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ANTIOXIDANT ROLE OF PROPOLIS ON SODIUM FLUORIDE HEPATO-RENAL TOXICITY IN ALBINO RABBITS: PATHOLOGICAL STUDY

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ABESTRACT

Fluoride anion is an agent which contributes to the dental protection and prevents osteoporosis in small doses, but in case of excessive exposure, it can interfere with metabolic pathways involving lipids, carbohydrates and proteins. Propolis is a compound formed by honeybees and considered as a common antioxidant. This study was designed to investigate the beneficial role of propolis as natural antioxidant against chronic sodium fluoride hepato-renal toxicity in albino rabbits. Four experimental groups receiving a combination of sodium fluoride (10 mg/ kg body weight/day) and/or propolis (25 mg/ kg body weight/day) for 60-day was divided as follows: no treatment (control), sodium fluoride alone, propolis alone and sod fluoride + propolis. Histopathological and histochemical results revealed that tissue alterations in both liver and kidney were present only in fluoride treated group. There was hepatocellular necrosis, extensive vacuolization and inflammatory cell infiltrations in the liver. However, the kidneys exhibited increasing amounts of cloudy swellings, degeneration of tubular epithelia, tissue necrosis, and extensive vacuolization in renal tubules as well as atrophy of glomeruli, interstitial oedema and interstitial nephritis. These hepato-renal toxic disturbances induced by fluoride reflect functional and structural alterations in the tissues. On the other hand, administration of propolis either alone or combined with sod fluoride pronounced or even complete recovery this hepato-renal toxicity. In addition to, the morphological analysis of apoptosis of liver and kidney tissues showed massive necrosis and increased rate of apoptosis in sodium fluoride only treated group. While in Propolis only and /or fluoride treated groups, there was low level in apoptosis. The conclusion of the present study suggests that the propolis is strong antioxidants and free radical scavengers that ameliorated the chronic hepato-renal toxicity induced by sodium fluoride in the albino rabbits.

Key words: Sodium Fluoride, Propolis, Hepato- renal toxicity, Apoptosis, Histopathology, Histochemical, Rabbit.

INTRODUCTION

Although fluoride intake is necessary for the development of teeth and body skeleton but its requirements are in traces. It can occur naturally in surface water as a result of atmospheric deposition of fluoride particles as well as fluoride-containing rocks as leaching from rocks and soils. Ground water and chemical manufacturing waste products can pollute water sources with excess fluoride. (ATSDR, 2003 and Bhatnagar *et al*., 2011). High levels of fluoride in drinking water have been a potential hazard all over the world as a result of increased in dustriazation and environmental pollution (Susheela *et al.,* 2013). Egypt is one of about 21 developing nations that have problems with endemic fluorosis, where the main pathway of fluorosis, is the ingestion of tap water from contaminated ground water sources.

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The fluoride concentration in industrial waste water samples collected from Abu Zabaal and Ahlia areas around Cairo vary from 1.13 to 7.10 mg/L, significantly exceeding the World Health Organization recommended maximum 1mg F/L (Helaland El Dakdoky, 2006). The source of drinking water in Marsa Matrouh and Arish governorates, Egypt is groundwater that coming from artesian wells and contained higher levels of fluoride with an average of 0.761 and 0.926 mg/L, respectively (Ibrahim *et al.,* 2013).

However, fluoride toxicity pathogenesis resulting by crosses the cell membrane very rapidly and distributed from the plasma to all tissue and organs which exerts an oxidative stress leading to generation of frees radicals of reactive oxygen species (ROS) and alterations in antioxidants or scavenging enzymes and finally resulting in histopathological alterations and apoptosis indifferent tissues (Bouaziz *et al*., 2006; Khandare *et al*., 2011 and Agha *et al.*, 2012). Apoptosis is the maintenance of tissue homeostasis involves in the removal of superfluous and damaged

cells. Apoptic cells eventually were swollen due to degeneration then nucleus fragmentation with condensation of the cytoplasm to produce membrane-bound apoptotic bodies that are phagocytosed by macrophages (Reed, 2001).

Propolisis natural sticky substances produced by honey bees by mixing their waxes with resinous sapand also known as bee glue (Yoshimi *et al*., 2009 and Alqayim, 2015). Nowadays propolis is reported to inhibit the generation of superoxide anion. Furthermore, propolis has been determined to reverse the consumption of glutathione, which is synthesized in the liver and has radical scavenging activity (Survswaron *et al*., 2007and Khalil and El-Sheikh, 2010). As well as propolis has an anabolic effect as it is rich in essential amino acids, protein, unsaturated fatty acids and also improves the digestive utilization of calcium, phosphorus and magnesium which contribute to the health effects (Campos *et al*., 2003). In modern times, it has been found to have a wide range of biological activities, such as being antibacterial (Oris *et al*., 2005), anti-inflammatory (Yoshizumi *et al*., 2005), anticarcinogenic (Aliyazicioglu *et al*., 2005), antioxidative (Kanbur *et al*., 2009) and immunomodulatory effects (Sforcin, 2007).

This study was designed to investigate the beneficial role of propolis as natural strong antioxidant against chronic sodium fluoride hepato-renal toxicity in albino rabbits.

MATERIALS AND METHODS

I - Animals:
Twenty healthy adult New Zealand white male rabbits of the same age, with a weight range of 2–2.5 kg was used for this study. They were housed in a well-ventilated animal house and each group caged separately, at a temperature of 29-32°C. The animals exposed to 10–12 h of daylight under proper hygienic conditions and received food and water *ad libitum*. All animals were acclimatized for one week before being dosed. The present study was carried out in Animal Reproduction Research Institute (A.R.R.I.).

II - Chemicals:
1- Sodium Fluoride (NaF): Crystalline powder natriumfluoride (Sigma, Germany) was dissolved in tap water from El Gomhoria Company for Chemical and Medical Trading, Egypt.

2- Propolis preparation: Egyptian propolis was commercial purchased beeswax honeycomb processing. The propolis adjuvant was prepared as previously described (Shaapan *et al*., 2014). The dose of the drug was determined by LD50 test according to Purohit *et al*. (2013).

III - Animal groups and dose administration:
It was applied according to Khandare *et al*. (2011). The rabbits were divided into four equal groups of five each.

1 - Control group (GP I): Animals were given water without any treatment.
2 - NaF group (GP II): Animals were provided 10 mg NaF/kg b.w.
3 - Propolis group (GP III): Animals were given 25mg propolis /kg b.w.
4 - NaF+ propolis group (GP IV): Animals were given 10 mg NaF/kg b.w.+ 25mg propolis /kg b.w.

All experimental animals were given different treatment daily in drinking water for 60-day. At the end of experiment, animals were sacrificed and liver and kidneys were taken for histopathological and histochemical studies as well as morphological assessment of apoptosis.

IV - Pathological studies:
1- Histopathological examination: liver and kidneys were taken and preserved in a 10% neutral formalin solution for fixation, then dehydrated through ascending grades of alcohol, cleared in xylene and embedded and blocked in paraffin. Sections of 3–5-µm thickness were taken and stained with hematoxylin and eosin then examined under the microscope. All these procedures were applied as previously described by Suvarna *et al.* (2013).

2- Histochemical examination: Periodic acid schiff (PAS) reaction was applied to demonstrate carbohydrates and Masson's Trichrome stain was used for connective tissue proliferation demonestration. All these procedures were applied as previously described by Suvarna *et al.* (2013). As well as Bromophenol, blue stains were performed according to Mazia *et al.* (1953)

3- Morphological assessment of apoptosis: Paraffin tissue sections were fixed on positive charged microscope slides, stained with an Acridine orange (A.O) / Ethidium bromide (E.B) mixture and viewed under a UV microscope as described by Dhama *et al.* (2002), the viable cells and early apoptotic cells appeared fluoresce green, while the nuclei in necrotic cells appeared fluoresce orange red.

RESULTS

I - Clinical signs:
Chronic exposure to fluoride for a period of 60 days induced deleterious impacts in animals under experiment. First symptoms of chronic fluoride toxicity are reduced feed intake as a result of loss of appetite that leading to emaciation. Also, transient diarrhea and/or constipation were seen. In addition to, nervous manifestation included muscle tremor, weakness, pupilary dilatation and hyperesthesia as

well as lethargy. While groups treated with propolis with fluoride and propolis only didn't show any of these symptoms.

II - Histopathological Examination:

1- Liver:
In the present study, the gross examination of the rabbit's liver of GP II(10mg NaF group) treated groups was pale enlarged with pen headed white foci on them comparing with the normal appearance of normal group. Microscopically, liver of control group (GP I) revealed that normal lobular pattern of hepatic cords. Comparing to the control group, hepatocytes ballooning degeneration with numerous centrilobular necrotic foci in addition to, moderate leukocytic cell infiltration was also observed in certain areas (Fig. 1).The central vein and hepatic sinusoids appeared dilated as well as bile duct proliferation and periportal fibrosis was also observed (Fig. 2). Periportal fibrosis were detected with Massontrichromestain (Fig. 3).

On the other side, nomacroscopical or microscopical histopathological changes were noticed in the examined livers of GPIII (Propolisonly treated) rabbits comparable to the control group except a very slight dilatation of blood sinusoids that denotes increased blood flow in these vessels. On the other hand, the macroscopic examination of liver of GP IV (Propolis with sod fluoride group) showed nearly normal size and color without any foci on their surfaces. Microscopically, animals showed no evidence of ballooning or degeneration but Kupffer cell proliferation was observed around the dilated sinusoidal vessels. Moreover, hepatic focal cell necrosis, and periportal mononuclear cell infiltration or fibrosis was absent compared to GP II (NaF treated group) (Fig. 4).

2- Kidney:
In this work, the gross examination of the kidneys in the fluoridated rabbits (Gp II) appeared congested and moderately enlargement comparing to control group kidneys. Histopathologically, a pronounced cellular degeneration with vacuolated cytoplasm and necrosis in the epithelial lining of convoluted tubules were seen (Fig.5). The glomeruli and their capsules appeared atrophied with interstitial nephritis and associated with perivascular oedema (Fig.6).

Concerning with the effect of propolis supplementation, it was cleared that, propolisdoes not have a protective effect against sod fluoride induced nephrotoxicity, gross nor microscopic lesions were detected in the examined rabbit kidneys of both GPIII and GP IV except mildcongestion with focal vacuolar tubular degeneration were seen in Gp IV (Fig.7).

III - Histochemical Examination:

1- Liver: Histochemically, we detected PAS-positive material was diffusely distributed in the form of purple granules of glycogen in the hepatic cells in control Gp I. Comparing to GP I, hepatocytes appeared markedly with less PAS-positive matter of glycogen particles in GPII (Fig. 8). On the other hand, in GP III and GP IV the polysaccharides appeared more or less like control (Fig. 9).Moreover, bromophenol blue stain in GPII revealed that fluoride caused a noticed decrease in the protein content in cytoplasm of hepatocytes (Fig. 10) while in GP III and GP IV, propilais showed a marked a preservative effect on protein content of hepatocytes (Fig. 11).

2- Kidney: The histochemical examination of the kidney of Gp I, III and IV rabbits in this work showed the presence of polysaccharides in the form of PAS positive materials in capillaries of the glomeruli, the basement membrane of the proximal and distal convoluted tubules which indicated that the polysaccharides of kidneys appeared more or less as control (Fig. 12). While fluoridated rabbits (Gp II) showed an decrease in the PAS +ve material as well as the basement membranes of the proximal and distal convoluted tubules appear thicker as compared with the control one (Fig. 13). In addition to, bromophenol blue stain in GPII showed a noticed decrease in the protein content in cytoplasm of tubular cells (Fig. 14) comparing to GP III and GP IV, which revealing that propilais have a preservative effect on protein content of renal tubular cells (Fig. 15).

IV - Morphological analysis of apoptosis:
The treatment by A.O. and E.B. described here, by which necrosis and apoptosis can be recognized. Early apoptotic cells excluded the ethidium bromide, but were permeable to acridine orange, that gave DNA green fluorescent. Early apoptotic cells contain bright dots of characteristic condensed chromatin in their nuclei. While in late apoptosis with loss of membrane integrity, both dyes enter the cell and the nucleus is stained orange-red. Necrotic cells also stain in orange, but nuclear morphology resembling that of viable cells.

Tissue examination in GpII showed marked increased levels of apoptosis associated with severe necrosis among the hepatic tissue with increased numbers of apoptotic hepatocytes in the periportal regions, the nuclei of these cells were enlarged, hyperchromatic and pleomorphic with a coarse chromatin pattern (Fig.16). Moreover, there was obvious apoptosis of renal tissues as represented by marked apoptosis of epithelial cells lining tubules and glomeruli (Fig: 17). On the other hand, both GpIII (Fig. 18) and GpIV showed hepatic and renal protection approaching to the control group which was reflected as green florescence color of tissues (Fig: 19&20).

FIGURES

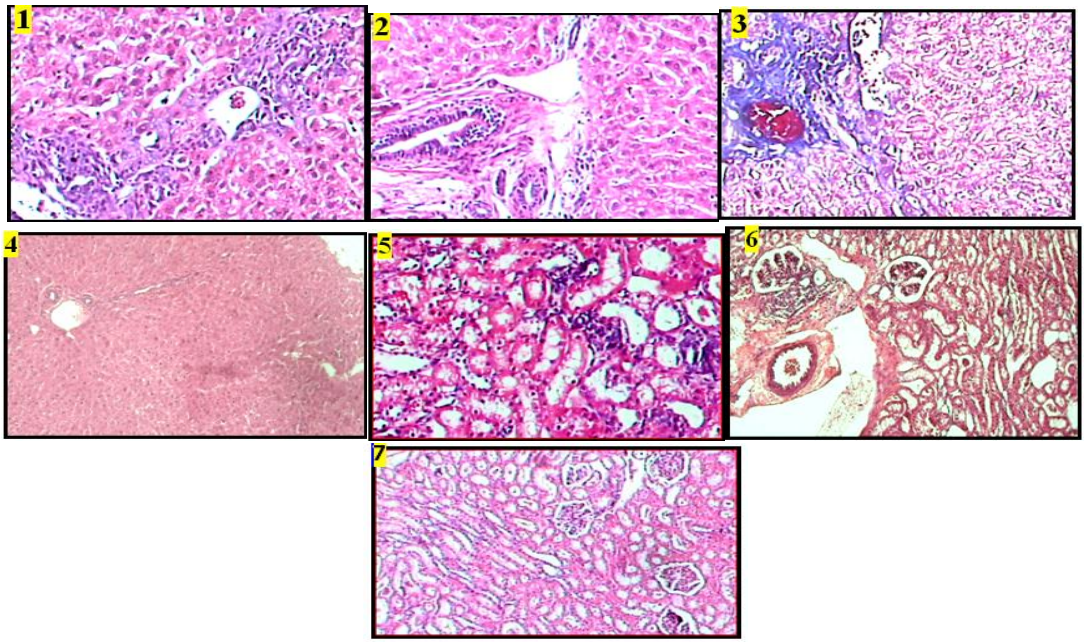

Fig. 1: Rabbit liver (GII) showing, Focal lymphocytic infiltration replaces the hepatic parenchyma (H&E X10).
Fig.2: Rabbit liver (GII) showing peri portal fibroblastic proliferation with bile duct epithelial lining hyperplastic changes associated with lymphocytic exocytosis (H&E X10).
Fig. 3: Rabbit liver (GII) showing perivascular and periportal blue band of Collagen fiber (Masson-trichrome stain X10)
Fig. 4: Rabbit liver (G IV) showing showed prominent hepatic tissue restoration except mild hepatic cords distortion without congestion as well as few limited hepatocyticvacculation (H&E X4).
Fig.5: Rabbit kidney (GpII) showing glomerular and tubular degeneration with mononuclear cell infiltrations (H&E, X10).
Fig. 6: Rabbit kidney (Gp II) showing vascular congestion with glomerular shrinkage and perivascular edema (H&E, X10).
Fig. 7: Rabbit kidney (G IV) showing mild congestion with focal vacuolar tubular degeneration (H&E, X10).

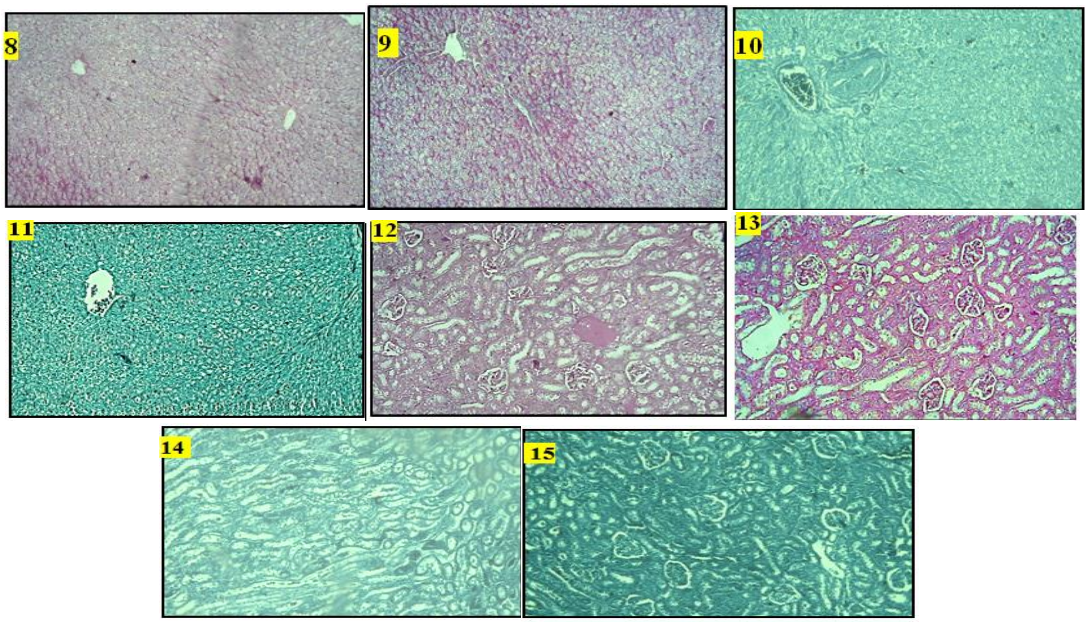

Fig. 8: Rabbit liver (GII) hepatocytes appeared markedly with less PAS-positive matter of glycogen particles showing (PAS, X10).
Fig. 9: Rabbit liver (GIV) PAS-positive material of glycogen particles distributed diffusely in the form of purple granules of glycogen in the hepatic cells (PAS, X10)
Fig. 10: Rabbit liver (GpII) showing a noticed decrease in the protein content in cytoplasm of hepatocytes (Bromophenol blue stain X10).
Fig. 11: Rabbit liver (GpIV) showing showed the marked preservative effect of propilais on protein content of hepatocytes (Bromophenol blue stain X10).
Fig. 12: Rabbit kidney (GpIV) showed the presence of polysaccharides in the form of PAS positive materials in capillaries of the glomeruli, the basement membrane of the proximal and distal convoluted tubules (PAS, X10).
Fig. 13: Rabbit kidney (GpII) showed decrease in the PAS +ve material as well as the basement membranes of the proximal and distal convoluted tubules (PAS, X10).
Fig.14: Rabbit kidney (GpII) showed the decrease in the protein content in cytoplasm of tubular cells (Bromophenol blue stain X10).
Fig. 15: Rabbit kidney (Gp IV) showing that propilais have a preservative effect on protein content of renal tubular cells (Bromophenol blue stain X10).

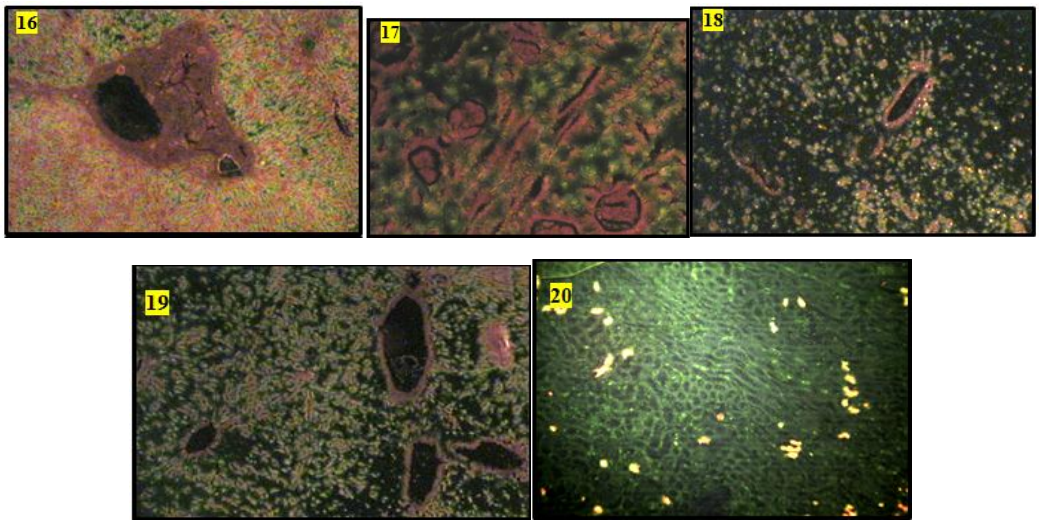

Fig.16: Rabbit liver (Gp II) showing obvious apoptosis in hepatic cells (strong orange fluorescent color) accompanied with necrotic and early apoptotic hepatocytes (green fluorescent for viable cells) (A. O and E. B., X 4).
Fig.17: Rabbit kidney (Gp II) showing prominent apoptosis of in most tubules of renal tissues and glomeruli as well as necrotic cells (A. O and E. B., X 10).
Fig.18: Rabbit liver (Gp III) showing less apoptosis level and necrotic hepatocytes (A. O and E. B., X 4).
Fig.19: Rabbit liver (Gp IV) showing less apoptosis level and necrotic hepatocytes (A. O and E. B., X 4).
Fig.20: Rabbit kidney (Gp IV) showing less apoptosis of in some tubules renal tissues (A. O and E. B., X 4).

DISCUSSION

Fluoride intake (fluorosis) usually occurs in two forms; endemic fluorosis, related to intake of drinking water with high fluoride contents and industrial fluorosis, which has been found to cause severe side effects, to the soft tissues like liver and, kidney (Wang and Li, 2002). Various studies demonstrated that elevated levels of serum hepatic and renal enzymes have been found following fluoride intoxication that indicating degenerative and inflammatory damages to the liver and kidney (Wang *et al.,* 2004 and Anjum *et al*., 2014).

Numerous reports indicated that excessive fluoride and chronic fluorosis can enhance lipid peroxidation and inhibits the oxidative enzymes in the body organs that resulting in metabolic, functional and structural damages and apoptosis as a result of massive cellular damage in many tissues, including liver (Grucka-Mamczar *et al*., 2009) and kidney (Shashi *et al*., 2001 and Nabavi *et al*., 2013), Also, this toxicity is accompanied by a decline in activities of antioxidant enzymes, as well as reducing substances like glutathione and ascorbic acid (Helal and El Dakdoky , 2006). It is evidently indicated that fluoride can disturb the metabolism of proteins and impair the activities of a series of enzymes (Agha *et al*., 2012 and Khudiar and Aldabaj, 2015). Mentioned signs in this work were agreed with the finding of Bataineh and Nusier (2006); Lohakare *et al.* (2010) and Bharti *et al*. (2017).

In this study, the noticed body weight reduction might be attributed to the decrease in feed consumption and transient diarrhea occurred which considered as a first symptoms of chronic F toxicity. Moreover, Ulemale *et al.* (2010) returned the decrease in feed consumption to gastroenteritis as a formation of hydrofluoric acid.

Liver is a critical organ of vital importance and very active site of metabolism, in which most of accumulated metals and toxins harmful effects can be detoxicated, especially fluoride toxicity (Chinoy *et al.*, 2004). Earlier studies showed that fluoride can produce abnormalities in the liver including degenerative and inflammatory changes (Shashi and Thapur, 2001). Fluoride consumption for a long period of time has many pathological effects as a result of increased oxidative stress on soft tissues like liver and endocrine organs by simple diffusion (Sahu *et al.,* 2015).

Noticed histopathological changes in fluoride-treated rabbits GP II Liver in the present study nearly similar to those previously reported by Shashi and Thapur (2001) and Ersan *et al.* (2010) in the liver of mice exposed to 10 ppm NaF and Podder *et al*. (2011) who used15 mg NaF/L for 30 days in mice. Whilethe noticed histopathological changes in both GP III and GP IV correlated to those observed by Mathivanan *et al.* (2013) and Barakat *et al.* (2015) who administrated propolis as a hepato-protective against hepatoxic effects of alcohol and boldenoneundecylenate in mice and rats, respectively.

Moreover, Abdel-Wahab (2013) used thymoquinone against hepatotoxicity and oxidative stress of sodium fluoride in rats.

Kidneys are the primary organs concerned with excretion and for retention of fluoride so they are the target organs for chronic fluoride toxicity. They are the most sensitive body organs in their histopathological and functional responses to excessive amounts of fluoride (Shashi *et al*., 2001 and Inkielewicz and Krechniaka, 2008). Their toxicity appeared as tubular degeneration, inflammation, fibrosis, parenchymatous nephritis, and dilatation of convoluted tubules in rats ingested high fluoride water (Ersan *et al*., 2010).

The gross and microscopic changes were noticed in kidneys of (Gp II) in this work were similar to those previously observed by Shashi *et al.* (2002) in rabbits and Zhan *et al*. (2006) in pig and Podder *et al.* (2011) who used15 mg NaF/L for 30 days in mice Similar results obtained by Barakat *et al.* (2015) and Osman and Tantaway (2013) who used propolis to improving the renal damage induced by boldenoneundecylenate and gentamicin in rats, respectively. In addition to, flouride nephrotoxicity explained by Kim *et al.* (2008) and El-Masry *et al*. (2011) as propolis increases the levels of both vitamin E and vitamin C which are increasing the antioxidant capacity of the cells and inhibiting the ROS generation, resulting in a decrease in both lipid peroxidation and protein oxidation. Our findings parallel with Kołodziejczyk *et al*. (2004) who used flavonoid Chrysin for amelioration of structural changes in the liver and kidneys of rats subchronically exposed to sodium fluoride. Błaszczyk *et al*. (2008) mentioned methionine and vitamin E were used as antioxidant against fluoride renal toxicity in rat kidney. Similar, Khudiar and Aldabaj (2015) used grape seed extract as anti-oxidant to rabbit treated with 100 ppm NaF for 60 days. Approaching of our results, Tavakkoli *et al.* (2017) used black seed as a protective agent against sodium fluoride.

In the present study, the histochemicalexamination of both livers and kidneys were correlated with Abou El-Soud and Khalil (2010) and El-Khayat *et al.* (2010) results who were administrated onion oil and garlic oil as heap to renal protective in diabetic rats. Which explained as cytotoxic agents as fluoride may lead to severe oxidative damage of the liver cells cellular components like cell membrane, lipids, proteins and DNA (Wang *et al*., 2005).

Different studies confirmed the mechanisms of antioxidant action may include suppression of ROS formation, scavenging of reactive oxygen species and protection of antioxidant defenses, so they have a preventive action which could reduce cell death and blocking apoptosis in different cellular structures caused by oxidants and free radicals that induced by sodium fluoride as recorded by Montoro *et al.* (2005) and Agha *et al*. (2012). So, propolis showed noticeable alleviation in liver and kidneys histopathological and histochemical changes which caused by sodium fluoride. As fluoride interacts and alters the metabolism of calcium and magnesium, the decrease in serum calcium related to decrease of intestinal absorption of calcium by fluoride (Xin *et al*., 2006). The antioxidant and ROS scavenging properties of propolis referred to its contents of considerable amounts of flavonoids, phenolic acids and polyphenol substances as caffeic acid phenyl ester (CAPE) which act as potent antioxidant that plays a role in scavenging of ROS, metal ion chelation and synergistic action with other antioxidant compounds (Wagh, 2013 and Kurek-Górecka *et al.*, 2014). So, propilis have renal and hepatic protective role as well as some regenerative properties as recorded previously by Sales *et al.* (2006) and Chandna *et al.* (2014).

CONCLUSION

The conclusion of the present study suggests that the propolisas strong antioxidants and free radical scavengers, ameliorate the toxic effect of sodium fluoride that induce histopathological changes in both liver and kidneys of albino rabbits.

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دور شمع العسل المضاد للاكسدة على التاثير السمى لفلوريد الصوديوم على الكبد والكلى الارانب البيضاء: دراسات هستوباثولوجية

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اجريت هذه الدراسة باستخدام عشرون من الارانب البيضاء البالغة لدراسة الدور المفيد لمستخلص شمع العسل كمضاد للاكسدة على تسمم الكبد والكلى الناتج عن التأثير المزمن لفلوريد الصوديوم. وقد تم تقسيم التجربة إلى أربعة مجاميع (بكل مجموعة خمسة أرانب) واستمرت لمدة ستون يوما ثم ذبحت. ١ - المجموعة الاولى: كمجموعة ضابطة أخذت ماء الشرب ٢- المجموعة الثانية: تم إضافة فلوريد الصوديوم الى ماء الشرب المستخدم للحيوانات بتركيز(١٠ملجم / لتر ماء/ كجم من وزن الجسم) ٣- المجموعة الثالثة: تم إضافة مستخلص شمع العسل الى ماء الشرب المستخدم للحيوانات بتركيز (٢٥ ملجم / لتر ماء/ كجم من وزن الجسم) ٤- المجموعة الرابعة: تم اضافة كل من فلوريد الصوديوم ١٠ملجم / لتر ماء/ كجم من وزن الجسم) مع مستخلص شمع العسل الى ماء الشرب المستخدم للحيوانات بتركيز (٢٥ ملجم / لتر ماء/ كجم من وزن الجسم) بعد ستون يوما ذبحت الأرانب فى المجموعات الأربعة وتم فحص أنسجه -الكبد والكلى فى الأرانب ظاهريا وهستوباثولوجيا. أظهرت الارانب فى المجموعة الثانية علامات تسمم تراوحت من متوسطة الى شديدة الحدة تبعا للجرعة المعطاه وشملت أعراض عصبية مثل ارتجاف العضلات ، مع ضعف عام ، وتوسع الحدقة وفرط الحس. بعد ستة أسابيع ، ظهرت أعراض إمساك أو إسهال على فترات متقطعة مع فقدان الشهية. في نهاية التجربة أظهرت معظم الأرانب قرح في أقدامهم مع تهتك الجلد بدرجات مختلفة ثم انتهت بشلل فى الأطراف. **- الكبد:** الفحص الظاهري اظهر تضخم مع احتقان أو شحوب متوسط الى شديد الحدة وفقا للجرعة ، حيث ظهرت بؤر بيضاء صغيرة الحجم فى أكباد الأرانب اما هستوباثولوجيا فقد تتميز بتحلل بالونى مع تنكرز فى الخلايا الكبدية وتليف حول القناة الصفراوية والأوعية البابية. بالإضافة إلى وجود خلايا التهابية. **-الكلى:** في هذا البحث، تنوعت التغيرات الباثولوجية الكلوية من متوسط الى شديد الحدة وفقا لتركيز الفلوريد حيث اظهرت تورم غيمى في الخلايا الطلائية المبطنة للانابيب الملتوية مع التهاب وتنكرز للانسجة البينية. وكذلك أظهرت تغيرا هستوكيميائيا فى كلا من الكبد والكلى فى بروتين وكربوهيدرات الخلايا بصبغات الباس والبروموفينول كما أظهرت موتا خلويا بصبغتى الاكريدين اورانج والايثيدومبروميد. اما حيوانات المجموعات الثالثة والرابعة تصحح للاعراض والتغيرات الباثولوجية والهستوكيميائية والموت الخلوى بصبغتى الاكريدين اورانج والايثيدومبروميد فى الكبد والكلى المذكورة سابقا فكانت قريبة نسبيا للمجموعة الضابطة **واستنتج من هذه الدراسة:** ان لشمع العسل دور مصحح ومضاد للاكسدة طارد للشوارد المؤكسدة الناتجة عن التسمم المزمن بفلوريد الصوديوم والمؤدى لتغيرات الباثولوجية فى أنسجة الأرانب حيث ظهرت قريبة نسبيا للمجموعة الضابطة.