatic diseases i. e., BPH or prostate cancer. Also, the histopathological alternations in PVL by naproxen could not prove of prostatic growth in the structure of prostate ventral lobe. Therefore, a high PSA concentration in group2 were maybe due to the fortified impact of naproxen either on the enzyme activity that involving in this glycoprotein synthesis or through accelerating of protein expression.

\textbf{Biochemical markers}

\textbf{Total protien tissue}

It was observed that, in rats with naproxen as compared with to control showed a significant (\(P\leq 0.05\)) declining in the prostatic total protein tissue (Figure4). This declining could be correlated with the reduction in serum testosterone concentration in this group. Wherein, it was well known that androgen fundemently testosterone had the important role in promotion of protein synthesis, hence growth of tissue through androgen receptors\(^{1}\).
Additionally, \(^{22}\) elucidated that, testosterone\(^{21}\) had androgenic and anabolic influences. Also, \(^{23}\) mentioned that, prostate growth and its homeostasis was controlled by androgen through interaction with nuclear androgen receptor. It was demonstrated that, the elevation of mRNA in prostate occurred in the presence of sustained elevation in the serum testosterone levels\(^{24}\). Ditto, \(^{25}\) reported that, testosterone reduced irreversible oxidative protein loss and increased protein synthesis, the other hand, we could presume that, the reduction in prostatic total protein of rats with naproxen could be account for lower in their enzymes activities which including in protein synthesis process as a result of lowering in the serum T concentration or/and by the oxidative damage on the histostructure of PVL in this group.

**Oxidative stress**

As shown in Table 3, it seemed that, the treatment with naproxen "Group2" induced oxidative stress in the tissue of the prostatic ventral lobe through an imbalance between MDA and anti-oxidant agents i. e., GSH and catalase (CAT) enzyme, given there were a significant (P \(\leq 0.05\)) increment in the MDA levels and a significantaly (P \(\leq 0.05\)) decrement in both of GSH level and in the activity of CAT enzyme compared to those in control "Group1". It was known that, the oxidative stress resulted from the production of oxygen radicals in excess than to anti-oxidant capacity of the stressed tissue\(^{26}\). Many previous reports elucidated that MDA was a well known of the lipid peroxidation indicator. Furthermore, it considered as end product derived from peroxidation of polyunsaturated fatty acid and related esters\(^{27; 28}\).

It was reported that, oxidative stress occurred as out come of imbalance between ROS and anti-oxidant at over production of ROS against anti-oxidant levels\(^{27; 29}\). Moreover, many endogenous anti-oxidant agents such as superoxide dismutase, glutathione peroxidase and glutathione-S-transferase enzyme, Catalase and GHS involved in scavenging ROS and detoxification of chemical compounds\(^{30}\). Whereby, \(^{31}\) confirmed that there was inversely relationship between the content of GSH "tripeptide foundin the cell cytosol"and its content and proxidative activity.

It was reported that, catalase played important role as anti-oxidant agent via its potency on H\(_2\)O\(_2\) dissociation to H\(_2\)O and O\(_2\)\(^{30}\). The pro-oxidant "ROS" was endogenously during aerobic metabolism, as well as, it was formed by exogenous sources\(^{32}\). Additionally, many exogenous antioxidant agents were widely used for detoxify the excess ROS\(^{33}\).

**Testosterone concentration**

Naproxen treated rats "group2" showed a significant (P \(\leq 0.05\)) decrement in their serum testosterone concentration compared to values in control group (Figure5).This result was in accordance with the result of\(^{34}\) who reported that stimulation Leydig cells from indomethacin "one membere of NSIAD"-treated rats with LH hormone produced significantly lower testosterone compared with value of control group. In other study, \(^{7}\) observed a marked decrement in the number of Leydig cells in indomethacin- treated rats.

In this study, it was obviously observed that naproxen interfered with androgen production via causing OS process, wherein the administration of naproxen caused to appear of oxidative stress in the testicular tissue which indicating a high testicular ROS levels. According to many previous studies ROS had ability to incidence a deleterious effects on the plasma membrane, proteins, and DNA molecules. Also, it was reported that, oxidative strees was related to an increment rate of cellular damage induced by reactive oxygen species\(^{35; 36}\). On the other study, an increment of ROS "free radicals" induced lipid peroxidation causing a deterioration by oxidizing polyunsaturated fatty acid\(^{37}\).
Therefore, the declining in the serum testosterone levels could be ascribed to the peroxidation of Leydig cells or to passive effect on their DNA and protein for leading to the inhibition in the activity, e., disruption of their steroidogenic enzyme followed by T production.

**The weights and histometrical alternations**

The present data showed that, treating rats with naproxen, Group 2 caused a significant (P ≤ 0.05) reduction in the mean weights of prostatic ventral lobes "PVL" compared to those values of control; Group 1. Whereas, the rats body weights of all group were uninfluenced (Table-1). In regarding, the secretory portion of PVL "acini" and excretory "duct system", naproxen caused a significant lowering in their mean diameter and in their height of the lining epithelial cells compared to control; Group 1 (Table-2). It was clear that, this reduction in PVL weight was as a reflection of passive changes on the histometrical aspects. Also, the reduction in the PVL weights and in histometrical profile were accompanied to the significant (P ≤ 0.05) lower in the PVL tissue total protein of Group 2 as observed in the current study (Figure 4).

We could suggested that, all these passive alternations were mediated by a significant (P ≤ 0.05) lower in the serum T concentration of Group 2 "rats with naproxen" (Figure 5). In previous studies many researchers confirmed the physiological role of T hormone in the subsidizing of the prostate development and its function (38-40).

It was known that, androgens were essential for normal development, growth and function maintenance of the prostate gland, and the androgen receptors were found in the epithelial cells and stroma of prostate (2). Ditto it was demonstrated that, the androgen receptors mediated the action of androgen on its target cells (41, 42) observed that, exogenous testosterone administration (3 mg.Kg⁻¹ b. w.) in castrated rats led to a significant weight gain and affected ventral growth of prostate. Also, (43) demonstrated that, the treatment of experimentally induced diabetes mellitus rats with testosterone (100 mg.Kg⁻¹ b. w. day) plus 5 IU of insulin caused a significant increment epithelial height lining of prostatic acini. In addition, (44) demonstrated that, the conversion of testosterone to dihydrotestosterone within the prostate induced growth of the prostate to its adult size.

**Histopathological evaluation of PVL**

Our finding demonstrated that, naproxen orally administration resulted in some toxic symptoms in a histo-architecture of PVL of Group 2 compared to Group 1 "control". These histopathological symptoms were represented by clear defect alternations in both prostatic ventral secretory portion "acini" and in the excretory portion "ducts". Therefore, it was noticed a markedly decline in the height of ventral prostatic acini epithelial cells and diverting to squamous cells also, these acini seemed with shrinkage secretion than those filled acini with homogenous secretion and lined with low cuboidal epithelial cells in control. Additionally, the stroma components were less dense in Group 2 compared to control (Figures 6, 7, 8 and 9). Regarding the histopathological indicators were represent by necrosis "cell death" in the most cells of simple columnar lining and in their folds, the appearance of vacuoles in the cytoplasm of columnar cells and between them. What is, sloughing of smooth layer from the prostatic acini wall and the congested vessels in the stromal compartments were noticed (Figures 12, 13, 14 and 15). It was repoted that, the treatment of naproxen and meloxicam drugs could cause a damage effect on the other tissue i. e., seminiferous tubules (45).

We could explain these passive alternations in the PVL histostructure based on a high production of ROS that acted directly on it according to the assurance of oxidative stress status in Group 2. Whereby, (46) mentioned that, lipid peroxidation was a well determinable mechanism of cellular injury at animal. In addition, unequalibrium
between oxidant and antioxidant agents produced excess ROS followed by oxidative damage in unsaturated fatty acid in the membranes, thiol groups in proteins, and nucleic acid bases in DNA. In other study, OS was considered as a reason to induce vascular tissue damage, protein structure and function damage, and causing apoptosis. Additionally, above mentioned harmful changes could be indirectly mediated by the declining in the serum T concentration that induction by OS. Previously, it was revealed that, chemical substance could affect structure of reproductive organs.

Phitofert competency against naproxen toxicity

In this study, the co-administration of phitofert with naproxen "Groups3" revealed its complete capacity to enervate the negative impacts of naproxen alone on the various studies parameters.

In regarding, mean weights, and histometrical "diameter and thickness of acini and acini epithelium" of PVL phitofert "Group3" resulted in a significant (P ≤ 0.05) elevation in their values compared to those in rats treatment with naproxen alone "Group2". Moreover these values were comparable to the normal values that observed in control; Group1 (Tables 1 and 2). Also, the same positive role of phitofert was reflected on the histostructure of PVL, so the histopathological sings were markedly disappearance and it seemed to like normal (Figures 10, 11, 16 and 17).

In relative to the PVL malfunctions that observed in Group2, the administration of phitofert to the naproxen-treated rats "Group3" could able to modify the PVL zinc concentration and PVL and serum PSA concentration whereby, their values significantly (P ≤ 0.05) increased compared to the values of rats with naproxen alone; Group2. Furthermore, the values of Group3 were similar to those in control; Group1 (Figures 2 and 3). Meanwhile, phitophert could attenuate the naproxen toxicity on PSA production by PVL via restore in the PVL tissue and serum to the nearly normal level compared to control; Group1, wherein it caused a significant reduction in concentration of both parameters (P ≤ 0.05) compared to Group2.

On, the potency of phitofert to recover some biochemical markers such as the resumption of PVL total protein production to their normal levels, phitofert resulted in a significant (P ≤ 0.05) increment in the PVL total protein concentration compared to those in rats with naproxen alone; Group2 and its concentration in Group3 were comparable to those in control; Group1 (Figure 4).

Concerning, the other biochemical markers i.e., oxidative stress markers, it was noticed that, the administration of photifert "Group3" to rats with naproxen caused to abrogate the oxidative stress status that induction by naproxen Group2. Obviously, this attenuation of OS was via a significant (P ≤ 0.05) reduction in the prostatic ventral lobe MDA levels and through the significant (P ≤ 0.05) elevation in the GSH concentration and CAT activity compared to those values of rats treatment with naproxen alone "Group2".

Furthemore, these values of Group3 were similar to those in control; Group1 (Tables 3). This potency of both phitofert to attenuate the OS via adusment of antioxidant markers against MDA was in agreement with the result of previous studies, wherein reported the phitofert abilitiyon attenuation of oxidative stress and impovment of antioxidant status in hyperprolactinaemic rats. As shown in Figure -5, the phitofert amdinistration to rats with naproxen "Group2" led to improve in the serum T concentration compared to those in rats recived naproxen alone "Group2" and the values were similar to those in control; Group1.

Evidently, all above improved findings and of their values to nearly normal levels could be associated with two mechanisms i.e., the direct attenuation of passive impact of naproxen-induced pro-oxidants "ROS" on the...
cellular component of PVL and indirect by abrogate the inversed impact of naproxen induced ROS on the Leydig cells "the main source of T production". Our results revealed that, the phitofert possessed completely competenceto eliminate all the toxic effect of naproxen on the histostructural and functional PVL. This capability of phitofert could be due to its ingredients because it consisted from several antioxidant substances such as maca, genesing, withanians, vitamins (including vitamin C, niacin, B_{12}, riboflavin i.e B_2), glutathione and coenzyme Q_{10} and some of these components had the potency to elevate T hormone production. Additionally, enormous previous studies reported the antioxidant properties of these components. Wherein,^{(51)} revealed that, maca had a significant inhibition of lipi peoxidation. Also, it was reported that, the antioxidant activity of maca was through its bioactive compounds such as alkaloids and phenols, and the antioxidant impact of alkaloids was higher than that of phenol^{(52)}.

Meanwhile,^{(53)} observed that a significant increase in the serum T levels of rats treated with 50 mg of maca negro plus 1.0 g of fagra extract, and this increment attributed to the interaction between the components of two plants. Also,^{(54)} demonstrated that, the feeding of male rat with hydroalchoholic extract of maca for 6 weeks resulted in a significant increased in the serum T concentration. Meanwhile, ginseng the other components of phitofert had ability to reduce lipid peroxidation and restore antioxidant capacity by suppressing aging rats- associated oxidative stress^{(55)}. And, ginseng appeared to be beneficial for attenuation a disease associated symptoms through its antioxidant activity so, it was able to prevent oxidative stress associated chronic disease^{(56)}.

In relative to Withanian somnifera the other ingredients of phitofert showed a significant cellular protection aganist oxidative damage via its antioxidant and anti-inflammatory properties^{(57; 58)}. On the other hand, many previous studies confirmed that the vitamins collection of phitofert drug had antioxidant role. Whereby, vitamin C caused a significant improvements in the stress-induced reproductive infertility via its ability to elevate the testosterone level and antioxidant effect^{(59)}.

Concerning, the other ingredient of phitofert i. e., glutathion "GSH" many previous studies reported the role of it in the protective defense against the damaging effect of oxidative stress^{(67; 68)}. Additionally, the coenzyme Q_{10} as one components of phitofert was also reported its having the antioxidant activity via scavanging ROS against oxidative stress for protecting the cells in many diseases^{(69; 70; 71)}. ©Annals of Tropical Medicine & Public Health S482
Table 1: The change in weight of animal bodies and ventral prostate of all experimental groups.

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Body weight (g)</th>
<th>Ventral prostatic weight (mg/100g b.w.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups 1 control (treated with d.w.)</td>
<td>303.75±5.79a</td>
<td>83.50±13.4a</td>
</tr>
<tr>
<td>Group 2 (Treated with naproxen)</td>
<td>286.25±8.71a</td>
<td>64.67±2.83b</td>
</tr>
<tr>
<td>Group 3 (Treated with naproxen+Phitofert)</td>
<td>292.50±6.80a</td>
<td>80.69±9.90a</td>
</tr>
</tbody>
</table>

The vertical different letters refer significant (P≤0.05) difference between the values of all experimental groups. The similar letters refer non-significant (P>0.05) difference between the values of all experimental groups.

Table 2: The histometric changes in the ventral in the ventral prostate lobe for all experimental groups.

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Acini portion (µm)</th>
<th>Excretory portion (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Epithelial height</td>
<td>Diameter</td>
</tr>
<tr>
<td></td>
<td>mean±S.E</td>
<td></td>
</tr>
<tr>
<td>Groups 1: control (treated with d.w.)</td>
<td>19.62±0.6 a</td>
<td>241.00±7.56 a</td>
</tr>
<tr>
<td>Group 2: (Treated with naproxen)</td>
<td>9.00±.46 b</td>
<td>113.25±2.31 b</td>
</tr>
<tr>
<td>Group 3: (Treated with naproxen+Phitofert)</td>
<td>17.25±0.46 a</td>
<td>298.12±4.08 a</td>
</tr>
</tbody>
</table>

The vertical different letters refer significant (P≤0.05) difference between the values of all experimental groups. The similar letters refer non-significant (P>0.05) difference between the values of all experimental groups.
Figure 1: The ventral prostatic tissue zinc concentration for all experimental groups. The different letters refer a significant (P≤0.05) difference between groups. The similar letters refer non-significant (P>0.05) difference between groups.

Figure 2: The ventral prostatic tissue-PSA concentration for all experimental groups. The different letters refer a significant (P≤0.05) difference between groups. The similar letters refer non-significant (P>0.05) difference between groups.
**Figure 3:** The serum PSA concentration for experimental groups.
The different letters refer a significant (P≤0.05) difference between groups.
The similar letters refer non-significant (P>0.05) difference between groups.

**Figure 4:** The ventral prostatic tissue-total protein concentration for all experimental groups.
The different letters refer a significant (P≤0.05) difference between groups.
The similar letters refer non-significant (P>0.05) difference between groups.
Figure 5: The serum testosterone concentration for experimental groups.
The different letters refer a significant (P<0.05) difference between groups.
The similar letters refer non-significant (P>0.05) difference between groups.

Table 3: The ventral prostatic oxidative markers for all experimental groups.

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Oxidative markers</th>
<th></th>
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<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>MDA</td>
<td>GSH</td>
<td>CAT</td>
<td></td>
</tr>
<tr>
<td>Groups 1 (treated with d.w.)</td>
<td>22.13±3.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>63.64±9.63&lt;sup&gt;a&lt;/sup&gt;</td>
<td>38.28±0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Group 2 (Treated with naproxen)</td>
<td>42.23±7.55&lt;sup&gt;b&lt;/sup&gt;</td>
<td>37.93±1.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>21.95±0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Group 3 (Treated with naproxen+phitofert)</td>
<td>28.09±0.44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>62.52±1.80&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36.49±0.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

The vertical different letters refer significant (P<0.05) differences between the values of all experimental groups.
The similar letters refer non-significant (P>0.05) differences between groups.
Figure 6: Acini of PVL in control rats, E: epithelial cell, S: stroma, PF: prostatic fluid (H&E, 10x).

Figure 7: Acini of PVL in control "G1", E: epithelial cell, S: stroma, PF: prostatic fluid (H&E, 40x).

Figure 8: Acini of PVL in naproxen-treated rats "G2", E: epithelial flattened cell, S: loose stroma, PF: receded prostatic fluid (H&E, 10x).

Figure 9: Acini of PVL in naproxen-treated rats "G2", E: epithelial flattened cell, S: loose stroma, PF: receded prostatic fluid (H&E, 40x).

Figure 10: Acini of PVL in rats treated with naproxen and phitofert "G3", E: epithelium, S: stroma, PF: prostatic fluid. (H&E, 10x).

Figure 11: Acini of PVL in rats treated with naproxen and phitofert "G3", E: Epithelium "cuboidal cells", S: dense stroma, IPF: invasive prostatic fluid (H & E, 10x).
Figure 12: Excretory portion of PVL control "G1", FE: folded epithelium, CE: cuboidal epithelium, L: lumen, S: stroma (H&E, 10x).

Figure 14: Excretory portion of VPL in naproxen-treated rats "G2", FE: folded epithelium, L: lumen, S: stroma (H&E, 10x).

Figure 16: Excretory portion of VPL in rats treated with naproxen and phitofert"G3", FE: folded epithelium, L: lumen, S: stroma (H&E, 10x).

Figure 17::Excretory portion of VPL in rats treated with naproxen and phitofert"G3", FE: folded epithelium, L: lumen, S: stroma (H&E, 10x).
CONCLUSION

Our findings revealed that, naproxen had toxic impact on histostructural and functional of prostatic ventral lobe as well as on the testicular function through the marked reduction in the serum T concentration. Whereas, phitofert drug was completely able to enhance all passive alternations of PVL.

ETHICAL CLEARANCE

The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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