

Prophylactic Role of *Curcuma Longa* and *Zingiber Officinale* on Histological Changes of Testis and Kidney of Male Rats Treated with Hydrogen Peroxide

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ABSTRACT

The present study aimed to investigate the prophylactic role of *Zingiber officinale* and *Curcuma longa* on histological changes of testis and kidney of male rats treated with hydrogen peroxide the male rats *Rattus norvegicus* divided in to four groups as follow the first group treated orally with 0.5 ml of physiological saline as a control group. The second group treated orally with 1 ml of hydrogen peroxide, the third group treated orally with 1ml hydrogen peroxide, and then treated orally with 0.5 ml of 200 mg/kg of *Curcuma longa*. The fourth group treated orally with 1 ml of hydrogen peroxide, and then treated orally with 0.5 ml of 200 mg/kg of *Zingiber officinale*. The duration of treatment was 30 days. Degenerative changes in various degrees were observed in microscopic examination of testis in animal group treated with hydrogen peroxide. In group treated with H₂O₂, testis showed inhibited spermatogenesis, testicular tubular degeneration and necrosis, obstruction of tubular lumen and lymphocytic infiltration ,the histological of kidney showed edematous glomerulus, necrosis ,congestion in blood vessel , degeneration in glumoular and increased numbers of nuclei. In the third group there was a decreasing of testis damage and area of lesion, degeneration, necrosis and, inflammatory infiltration compared to the second group, also in the section of the kidney we observed a few from inflammatory cells. A decreasing in degenerative changes was noticed in animals treated with hydrogen peroxide and 200 mg/kg of *Zingiber officinale*. The fourth treated group showed improve in histochange in testis . The section of testis was similar appearance with control group and in the section of the kidney a few of mononuclear cell.

Keywords: Hydrogen peroxide, *Curcuma longa*, *Zingiber officinale*, *Rattus norvegicus*.

INTRODUCTION

Oxidative stress is determined by the balance between the generation and degradation of reactive oxygen species (ROS) within a tissue (1, 2). Because many of the known triggers of apoptosis are oxidants or stimulators of intracellular generation of reactive oxygen intermediates (ROI), such as superoxide anion, hydrogen peroxide (H₂O₂) and hydroxyl radicals (OH[•]), ROI are commonly held to be mediators of apoptotic cell death (3). Several studies have shown that H₂O₂ exogenously added or produced by sperm, is toxic to mammalian spermatozoon causing damage to spermatic cell, including inhibition of motility and decline in energy metabolism (4- 6). Once produced, the H₂O₂ is removed by antioxidant enzymes such as catalase, peroxiredutases and glutathione peroxidase peroxiredutases that catalyze their reduction to O₂ and water (7). Moreover, the increasing use of plant extracts in the food, cosmetics and pharmaceutical industries suggests that in order to find active

compounds, a systematic study of medicinal plants is very important (8,9). Ginger rhizome (*Zingiber officinale* R., family: zingiberaceae) is used worldwide as a spice both antioxidative (10) and androgenic activity (11) of *Z. officinale* were reported in animal models. All major active component which acts as a potent antioxidant of *Z. officinale* such as zingerone, gingerdiol, zingibrene, gingerol and shogaols have antioxidant activity (12). Besides, other researchers showed that ginger oil has dominative protective effect on DNA damage induced by H₂O₂ and might act as a scavenger of oxygen radical and might be used as an antioxidant (13). Recent study supported that *Zingiber officinale* has the protective nutraceutical effect against oxidative stress and also reproductive toxicity (14). Among main active phytochemicals in *Zingiber officinale* such as gingerols, gingerdiol, shogaols, zingerone, and zingibrene are claimed to have antioxidant activity, these antioxidants are important protection against oxidative stress due to their ability to detoxify free radicals, such as reactive oxygen species (ROS) (15). In the another hand, numerous studies have shown that *Curcuma longa* has broad biological functions particularly antioxidant and anti inflammatory. actually, it been known either established that *Curcuma longa* is a bifunctional antioxidant; it exerts antioxidant activity in a direct and an indirect way by scavenging reactive oxygen species and inducing an antioxidant response, respectively. *Curcuma longa* has the capacity of lowering cholesterol, fatty acids, and triglycerides in an alcohol-induced toxicity (16). Therefore, the present study was designed to investigate the protective effects of *Zingiber officinale* and *Curcuma longa* rhizome on toxicity of H₂O₂ on testis and kidney of male rats.

MATERIALS AND METHODS:

Animals and experimental design:

Forty albino male rats (12 weeks old, weighing between 120 to 200 gm) kept under standard conditions of temperature, humidity and living regimens, with *ad libitum* access to food and water were maintained for one week accommodation period before conducting this experiment. Rats supplied from the animal house Department of Biology, Collage of Science, University of Thi-Qar, Iraq . The animals randomly divided into four equal groups (N=10 animals), The first group (control group) (G1) was treated with free drinking water. The second group (G.2) received orally 1ml of 1% H₂O₂. The third group (G3) received orally 1 ml of 1% of H₂O₂ and then treated with 0.5 ml 200 gm/kg of *Curcuma longa*, and (G.4) received 1ml of hydrogen peroxide and then treated with 0.5 ml of 200 mg/kg of *Zingiber officinale*. The duration of treatment was 30 day consequence. After then animals from each group were anesthetized. Testis and kidney isolated, in brief the routine sequence of events according to (17).

RESULTS

Histological Changes

In control group the testis was within the normal limits. The seminiferous tubules have normal structure as evidenced by well-organized distribution of spermatogenic and leydig cells (fig.1). In the animals that treated with dose of hydrogen peroxide there were degenerative changes in the testis with evidence of distortion and confluence in all the seminiferous tubules,

leydig cells degeneration and cellular debris were also observed in testis of hydrogen peroxide-treated congestion in blood vessel(fig.2). In the dose animals of *Curcuma longa* showed improve in histochange in testis expect minimal of congestion(fig.3), (fig. 4), the fourth-treated group with hydrogen peroxide and *Zingiber officinale* showed no testis damage,. It was noticed that this group has the same histological appearance as the control group.

The kidney of control rat (especially cortical tubules and glomeruli) with normal limits(fig.5), but, after exposure the rat to (1 ml for H₂O₂) for one month the kidneys showed histological changes included edematous glomerulus, necrosis ,congestion in blood vessel, degeneration in some of glumoular and increased numbers of nuclei (fig.6). In the section of the kidney in the third group there was a decreasing of kidney damage and area of lesion, such as degeneration, necrosis and inflammatory infiltration compared to peroxide group also in the section of the kidney we observed a few from inflammatory cells, increased numbers of Nuclei, the diameter of distal tubule and space of glomerulus compared with animals of control group(fig. 7). While the improve in histological change for kidney in the fourth group. We showed in the section of kidney minimal change in kidney such as a few of mononuclear cell and increased numbers of nuclei compared with animals of treated with hydrogen peroxide only (fig 8).

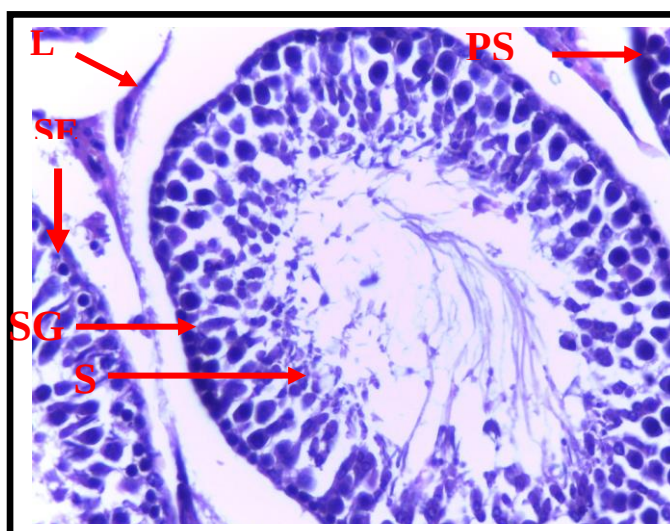


Fig. (1). Section of the testis, seminiferous tubules in control group. Note the spermatogenic cells at different stages. Leydig cells (L), spermatogonia (SG), primary spermatocyte (PS), spermatid (S), sertoli cell (SE).H&E staining 800x

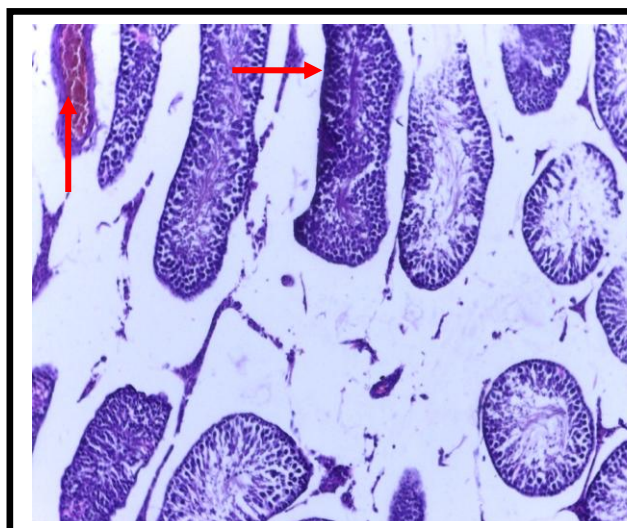


Fig. (2): Section of the testis from the animals in group 2 treated with hydrogen peroxide., (H & E 200x (apoptotic germ cells, congestion in blood vessel, degeneration in spermatogenic epithelium and necrosis.



Fig. (3): Section of the testis from the animals treated with hydrogen peroxide and *curcuma longa* . (H & E 200x) (G=, N= Increased numbers of Nuclei and infiltration inflammatory cells.

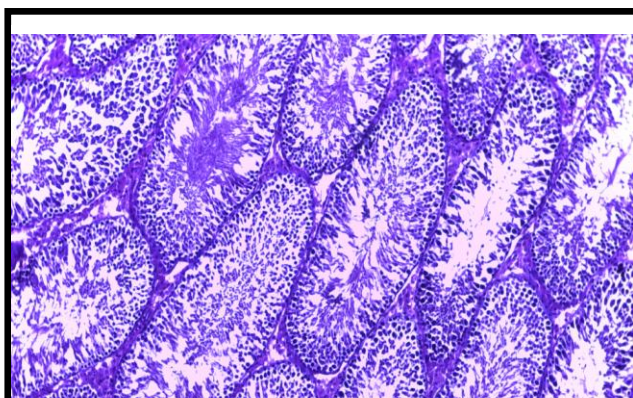


Fig. (4): Section of the testis from the animals in treated with hydrogen peroxide and. *Zingiber officinale* showed , there are no apoptotic germ cells . H&E staining 200x

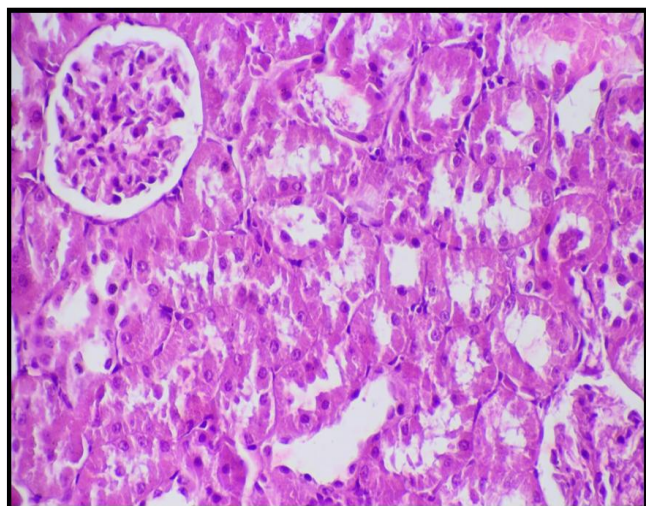


Fig. (5): Section of kidney of control group with normal limits. (especially cortical tubules and glomeruli). (H & E 800x).

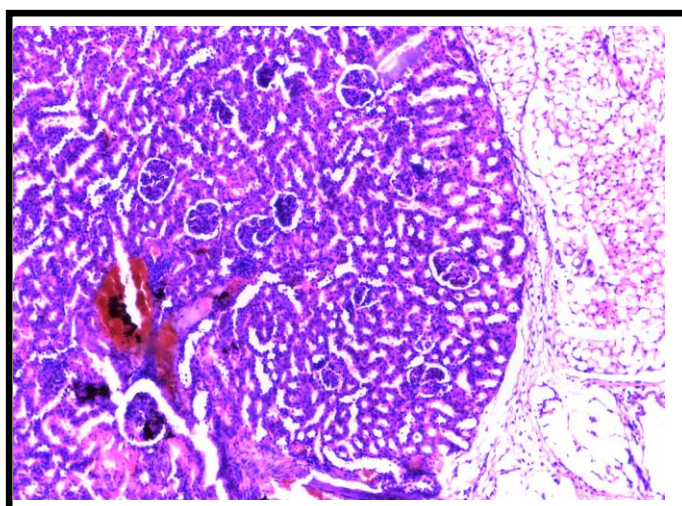


Fig. (6): Section of the kidney from the animals in group 2 treated with hydrogen peroxide (EG=edematous glomerulus, necrosis ,congestion in blood vessel , degeneration in glumoular and N= Increased numbers of nuclei (H & E 200x).

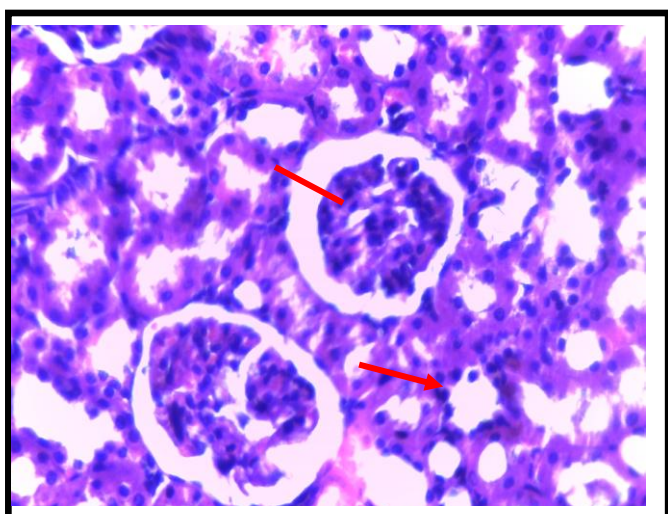


Fig. (7): Section of the kidney from the animals in group treated with hydrogen peroxide and *curcuma longa* . (G=, N= Increased numbers of nuclei, the diameter of distal tubule and space of glomerulus compared with animals of control group. (H&E 800X)

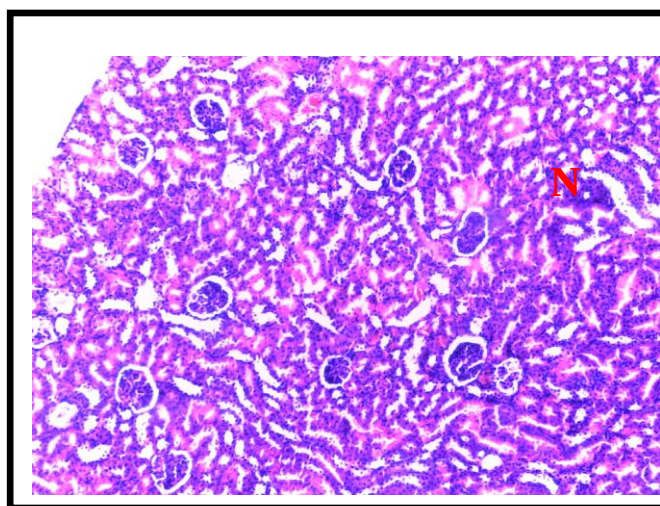


Fig. (8): Section of the kidney from the animals treated with hydrogen peroxide and *Zingiber officinale* showed , N= increased numbers of nuclei (H&E 200X)

DISCUSSION

The results of this study demonstrate that *Curcuma longa* and *Zingiber officinale* extracts is effective in protecting against oxidative damage has been shown on histological changes of testis and kidney,hydrogen peroxide which caused oxidative stress. We were observed in microscopic examination of testis and kidney in animal group treated with hydrogen peroxide inhibited spermatogenesis, testicular tubular degeneration and necrosis, obstruction of tubular lumen and

lymphocytic infiltration, the histological of kidney showed edematous glomerulus, necrosis, congestion in blood vessel, degeneration in glomerular and increased numbers of nuclei. The result of the study revealed that the level of oxidative stress markers have changed after H₂O₂ administration and represented an oxidative stress status. The effect of H₂O₂ -induced oxidative stress on histological structure of testes and kidney The other researchers finding were showed that, histological architecture of testes was affected by correlate with the BSO-induced oxidative stress. BSO administration and cellular damage was obvious. As it is known, testosterone is a hormone which is secreted by leydig cells and is necessary for spermatogenesis. Decreasing level of testosterone in the study well correlate with the findings that indicate a spermatogenesis disturbance. In agreement with our results, structural damages of the histological structure under the effects of oxidative stress has previously been reported (18;19). Plants of ginger is considered a safe herbal medicine with only few and insignificant adverse/side effects, and compounds isolated there include immuno-modulatory, anti-tumorigenic, , anti-apoptotic, anti-inflammatory, anti-lipidemic ,anti-hyperglycemic, and anti-emetic action. Ginger is a strong antioxidant substance and may either reduce or prevent generation of free radicals (20). The stage of spermatogenesis occur in semini-ferous tubule of testis, (21; 22). The sperm count is considered as important parameter assesses the effects of chemical on spermatogenesis (23). (24) found the oxidative damage, elevated lipid peroxidation and the alteration of membrane properties can lead to death of germ cell at different stages of development and decrease of the sperm count. Accordingly, some authors (25). (26) showed that ginger caused increase of spermatogenesis it is expected that antioxidant therapy acts as a protective defense against oxidative stress and improve fertility parameters also, oxidative damage has been shown by the ability of antioxidants such as ascorbic acid in semen to protect spermatozoa. The present study agreement with (27) which demonstrated that the administration of ginger can overcome reproductive toxicity of gentamicin, natural extract was also able to reduce apoptosis in testis ,Also, testicular toxicity .Previous results were supported by the finding of (28), who found that treatment of 50 mg/kg or 100 mg/kg/rat ginger for 20 days significantly increased sperm functions (sperm count, viability, motility), concentrations in rat, ginger have protective effects against oxidative stress in rat. In present study a dministration of Zingiber can protect testis from toxicity by hydrogen peroxide, the reason of the tissue damage back to free radicals are a group of highly reactive chemical molecules with one or more unpaired electrons that can oxidatively modify biomolecules they encounter. Reacting almost immediately with any substance in their surrounding area, they begin a chain reaction leading to cellular damage (29). Also in present study observed the administration of curcuma longa reduced of toxicity of hydrogen peroxide in testis ,this result agreement with Ramtej (30) which reported oral administration of aflatoxin for 45 days caused degenerative changes in the testis with evidence of distortion and confluence in all the seminiferous tubules, leydig cell degeneration and lumen filled with cellular debris were also observed in testis of aflatoxin-treated mice., all treatment-related changes were reduced on treatment with curcumin. Turmeric (*Curcuma longa*) and its active ingredient curcumin have been shown to scavenge the free radicals and thereby act as good antioxidants (31). The structure of curcumin shows ability as a chain-breaking antioxidant (32).Thus, curcumin proves to be highly potent component of turmeric ameliorating histological changes induced by hydrogen peroxide in testis of mice. In present study found rats which treatment with hydrogen peroxide occur histopathological in kidney such as degeneration in glumoular Bowman's capsule as well as the

diameter of distal tubule in mice treated with hydrogen peroxide compared to the control kidney. Furthermore, (33) reported that curcumin is a bifunctional antioxidant because of its ability to react directly with reactive species and to induce an up-regulation of various cytoprotective and antioxidant proteins. It is able to scavenge superoxide anion ($O_2^{\cdot-}$); H_2O_2 , (34). When Curcumin treated animals showed an improvement in renal function and showed less histological damage, the reason back to the mechanism of curcumin is attributed to its unique conjugated structure, which includes two methoxylated phenols and an phenol form of diketone(35). This study demonstrated that the administration of ginger can overcome reproductive toxicity and histopathology of kidney of hydrogen peroxide. This natural extract was also able to reduce apoptosis in testis and reduce the damage of kidney.

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