

Antioxidant Compounds from Rice Straw Extract and Their Effect on Diazinon Insecticide Hazard

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ABSTRACT

Rice straw is rich with antioxidants that suppress the naturally produced free radicals and delaying oxidative reactions. Diazinon is an organophosphorus pesticide and the toxicity of diazinon is due to blocking of acetyl cholinesterase activity. The current work was aimed to produce antioxidant from rice straw able to protect against hazardous of diazinon pesticide in vivo. The antioxidant compounds of the rice straw extract were determined and found that its main components are phenol and flavonoids at level 0.7mg/ml, 91.74 mg/ml but antioxidant activity attained 49.15 % respectively. The rice straw extract administrated at two doses 250 and 500 mg and their combination with diazinon and tested in vivo. The liver, kidney function and tumor marker as well as hisopathological examinations were tested. The obtained results indicated that the safety of the rice straw extract at two tested doses on the biochemical and histological parameters of experiments rats and the same parameters were increased to unsafe limit in diazinon group. It is worthy to report that the rice straw extract succeeds in protecting liver and kidney damages due to diazinon exposure and rice straw extract has the ability on scavenging free radical from the body and could be used safely as antioxidant agents. Utilization of rice straw will help to increase the value of all the out puts and the expected success is very high. It will be capable to solve some environmental problems, as well as in large benefit of Egyptian rice straw residues through the production of bio active compounds use as antioxidant agents to improve human health in Egypt.

Key words: Antioxidants, rice straw, free radicals, Diazinon Insecticide Hazard

Introduction

Antioxidants are the major plant products that play a role as anticancer agents (Shimizu *et al.*, 2013) by acting as reducing agents, hydrogen donators, and singlet oxygen quenchers that suppress the naturally produced free radicals and delaying oxidative reactions such as lipid oxidation (Xuz, 2012). Further, antioxidant studies have suggested associations between the consumption of phenolic-rich food and beverage as well as reduced oxidative stress-related diseases (Scalbert and Williamson, 2000). The major plant antioxidants are phenolic compounds with their large known number exceeding 8000 compounds (Stalikas, 2010). Phenolics contain important groups such as flavonoids, which belong to polyphenols and include subclasses such as flavonols, flavones, catechins and anthocyanins (Bingham, *et al.* 1998)

Rice (*Oryza sativa* L.) is one of the principal food crops in the world. Large amount of rice straw residues are produced after harvest. A major portion of this residue is disposed by burning which cause environmental problems. Several allelochemicals belong to different classes of secondary metabolites detected in rice residues and root exudates. These include phenolics (Seal *et al.*, 2004), flavones (Macias *et al.*, 2006).

The use of chemicals in modern agriculture has significantly increased productivity. But it has also significantly increased the concentration of pesticides in food and in our environment, with associated negative effects on human health. Annually there are dozens of million cases of pesticide poisonings worldwide (Richter, 2002). Moreover, it is now better understood that pesticides have significant chronic health effects, including cancer, neurological effects, diabetes, respiratory diseases, fetal diseases, and genetic disorders. These health effects are different depending on the degree, and the type of exposure. Typically, the effects are different for farmers who are directly exposed to pesticides, compared to those for farmer's relatives or people living in rural areas who are less directly exposed. There are also effects on consumers through pesticide residues in food.

Diazinon is an organophosphate insecticide is the fifth most commonly used pesticide used by homeowners, with two to four million pounds used annually (Beyond, 2003). Most synthetic compounds are highly toxic and are powerful inhibitors of acetylcholinesterase, a vital enzyme involved in neurotransmission,

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in the form of acetylcholine substitutes (Bakry *et al.*, 2006). The current work interested in large benefit of rice straw to produce the antioxidant compounds and its use to moderate or eliminate hazard of diazinon pesticide.

Materials and Methods

Materials

Rice straw

Rice straw was purchased from the Egyptian farm Dakahlia, Egypt.

Kits

Biochemical analyses: Urea, Creatinine, UA, GPT, GOT, ALP, Bil, AFP kits were purchased from Biomeieux, Laboratory of Reagents and Products (France).

Methods

Preparation of rice straw extracts:

Antioxidants from rice straw was carried out as described by Kagale *et al.* (2004), with some modifications. Briefly, 100 g of the chopped rice straw was added to 100 ml of sterile distilled water, ethanol (1:1 v/v) and incubated at room temperature for 24 h. Thereafter, the slurry was filtered through filter paper. The water extract was concentrated using rotary evaporator under reduced pressure, and the residues were dissolved in 100 ml of sterile distilled water.

Determination of total phenolic extraction:

The total phenolic content was extracted according to the Folin-Ciocalteu procedure (Zilic *et al.*, 2012). Briefly, the extract (100 μ L) was transferred into a test tube and the volume adjusted to 500 μ L with distilled water and oxidized with the addition of 250 μ L of Folin-Ciocalteu reagent. After 5 min, the mixture was neutralized with 1.25 mL of 20% aqueous Na₂CO₃ solution. After 40 min, the absorbance was measured at 725 nm against the solvent blank. The total phenolic content was determined by means of a calibration curve prepared with gallic acid, and expressed as μ g of gallic acid equivalent (GAE) per g of sample.

Determination of total flavonoid purification:

The total flavonoid content was purified according to Zilic *et al.* (2012). Briefly, 50 μ L of 5% NaNO₂ was mixed with 100 μ L of extract. After 6 min, 500 μ L of a 10% AlCl₃ solution was added. After 7 min, 250 μ L of 1 M NaOH was added, and the mixture was centrifuged at 5000 g for 10 min. Absorbance of the supernatant was measured at 510 nm against the solvent blank. The total flavonoid content was expressed as μ g of catechin equivalent (CE) per g of sample.

Antioxidant activity determinations:

Determination of radical DPPH scavenging activity.

Free radical scavenging capacity of Rice straw extract was determined using the stable 1,1-Diphenyl-2-picryl-hydrazyl (DPPH•) according to Hwang and Do Thi (2014). The final concentration was 200 μ M for DPPH• and the final reaction volume was 3.0 mL. The absorbance at 517 nm was measured against a blank of pure methanol at 60 min. Percent inhibition of the DPPH free radical was calculated by the following equation:

$$\text{Inhibition (\%)} = 100 \times (\text{A control} - \text{A sample}) / \text{A control}$$

Where:

- A control is the absorbance of the control reaction (containing all reagents except the test compound).
- A sample is the absorbance with the test compound.

Experimental animals:

Two months old, mature male rats were purchased from the Animal House Colony, National Research Center, Giza, Egypt.

Experimental Design:

Animals were divided into six groups (7 rats/group) and housed in filter-top polycarbonate cages and were maintained on their respective extract for 4 weeks as follow:

Group1: Normal control animals which fed on basal diet and water without any treatment. Group2 (DZ): Fed on basal diet and diazinon dissolved in corn oil. Group3 (EX1): Fed on basal diet + rice straw extract (250 mg/day). Group4 (EX2): Fed on basal diet + rice straw extract (500 mg/day). Group5 (EX1+DZ): Fed on basal diet and diazinon + rice straw extract (250 mg/day). Group6 (EX2+DZ): Fed on basal diet and diazinon + rice straw extract (500 mg/day). The animals were observed daily for signs of toxicity and weighted as well. At the end of experimentation period (i.e. day 28), blood samples were collected from all animals from retro-orbital venous

plexus for biochemical analysis. Then all animals were killed and samples of the liver and kidney tissues of each animal were removed and hydrated in ascending grades of ethanol, cleaned in xylene and embedded in paraffin.

Histopathological Examination:

All histological analyses were performed in routinely processed formalin-fixed, paraffin embedded tissue sections of 5 mm-thickness. They were stained with hematoxylin-eosin stain and the slides were examined with light microscope. Randomly selected fields were evaluated for cellular and tubular structures. Degeneration in epithelium and interstitial spaces were also noted.

Results and Discussion

The results in table (1) showed the bioactive compounds of rice straw extract which was determined to explore its active compounds and found that it contained the phenol and flavonoids at level 0.7mg/ml and 91.74 mg/ml respectively. The antioxidant activity amounted to 49.15 % in the determination of radical DPPH scavenging activity. The major plant antioxidants are phenolic compounds with their large known number exceeding 8000 compounds (Stalikas, 2010). Phenolics contain important groups such as flavonoids, which belong to polyphenols and include sub-classes such as flavonols, flavones, catechins and anthocyanins (Bingham *et al.*, 1998) Flavonoids have proven anticancer activity via proteasome inhibition (Liu *et al.*, 2008). Tannins are another major group belonging to phenolics and have been associated with human health through improving the immune system (Alonso-Amelot, 2011). Specific flavonoids showed cytotoxic activities against human cancer cells, such as the delphinidin which is a flavonoid pigment found in the peel of Solanum melongena and inhibited matrix metalloproteinase (MMPs) which degrade the extracellular matrix during the invasion of tumor cells (Gutierrez, 2007).

Table1: Bioactive compounds of rice straw extract

Sample	Total phenols (mg/ml)	Total flavonoids (mg/ml)	Antioxidant activity (%)
Rice extract	0.70	91.74	49.15

Furthermore, other flavonoids such as the luteolin was isolated from several plants and showed inhibition of various human cancer cells (Gutierrez, 2007). Specific phenolic compounds such as rosmarinic acid, β -sitosterol, apigenin, carnosic acid and myretenal inhibited the growth and proliferation of diverse cancer cells, including skin and lung cancers and had been reported in different Lamiaceae family members, including basil (Baliga *et al* 2013) and mint (Jain *et al.*, 2011). Several allelochemicals belong to different classes of secondary metabolites detected in rice residues and root exudates. These include phenolics (Mattice *et al.*, 1998; Seal *et al.*, 2004), flavones, diterpenes, steroids (Kong *et al.*, 2004; Macias *et al.*, 2006) and momilactone B (Kato-Noguchi and Ino, 2005).

The results in table (2) showed the effect of diazinon pesticide on biochemical parameters in rats and trial to eliminate or moderate its negative effect using rice straw extract and in this respect we studied the liver and kidney function as well as ALP, Bil. and AFP as a tumor marker. In general the control group of rats appeared healthy and its biochemical parameters were in normal range, while the diazinon group was on the contrary and all the biochemical parameters were increased. It is worthy to report that administration of rats with rice straw extract alone at two doses and when combined with diazinon restore all biochemical parameters to reach the normal control group. The tumor markers were increased due to administration of diazinon pesticide which induced degeneration in the liver and kidney tissues, while rice straw extract regenerated the tissues to normal situation and the tumor markers were decreased towards the safe range. It is mean safety of the rice straw extract as natural very cheap antioxidants able to protect the body from the hazard of diazinon pesticide.

Table 2: Effect of rice straw extracts on the biochemical parameters of rats

Tests	Treatment groups *					
	Control	EX1	EX2	DZ	EX1+DZ	EX2+DZ
Urea (mg/dL)	15.25±1.71a	17.50±1.0a	16.0±2.16a	53.25±2.21c	24.75±0.96b	22.0±2.94b
Creatinine (mg/dL)	0.80±0.13b	0.58±0.04a	0.61±0.05a	1.22±0.12c	0.60±0.04a	0.55±0.9a
UA (mg/dL)	4.30±0.87a	4.60±0.53a	4.50±0.85a	6.70±0.73b	4.90±0.26a	4.20±0.4a
GPT (IU/L)	21.25±1.7a	21.50±1.75a	19.75±1.72a	36.50±4.1c	30.25±4.05b	23.25±2.75a
GOT (IU/L)	22.5±4.6a	22.75±3.3a	21.00±1.82a	35.50±4.04b	19.50±1.29a	20.75±3.3a
Bil (mg/dL)	0.48±0.14a	0.62±0.12a	0.49±0.09a	1.18±0.28b	0.53±0.05a	0.43±0.06a
ALP (IU/L)	83.50±6.9a	86.25±3.86a	89.00±6.97a	113.25±10.4b	94.25±4.27a	87.25±6.55a
AFP (ng/ml)	0.92±0.18b	1.19±0.24bc	1.00±0.14b	1.45±0.13c	0.96±0.21b	0.85±0.09a

Mean \pm SD Mean values in the row or column with the same letter are not significant different at 0.05 level.

The toxic effects of diazinon on animals were studied by some investigators (Abdou and ElMazoudy, 2010; Shah and Iqbal, 2010). Also, Ceron *et al.* (1996) reported that diazinon inhibits acetylcholinesterase

activity and other organic functions. Diazinon was also found to lead to alterations in blood factors, plasma testosterone and glucose levels in male rats (Alahyary *et al.*, 2008). Oral administration of diazinon to mice resulted in a decrease in splenic T-dependent antibody response to DNP ficoll and a dramatic thymus atrophy (Kump *et al.*, 1996). Other studies have indicated that diazinon has the capacity to disrupt reproductive function in animals (Rodriguez and Bustos-Obregn, 2000 and Yehia *et al.*, 2007). Gokcimen *et al.* (2007) reported that diazinon induced histopathological changes in the liver and pancreas of rats. Diazinon treatment induced hematological changes (Kalender *et al.*, 2006) as well as hepatotoxicity (Kalender *et al.*, 2005) in rats.

Natural antioxidants, either in the form of raw extracts or their chemical constituents are very effective to prevent the destructive processes caused by oxidative stress (Zengin *et al.* 2011) Antioxidants stabilize or deactivate free radicals, often before they attack targets in biological cells (Nunes *et al.* 2012). The role of free radical reactions in disease pathology is well established and is known to be involved in many acute and chronic disorders in human beings, such as diabetes, atherosclerosis, aging, immunosuppression and neurodegeneration (Harman 1998). Studies on herbal plants, vegetables, and fruits have indicated the presence of antioxidants such as phenolics, flavonoids, tannins, and proanthocyanidins. Liver diseases remain a serious health problem. It is well known that free radicals cause cell damage through mechanisms of covalent binding and lipid peroxidation with subsequent tissue injury. Antioxidant agents of natural origin have attracted special interest because of their free radical scavenging abilities (Osawa *et al.* 1990). The use of antioxidant constituents has been proposed as an effective therapeutic approach for hepatic damages (Govind, 2011). Several allelochemicals belong to different classes of secondary metabolites detected in rice residues and root exudates. These include phenolics (Seal *et al.*, 2004), flavones (Macias *et al.*, 2006). The biochemical analysis was followed by the histological examination to confirm the obtained results and cleared that the biochemical analyses at the same trend of histological picture and both confirm else. From the obtained histological, picture (1) found that liver of control rat showing the normal histological structure of hepatic lobule and the same pictures (3, 4) when rice straw extract was used at low concentrations since the histological picture gave no histopathological changes, while when the diazinon pesticide was used the histological picture (2) was changed to give focal hepatic necrosis associated with inflammatory cell infiltration. It is worthy to report that administration of rice straw at low concentrations combined with diazinon pesticide enhanced, eliminate and moderate the histological picture (5, 6) to give no histopathological changes such as the control group. The same trend is clear from the histological kidney examination since the control rat group showed the kidney normal histological structure of renal parenchyma as well as the groups of rice straw extract which gave no histopathological changes (picture a), and at the contrary the kidney of diazinon pesticide group showing protein cast in the lumen of renal tubules. It is worthy to report that the rats received diazinon combined with rice straw extract appeared excellent histological picture (e, f) and showing no histopathological changes. Gokcimen *et al.* (2007) reported that diazinon induced histopathological changes in the liver of rats. Diazinon treatment induced hematological changes (Kalender *et al.*, 2006) as well as hepatotoxicity (Kalender *et al.*, 2005) in rats. Sarhan and Al-Sahhaf 2011 reported that Diazinon induced blood vessel congestion, leucocytic infiltrations in the liver parenchyma in addition to cytoplasmic vacuolation, fatty degeneration and pyknotic nuclei in the hepatocytes. On the other hand, renal damage was observed in the kidneys of treated rabbits. Renal tissues showed hypertrophied glomeruli, destructive of its lining epithelia. Renal blood vessels were congested and the inter-tubular spaces were filled with red blood cells.

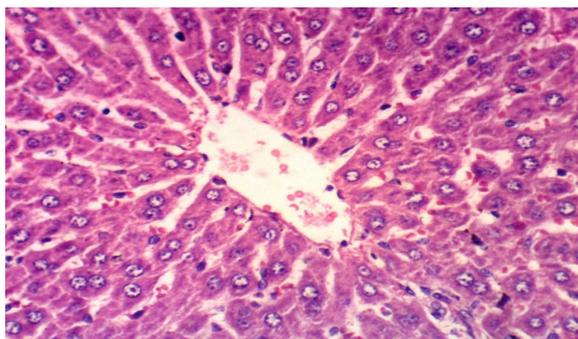


Fig. 1: Liver of control rats showing the normal histological structure of the hepatic lobule (H & E X 400).

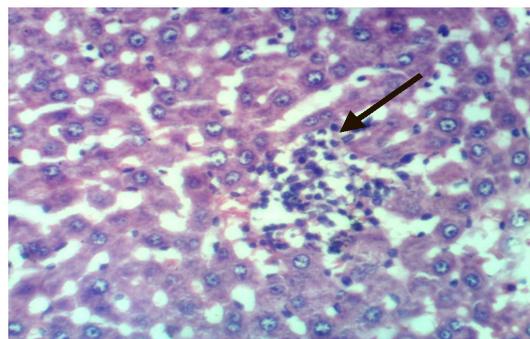


Fig. 2: Liver of rats treated with diazinon showing focal hepatic necrosis associated with inflammatory cell infiltration (H & E X 400).

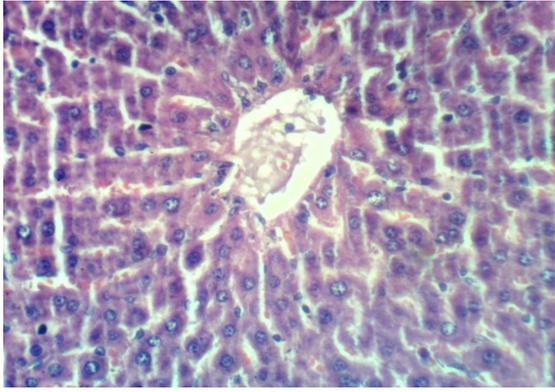


Fig.3: Liver of rats EX1 showing no histopathological changes (H & E X 400).

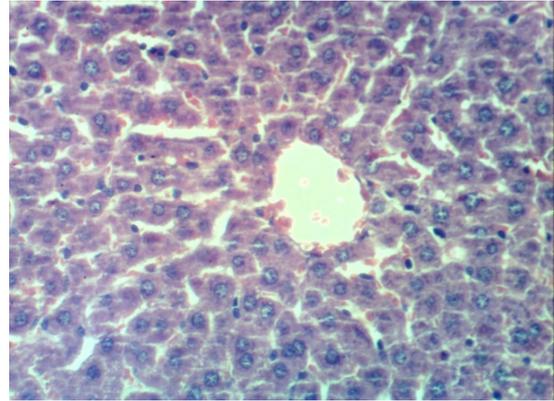


Fig. 4: Liver of rats EX2 showing no histopathological changes (H & E X 400).

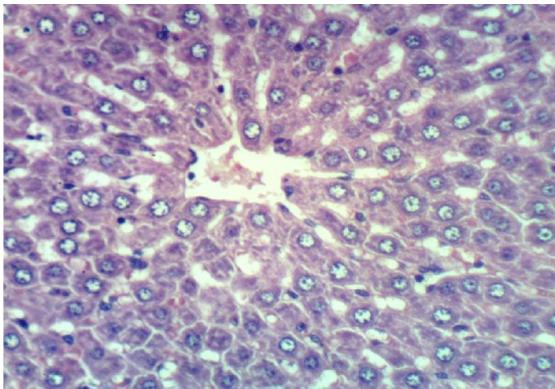


Fig. 5: Liver of rats EX1 + DZ showing no histopathological changes (H & E X 400)

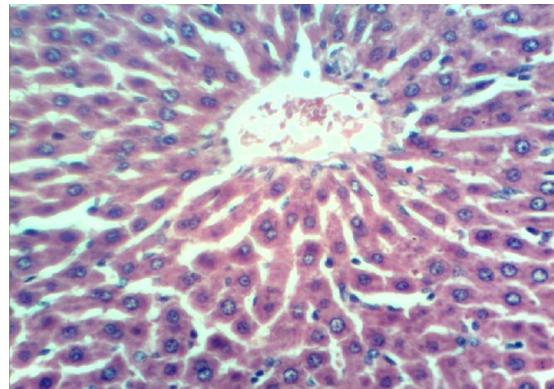


Fig. 6: Liver of rats EX2 + DZ. showing no histopathological changes (H & E X 400).

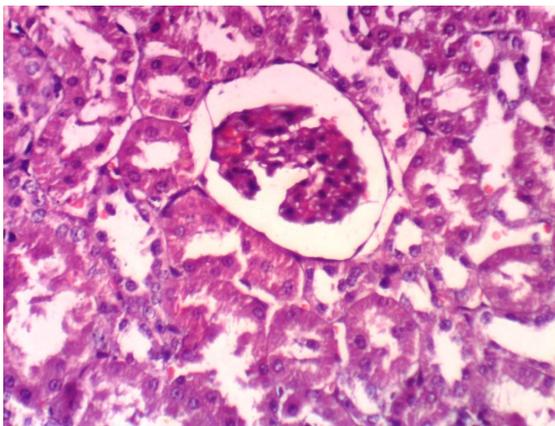


Fig. (a). Kidney of control rats showing the normal histological structure of renal parenchyma (H & E X 400).

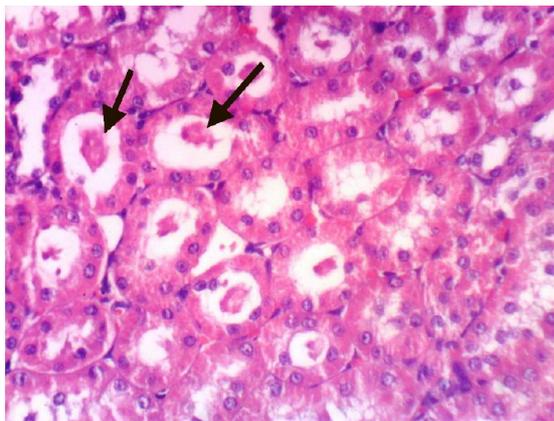


Fig. (b). Kidney of rats treated with diazinon showing protein cast in the lumen of renal tubules (H & E X 400).

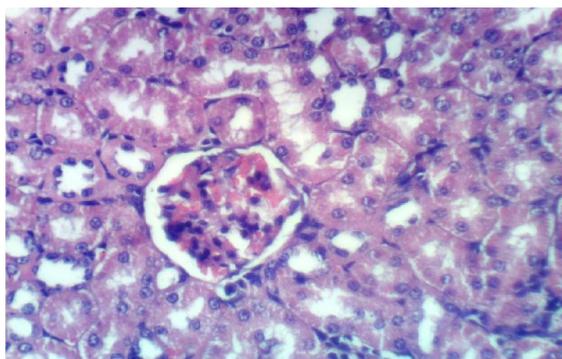


Fig. (c): Kidney of rats EX1 showing no histopathological changes (H & E X 400)

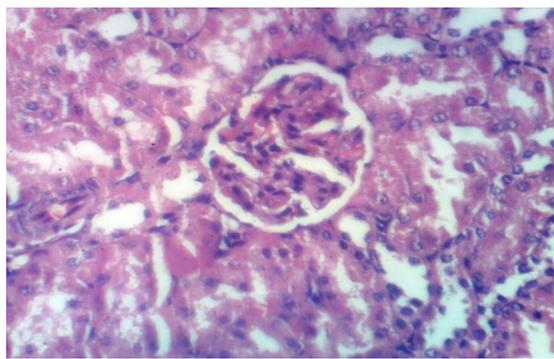


Fig. (d): Kidney of rats EX2 showing no histopathological changes (H & E X 400).

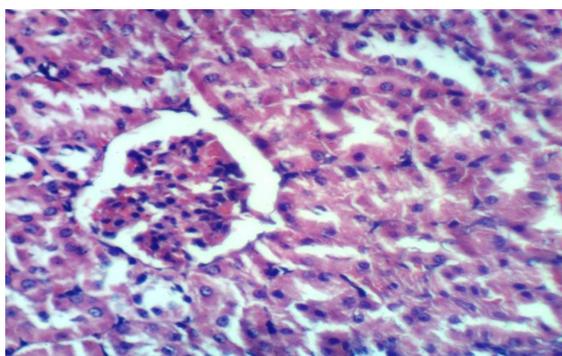


Fig. (e): Kidney of rats EX1 + DZ showing no histopathological changes (H & E X 400).

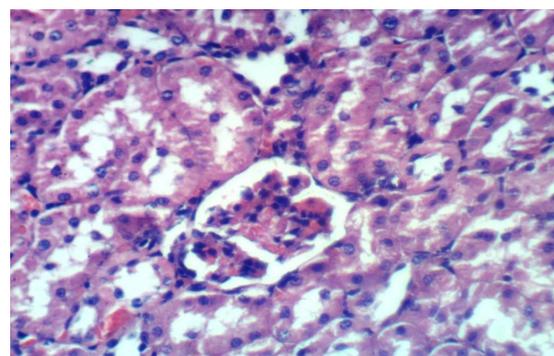


Fig. (f): Kidney of rats EX2 + DZ showing no histopathological changes (H & E X 400).

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