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INHIBITORY ACTIVITY OF PROTECTED EDIBLE PLANTS ON OXIDATIVE STRESS INDUCED BY ORAL 1,4-DIOXANE

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Abstract

1,4-Dioxane (DX) with two oxygen atoms make it hydrophilic and infinitely soluble in water. As a synthetic organic compound, it used widely throughout industry as a solvent. Dioxane causes numerous human ailments such as liver damage and kidney failure. It has been shown in research to be carcinogenic to animals, and is a potential carcinogen to humans. Daily administration for 1,4-dioxane (100 mg/kg body weight) in drinking water for rats weighing 120 g, except for normal control group. Experimental animal for 42 days was followed through body weight, serum alkaline phosphatase, serum creatinine, malondialdehyde, and catalase enzyme activity; beside histological patterns for liver, kidney, brain and ovary sections. Protection treatment has been offered using oral injection N-acetyl cysteine (100mg/kg b.wt.), and fresh 200mg/kg b.wt. in diet meal for each of nabk, husk, and sycamore in separated groups. Body weight and CAT activity have decreased by 25.8, and 68.7%, respectively. While increase has found in MDA, ALP and creatinine values by 76, 48.9, and 67.3%, respectively. NAC showed improvement especially for MDA peroxidation marker and creatinine for kidney disorder.

On the other hand, nabk improved CAT activity and husk for ALP liver mutagenicity marker. Intoxicated DX showed edema, kupffer cell activation, atrophy of glomerular tuft, and necrosis of neurons in liver, kidney and brain sections. Obviously nabk showed highly improvement in liver toxicity which is the most sensitive organ to DX as found in research.

Keywords: Dioxane, Malondialdehyde, Catalase, Acetyl cysteine, Nabk, Husk, Sycamore.

Introduction

1,4-Dioxane ($C_4H_8O_2$) DX is a colorless highly flammable heterocyclic organic liquid with a faint sweet odor similar to that of diethyl ether (Stickney and Silberhorn, 2005).1,4-Dioxane has been widely used as a solvent for various organic synthesis reactions and as a reaction medium solvent in chemical manufacture products. It is used for wetting, dyes baths, stain, printing or polishing compositions, emulsions and detergent preparations. Moreover, DX was used in purifying drugs and in cosmetic products and as a stabilizer in chlorinated organic solvents (Chemical Daily, 2006). It is used also as a dehydrating agent in the preparation of tissue sections for histology (HSDB, 1995). Interesting to say that dioxane was found in manufactured food additives and some condiments (Sack and Steele, 1989). It has been frequently emitting by carpets and draperies as well (Bayer and Papanicolopulos, 1990). According to the Pollutant Release and Transfer Register Report from (Japan Ministry of the Environment, 2007), 95 and 79 tons of DX were released annually into the atmosphere and public water in the year 2005, respectively, primarily from chemical industry.

DX was absorbed rapidly following oral and inhalation exposure in rats (Stickney and Silberhorn, 2005). The metabolism is linear at the exposure level up to 10 $mg.kg^{-1}$ and at the higher oral or the intravenous doses, the metabolites (Beta-hydroxyethoxyacetic acid, HEAA) excretion was reduced and dioxane concentrations were increased in both the urine and breath. The liver and kidney toxicity was induced after doses large enough to saturate the processes

for detoxification and elimination. In recent years international concern has risen about the ubiquitous presence of 1,4-dioxane in the environment and the adverse health effects to its exposure. DX is classified by Environmental Protection Agency (EPA), The International Agency for Research on Cancer (IARC) and Agency for Toxic Substances and Disease Registry (ATSDR) as a probable human carcinogen. Researchers investigated thatreplicated exposure of DX administered in drinking water resulted in liver and nasal cavity tumors rats, liver carcinomas and adenomas in mice and liver and gall bladder tumors in guinea pigs (Stickney *et al,* 2003). 1,4-dioxane was tested in drinking water for rats and mice for 2 years up to 5000 and 8000 ppm, respectively. They provided clear evidence of carcinogenicity in rats and mice (Kano *et al,* 2009).

Husk tomato (*Physalispubescens*L) fruit belongs to the *Solanaceae* family. Physalis species are known to contain carbohydrates, lipids, minerals, vitamins, and phytosterols (Puente *et al,* 2011). They also are a major contributor of withanolide-type structures. The withanolides have attracted substantial recent interest due to their exhibition of significant biological activities, specifically the antimicrobial, antitumor, anti-inflammatory, immunomodulatory, and the insect-antifeedant activities (Misico *et al,* 2011; Chen *et al,* 2011).

The *Zizyphus spina-christi* (family: *Rhamnaceae*) locally known as Nabk, has very nutritious fruits that are usually eaten fresh. For a long time, in folklore medicine, Nabk has been used for the treatment of some diseases, such as digestive disorders, weakness, the liver complaints, obesity, urinary troubles, diabetes, skin infections, and loss of appetite, feverish, phyaryngitis, bronchitis, anemia, diarrhea, and insomnia (Han and Park, 1986; Kirtikar and Basu, 1984). *Z. spins-christi* extracts has also been reported protective effect against the aflatoxicosis (Abdel-Wahhab *et al,* 2007) and anticonceptive properties in the rat and have a calming effect on the central nervous system. Flavonoids, alkaloids, triterpenoids, saponins, lipids, proteins, free sugar and mucilage are the main important compounds characterized in this plant (Adzu *et al,* 2003).

The fruits of sycamore fig (*Ficussycomorus*L. family *Moraceae*) are rich in the antioxidants. The *Ficus* has been traditionally used for its medicinal benefits as metabolic, cardiovascular, respiratory, antispasmodic and anti-inflammatory remedy (Duke *et al,* 2002). *Ficus* species are an excellent source of minerals, vitamins and dietary fiber; they are fat and cholesterol-free and contain a high number of amino acids (Slavin, 2006). Moreover, Pande, and Akoh (2010) added that the *Ficus* species contain polyphenolic compounds and flavonoid, which act as antioxidants.

The effects of DX has been reported through many laboratory and field research studies and approved by the chemical and histological studies. The research focus on the importance of Nabk, Husk tomato and sycamore in decreasing the harmful effects of DX on biochemical and histological patterns comparing to the activity of N-acetyl cysteine NAC as common effective compound.

Subjects, Material and Methods

Biological experiment: Forty two clean laboratory bred female Sprague-Dawley rats, weighing 120±5gm were purchased from the Research Institute of Ophthalmology, Giza Governorate, Egypt. The animals were given two weeks acclimation period, during which they were fed *ad libitum* a standard rat chow diet, with alternated 12-h dark/light cycle, and the ambient temperature was held between 22±2°C. All these studies were performed in accordance with the Guidelines for The Care and Laboratory Animals Usage, as adopted and promulgated by the Research Institute of Ophthalmology, Giza on 2013.

Dioxane 1,4 DX: It was brought from the Adwic Company (Egypt), as well the as N-

acetyl cystein NAC was bought from Sedico Company (Egypt). Three fruits were purchased from Egyptian market, washed with distilled water and air dried. Minded powder fruits were Husk tomato (*Physalispubescens*; family: *Solanaceae*), Nabk (*Zizyphus spina-christi;* family: *Rhamnaceae*) and Sycomore fig (*Ficussycomorus;* family: *Moraceae*).

Animals: They were divided into six groups (7 rats each), as followed; Group (1) normal control group were fed with standard rat chow diet. Group (2) rats were fed the chow diet and gave 100 mg DX/kg body weight daily in drinking water. Group (3) rats fed with standard diet and administered oral NAC (100 mg/kg b.wt.) allowed before drinking DX in water as group 2. Group (4) rats fed with standard diet mixed with fresh Nabk (200 mg/kg b.wt.) daily and allowed drinking DX in water. Group (5) rats fed with standard diet mixed with fresh Husk (200 mg/kg b.wt.) daily and allowed drinking DX in water. Group (6) rats fed with standard diet mixed with fresh Sycomorefig (200 mg/kg b.wt.) daily and allowed drinking DX in water.

Assessment of activity: Food consumption was monitored daily and body weight was determined once a week. After the 7-week experimental period, food was forbidden for 12 hours. The fasting rats were sacrificed and blood samples were collected into clean centrifuge tubes. Blood samples were allowed to coagulate and centrifuge at 3000 rpm for 20 minutes to separate the blood serum. Separated serum was stored at -20°C for subsequent biochemical analyses.

Biochemical analysis: The activity of catalase (CAT) and thiobarbituric acid-reactive substances (TBARS) content were evaluated in liver tissue. CAT activity was measured using the method of (Aebi, 1974). The activity was defined as the micromoles of H_2O_2 decrease per milligram of protein per minute. TBARS are the markers of lipid peroxidation and their concentration were measured referring to assay by (Uchiyama & Mihara, 1978). The concentrations were determined using a malondialdehyde (MDA) as standard. The determination of serum alkaline phosphatase ALP was measured with colorimetric method (Belfield & Goldberg, 1971), while serum creatinine was performed (Larsen, 1972).

Histopathological study: Samples were taken from the rats in experimental groups. After that, samples were fixed in 10% formal saline solution for twenty four hours. In tap water, samples were washed and then serial dilutions of absolute ethyl alcohol were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56°C in a hot air oven for twenty four hours. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns thickness by slidge microtome. On glass slides, tissue sections were collected, deparaffinized and stained by hematoxylin and eosin stain for histopathological examination through the light microscope (Banchroft *et al,* 1996).

Statistical analysis: Using SPSS program (SPSS, 1998) means were calculated among 7 replicates, with their Standard Deviations (± SD) for each group. Statistical analyzed by independent "T" test, and the probability level of 0.05 or less ($P<0.05$) was taken as a significance level.

Results and Discussion

Biochemical study: Exposure to variety of dioxane levels by inhalation and direct skin-contact, exhibited lesions in the liver, kidneys, brain and respiratory system. However, the effects could not be easily separated from the effects due to high intake of alcohol (Johnstone, 1959). Air measurements indicated dioxane levels varied from 0.01 to 13 ppm. Clinical evidence of increased (i.e., abnormal) aspartate transaminase (also known as serum glutamate-oxalacetic transaminase or SGOT), alanine transaminase (serum glutamate pyruvate transaminase or SGPT), alkaline phosphatase ALP, and gamma glutamyltransferase activities (liver function) was noted (Thiess *et al,* 1979).

The concentration of exposed DX has been chosen from previous study. Exposure to dioxane by ingestion results in saturation of metabolism above 100 mg/kg given in single dose. Saturation of metabolism was also observed as low as 10 mg/kg if dioxane was administered in multiple doses. Dioxane itself is not cleared through the kidney. A decrease in metabolic clearance with increasing dose has been interpreted as the saturation of metabolism at the higher doses (Young *et al,* 1978).

Effect of DX on body weight, values for redox system in MDA the final product for lipid peroxidation, and CAT responsible for transforming H_2O_2 into water, ALP as a liver disorder marker and creatinine the mark for kidney function (Tab.1). DX significantly affected on body weight through decreasing the gain in 25.8 %. Improvement has been occurred significantly especially for NAC 18.6% followed by husk, sycamore then nabk. Inhalation to dioxane for 2 years (Torkelson *et al,* 1974) has increased mortality and decreased body weight gains, compared to unexposed control rats, were observed. Among the male rats, decreased blood urea nitrogen (kidney function), decreased alkaline phosphatase (Cholesteric liver function), increased red blood cells, and decreased white blood cells were also observed. According to the authors, exposure-related, non-cancerous tissue lesions were not observed during the 2-year period. Results are correlated with potential of dioxane (Giavini *et al,* 1985). DX administered by gavage at doses of 0, 0.25, 0.5, & 1.0 ml/kg-day, on gestation days 6-15, and observations continued through day 21. Dams exposed to the highest dose exhibited nonsignificant weight loss and a significant decrease in food consumption during the first 16 days. During the remaining 5 days, food consumption increased, but the weight gain reduction in the presence of dioxane continued. In the same time, redox system showed increasing in MDA value by 76%, and the most significant improvement was for NAC 63.7% compared to DX group, followed by husk, sycomore and finally nabk fruit powder. Reducing CAT activity was noticed by 68.7 in DX administration, while the significant enhancing was the best for nabk 48.4% comparing to DX group, followed by sycamore in closed ration and then NAC and husk.

Serum ALP value has been increased 48.9% by DX toxicity. Improvement in ALP values has been found for 34.2% in husk administration compared to DX, followed by NAC, nabk and sycamore. Serum creatinine as well has been increased 67.3% by affording DX to experimental animals, and the improvement was for NAC (47.7%) significantly compared to DX toxicity, followed by husk, sycamore and finally nabk administration. In inhalation study, rats were exposed to dioxane at levels of 0.15, 1.3, and 5.7 ppm (Pilipyuk *et al,* 1977). Frequency was not specified, but the duration is given as “90 successive days”. At the end of the 3-month exposure, increased SGOT activity at the two highest doses and increased SGPT activity at all doses were measured in the sera of the exposed rats. Research do not consider the hematologic and clinical changes of toxicologic importance. In particular, toxic manifestations are usually associated with increased blood urea nitrogen and alkaline phosphatase levels (Torkelson *et al,* 1974).

Histopathological study (Fig.1) investigated the patterns of liver section for control, intoxicated and protected groups.NC liver sections of rat from normal control group showed normal histological structure of hepatic lobule. While, DX liver section of rat intoxicated dioxanegroup showed portal oedema, dissociation of hepatic cords, Kupffer cells activation with small focal hepatic necrosis associated with inflamm-atory cells infiltration. On the other hand, NAC+DX Liver section of rat from NAC standard protected group showed apparent normal hepatic lobule. In plant protected groups, Nabk+DX liver section of rat showed no

histopathological changes. In liver sections, NAC and fresh nabk have the highest protection qualitatively. The reason might due to the correlation for histology with MDA and CAT enzyme level, respectively for NAC and nabk (Table 1). Husk+DX liver sections of rat from Husk group showed slight Kupffer cells activation, thickening in the wall of bile duct and slight activation of Kupffer cells. Husk gave significant improvement for ALP value determination (Table 1). In the same way, Syc+DX liver sections of rat from Sycomore group showed Kupffer cells activation and vacuolization of hepatocytes.

Table 1: Body weight gain, TBARS serum and creatininelevelsas well as ALP and CAT activities in normal and DX–administrated rats treated with nabk, husk tomato, sycomore and NAC*

Group	Body weight gain/g/rat	TBARS (μmol MDA/g tissue)	CAT (μM of H_2O_2/min/mg protein)	Serum ALP (IU/L)	Serum creatinine (mg/dL)
NC	73.75±1.77	5.84±0.27	816.77± 65.57	70.08±3.45	1.13±0.11
DX	54.75 [a] ±1.49	24.30[a]±1.23	255.60[a] ± 25.89	137.14[a]±6.13	3.46[a]±0.24
NAC+ DX	67.25 [b] ±1.98	8.82[b]±0.24	442.87[a,b]± 32.18	95.45[a,b] ±5.04	1. 81[b]±0.14
Nabk+ DX	63.62 [b] ±1.58	11.29[b]±0.45	495.45[a,b]± 33.24	95.69[a,b] ±3.55	2.33[a,b] ±0.17
Husk+ DX	66.75 [b] ±2.06	10.34[b]±0.33	406.86[a,b]±42.12	90.29[a,b] ±4.43	1.87[b]±0.12
Syc+ DX	66.59 [b] ±1.85	11.26[b]±0.43	493.18[a,b]± 43.24	113.29[b]±4.05	2.27[a,b] ±0.15

** Mean ± SD of 7 rats in each group. [a] Significantly different from normal control at $p < 0.05$, [b] Significantly different from dioxane (DX) at $p < 0.05$*

Liver tumors reported after chronic oral exposure in rodents. Dose-related non-neoplastic changes in the liver increased in the hypertrophic response of hepatocytes, followed by necrosis, inflammation and hyperplastic hepatocellular foci (Dourson *et el,* 2014).

The major metabolite of dioxane is hydroxyethoxyacetic acid (HEAA) and the kidney is the major route of excretion (Young *et al,* 1976). NC kidney section of rat from normal control group showed normal histological structure of renal parenchyma (Fig.2). DX kidney sections of rat from intoxicated group with dioxane showed vacuolation of endothelial lining glomerular tuft and epithelial lining renal tubules with interstitial oedema and atrophy of glomerular tuft. In the protection process, NAC+DX kidney sections of rat from group treated with NAC showed vacuolations of endothelial lining glomerular tuft and epithelial lining renal tubules. Nabk+DX kidney sections of rat showed atrophy of some glomerular tuft. Husk+DX kidney sections of rat from Husk group showed congestion of renal blood vessel. In the same trend, table (1) proved to show prospective role for NAC and Husk on creatinine levels. Syc+DX Kidney sections of rat from Sycomore group gave vacuolations of epithelial lining renal tubules and congestion of glomerular tuft. Human studies on the relationship between exposure to hydrocarbon solvents including dioxane and renal failure have reviewed, in particular rare glomerulonephritis (Yaqoob *et al,* 1994). They suggested that such solvents may play a role in renal failure. Of interest to the discussion on chronic exposure to dioxane suggested that disease mechanism process involves local autoimmunity with decreased in white blood cells.

In experimental animals, DX from oral exposures caused liver toxicity as evidenced by histological and biochemical changes (e.g., liver enzyme changes, centrilobular swelling, and necrosis) at time points of treatment (Kano *et al,* 2008). Brain section of rat from normal control group showed no histopathological changes. Brain sections of rat from dioxane group showed focal haemorrhage (Fig.3), necrosis of neurons and

neuronophagia of necrotic neurons. NAC+DX brain sections of rat from group NAC showed necrosis of some neurons. Nabk+DX brain sections of rat from Nabk group showed focal gliosis and neuronophagia of necrotic neurons. Husk+DX brain sections of rat from Husk group showed cellular oedema and necrosis of some neurons. Brain sections of rat from Sycomore with DX group showing pyknosis of neurons, cellular oedema and neuronophagia of pyknotic neurons. The severe effect of DX on brain sections was not overcome completely by using NAC or fresh fruit protection. The effect of oral exposure to 1,4-dioxane was evaluated on the regional neurochemistry of the rat brain (Kanada *et al,* 1994). 1,4-Dioxane was administered by gavage to male Sprague Dawley rats (5/group) at a dose of 1,050 mg/kg, approximately equal to one-fourth the oral LD_{50}.1,4-Dioxane exposure was shown to reduce the dopamine and serotonin content of the hypothalamus. Ovary section of rat from normal control group showed no histopathological changes (Fig.4). DX Ovary sections of rat from dioxane group have congestion of medullary blood vessels, hyperplasia of interstitial cells and well vacularized Corpora lutea with degeneration of corpora lutea. NAC+DX ovary sections of rat from NAC group showed follicles of different stages of development. Nabk+DX ovary sections of rat from group Nabk showed follicles of different stages of development with vacuolization of Corpus luteum. Husk+DX ovary section of rat from Husk group showed hyperplasia of interstitial cells with presence of follicles of different stages of development. Syc+DX ovary sections of rat from Sycomore group showed congestion of interstitial blood vessel and vacuolization of luteal tissue.

The effect of DX inhalation on free radical processes in rat ovary and brain was investigated. Female rats were exposed to 0, 10, or 100 mg/m^3 of DX vapor for 4 hours/day, 5 days/week, for 1 month. Inhalation of 100 mg/m3 of DX resulted in a significant increase in glutathione peroxidase activity, & activation of free radical processes was apparent in both the rat ovary and brain cortex (Burmistrov *et al,* 2001). US Environmental Protection Agency elucidated several possible modes of action for DX based on the oral route of exposure including a regenerative hyperplasia, especially for liver tumors (US-EPA, 2013). Chronic oral exposure to DX showed liver tumors in rodents (Dourson *et al,* 2014). No non-neoplastic lesions were reported in the livers of mice, at least at the high dose (NCI, 1978), while others reported swelling of the centrilobular hepatocytes, necrosis, and hyperplasia at comparable or lower doses (Kano *et al,* 2008; Kano *et al,* 2009). High acute occupational exposure to DX can result as appeared in our study in liver, kidney & central nervous system toxicity (Johnstone, 1959). No plant protection research found in DX intoxication field, so many researches needed to deal with DX oral or inhalation pollution. Analytical steps for potent compounds identification in plants need as well high potential research in scientific future. DX carcinogenicity was provided by oral administration of DX formulated drinking water to rats and mice (Kano *et al,* 2009). Nasal and hepatocellular tumors were used previously as data of relevancy to human risk exposed to DX through water drinking based on the in vivo genotoxic mode of action.

Conclusion

Clear evidence of toxicity was provided by a 7-week oral administration of 1,4-dioxane-formulated drinking water to female rats. The biochemical and histopathological investigations have been explained the importance of nabk, husk tomato and sycomor fig fruits powder treatment comparing to N-acetyl cysteine NAC in decreasing the dangerous effects of the DX administration.

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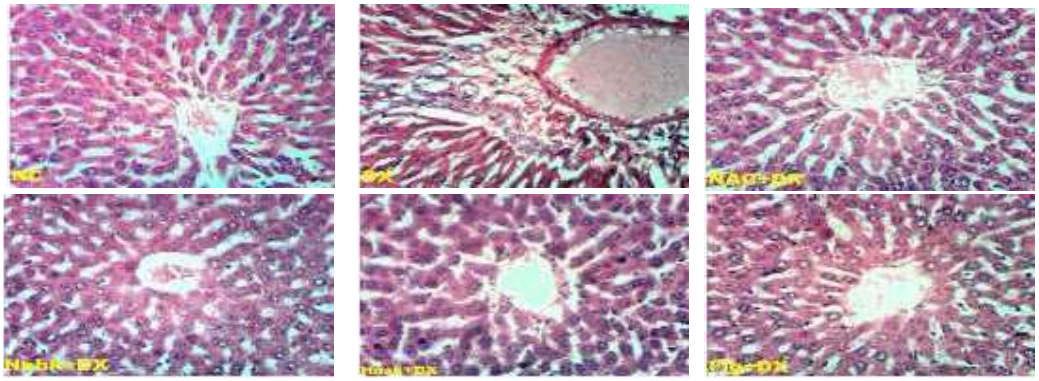

Fig.1: Liver sections of control, DX, NAC, Nabk, husk and sycamore fig protection groups, respectively.

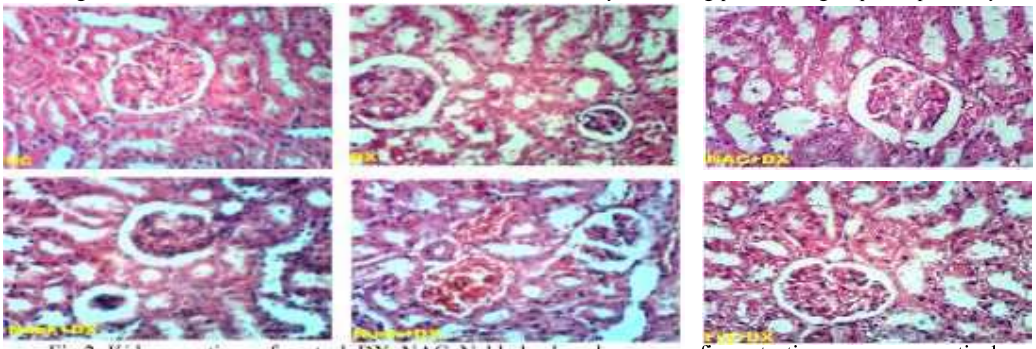

Fig.2: Kidney sections of control, DX, NAC, Nabk, husk and sycamore fig protection groups, respectively.

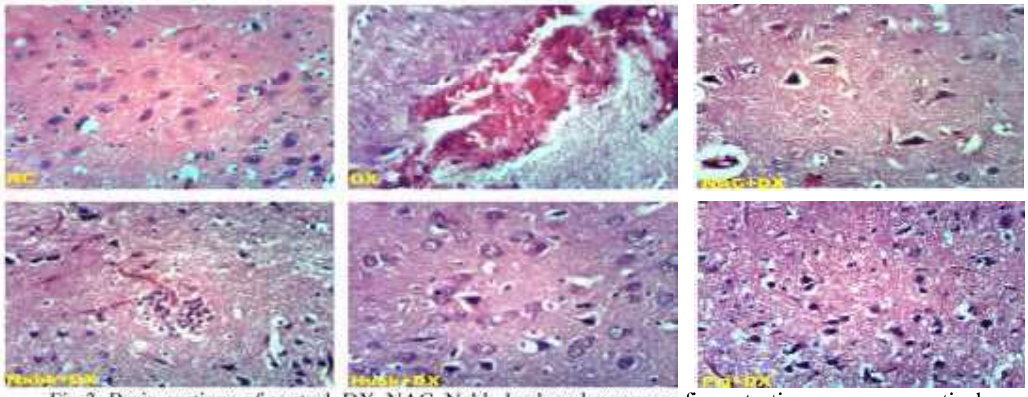

Fig.3: Brain sections of control, DX, NAC, Nabk, husk and sycamore fig protection groups, respectively.

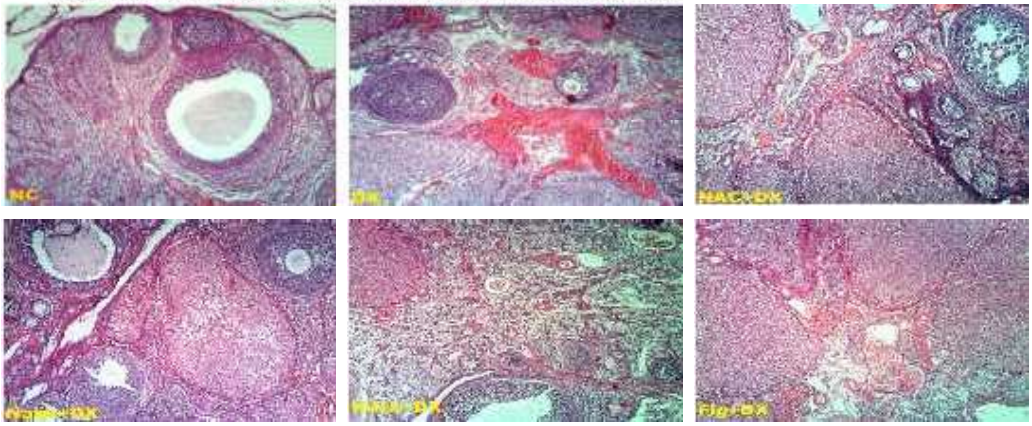

Fig.4 Ovary sections of control, DX, NAC, Nabk, husk and sycamore fig protection groups, respectively.