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Role of Pumpkin and Linseed Oils in Attenuating the Nephrotoxicity of Bisphenol-A in Mice: Biochemical, Genetic and Histopathological studies

Osama H. Elhamalawy[a]; Fathia S. Al-Anany[b]; Aida I. El makawy[c*]

[a]*Department of Environment and Bio-Agriculture, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt.*
[b]*Department of Biological and Environmental Sciences, Faculty of Home Economics, Al-Azhar University, Tanta, Egypt*
[c]*Department of Cell Biology, National Research Centre, 33 El Bohouth Street, Dokki, Giza, P.O. Box 12622, Egypt.*

Abstract

Edible plant oils have been made known to be exciting sources of bioactive compounds making them a suitable alternative in the management of some human diseases. Pumpkin and linseed oils are high in Omega-3 & Omega-6 are considered the utmost vital essential fatty acids that the body cannot create and are considered crucial for health. Bisphenol-A (BPA) plays a role in the development of chronic kidney disease, particularly in developing countries with high levels of pollution. The toxicity of BPA in mice kidney cells and the attenuating role of pumpkin and linseed oils against the deleterious effects of BPA were assessed using kidney function analysis, comet assay for DNA damage detection, and histopathological examination. In an experimental mouse model, pumpkin and linseed oils were administered orally by gavage before or together with BPA administration once a day for four weeks. BPA increased the levels of kidney function markers and the administration of pumpkin and linseed oils resulted in a successful recovery to the normal levels of these metabolites. Our results indicate that pumpkin and linseed oils can increase DNA integrity and ameliorate the histopathological defects in the kidney tissues of BPA-treated mice. The current study found that pumpkin and linseed oils have promising attenuating activity against the unwanted properties of BPA.

Keywords: Nutraceutical oils; Bisphenol-A; Kidney function; Genotoxicity; Histopathology.

1. Introduction

The kidney plays a vital role in the excretion of toxic substances and the maintenance of physiological homeostasis, and is the main target organ for exogenous toxic factors [1]. Nephrotoxicity is a fast deterioration in kidney function owing to the toxic effects of medicines and chemicals [2]. Recently, exposure to chemical substances has become part of ordinary life. Environmental pollutants, including bisphenol-A (BPA), are involved in the etiology of chronic kidney disease, mainly in developing countries where pollution is common [3]. BPA is an artificial organic compound obtained from the condensation of acetone and phenol. Owing to its potential to interact with the endocrine system, it has drawn an international interest in recent times [4]. It is commonly used in the manufacturing of food storage containers, including feeding and non-returnable bottles and other kitchen items, the inner coating of food cans, thermal paper, sunscreen lotions, facial lotions and cleansers, and nail polish [5&6]. BPA contaminants foods by leaching from beverage bottles and can coatings. Also, plastic supplies intended for food storage can release BPA during the processing stages [7]. Experiments have indicated that BPA can accumulate, and influence the integrity of a variety of

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organs [8]. Epidemiological studies in children and adults have evidence for a connection between high urine BPA rates and low-grade albuminuria [9]. These observations raised the possibility that BPA exposure in daily life may have harmful influences on the kidney [10].

As interest shifts toward natural products that have beneficial properties, pumpkin (Cucurbita pepo Linn, Family: Cucurbitaceae) is one of the most widely cultivated species [11]. Pumpkin seeds, called pepitas, are green consumable seeds that are frequently suggested for dietary enrichment and the treatment of stomach-related disorders [12]. Pumpkin seeds are rich source of proteins, vitamins, and oil, particularly omega-6 fatty acids. They contain considerable quantities of fatty palm, stearic, oleic, linoleic, and phenolic acids, and vitamin E [13]. Pumpkin oil is efficient in the treatment of benign prostatic hyperplasia [14]. In addition to its anti-inflammatory and hypolipidemic effects, it decreases oxidative damage caused by aflatoxins [15]. Animal studies found that pumpkin oil could be valuable for treating hypertension, arthritis, hypercholesterolemia, and diabetes. Consumption of pumpkin was correlated with reduced incidence of lung, stomach, breast, and colorectal cancers [16]. Previous studies have revealed that pumpkin oil has been used in traditional medicine for dealing with kidney, and urinary issues [17&18]. Also, pumpkin oil is a known source of phytosterols, that makes it a suitable alternative nutraceutical in the management of some non-communicable diseases in humans [19&20].

Linseed (Linum usitatissimum L.), also known as flaxseed, is a rich source of antioxidants, and can help to protect cells from oxidative harm. Linseeds contain high levels of fiber, glycosides, phenols, and tannins that might be useful in treating some diseases [21]. Linseed`s bioactive ingredients include strong antioxidants, omega-3 short-chain polyunsaturated fatty acids, lignans, and phytoestrogen SDG (secoisolariciresinol-diglucoside), which have antioxidant properties and suppress lipid peroxidation [22]. Biological and clinical studies into linseed have attributed its antioxidant properties to its high α-linolenic acid and lignan content [23]. Linseed oil has been shown to improve lipid and glucose profiles, mitigate cardiac, hepatic, and renal indicators, eliminate oxidative damage, and boost the antioxidant protection mechanism in metabolic

syndrome patients. It also inhibits the growth of cervical cancer in mice, and diminishes cell proliferation in a variety of human cancers [24&25].

The goal of the current investigation was to explore the nephrotoxicity of BPA using biochemical, genetical, and histopathological examination of the kidney of male mice. Besides to investigate the role of pumpkin and linseed oils in alleviating the toxicity of BPA in mouse kidney.

2. Experimental
2.1. Materials
2.1.1. Chemicals
Pumpkin and linseed oils were bought from EL Captin Company (Al Obour City, Cairo, Egypt). BPA (≥99%) was procured from Sigma-Aldrich Company (St. Louis, MO, USA).

2.1.2. BPA dose
The dose of BPA was designated using the National Toxicology Program (NTP) 1982 report of acute, subacute, and chronic toxicity study. The overall oral low observed adverse effect level (LOAEL) of BPA is 50 mg/kg bw/day in this document [26]. BPA was dissolved in absolute ethyl alcohol (95%) and diluted with corn oil [1:20 alcohol: corn oil (vehicle)] to obtain a final concentration of BPA.

2.1.3. Animals
Fifty-four male mice weighing 24 ± 5g were obtained from the animal house of the National Research Institute, Giza, Egypt. Animals were held in the animal house of the Environment and Bio-agriculture Department, Faculty of Agriculture, Al-Azhar University, under a 12 h light/dark period with a temperature of 23 ± 4 °C and relative humidity of 55 ± 10%. Animals were fed with rodent chow and tap water were given ad libitum. Prior to the beginning of the experiments, the animals were given a two-week acclimatization period. The experimental protocols of the study was conducted under the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals [27].

2.1.4. Animal grouping
The mice were divided into nine groups (n = 6), as follows: Control group: animals were orally given distilled water. Vehicle group: animals were orally

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gavage with ethyl alcohol-corn oil solution (1:20). Pumpkin and linseed groups: animals were orally administered pumpkin or linseed oils (1 mL/kg b.w/day) in line with Kaithwas and Majumdar [28] for 4 weeks. BPA group: animals were orally treated with the BPA 50 mg/kg/day for 4 weeks. Pumpkin oil and linseed oil prior to BPA groups: animals were orally given the same dose of pumpkin or linseed oil prior to the administration of BPA (50 mg/kg b.w. / day for 4 weeks). Pumpkin oil plus BPA and linseed oil plus BPA groups: animals were orally given the same dose of pumpkin or linseed oil concurrently with BPA (50 mg/kg b.w./day for 4 weeks).

2.2. Methods

2.2.1. Determination of relative kidney weight

Animals were sacrificed by cervical decapitation after the experiments. Kidneys were separated, the extra fat removed and weighed, and the relative kidney weight percentage was determined as follows:

Relative kidney weight =(Absolute kidney weight (g))/(Final body weight (g)) x100

2.2.2.. Biochemical investigations

Blood samples were collected from the retro-orbital sinus of each mouse via eye puncture in sterile test tubes for serum isolation. The blood was allowed to clot at room temperature for15–30 min then centrifuged at 3000 rpm for 10 minutes in a refrigerated centrifuge. Serum was collected in 0.5 mL aliquots, then stored at -20°C for use in the biochemical investigation. Serum urea and creatinine levels were measured using diagnostic kits (Cat. No. UR 21 10 & CR 12 50, Biodiagnostic Co., Giza, Egypt) according to the methodology of Patton and Crouch [29] and Henry et al. [30]. Serum uric acid was determined using standard Diagnostic kits (Cat. No. UA 21 20, Biodiagnostic Co., Giza, Egypt) with a clinical Jenway 6705 UV/visible spectrophotometer, at 510 nm according to Fossati et al. [31].

2.2.3. Comet assay in kidney cells

DNA integrity was investigated using the method described by Elhamalawy and El Makawy [32]. Briefly, cells were encapsulated with low melting point agarose and lysed in a pH 10 lysis buffer at 4°C for 2 h. DNA was unwound in an alkaline solution (0.3 M NaOH, 1 mM EDTA) at 4°C for 40 minutes, then electrophoresed using a 25 V 300 mA electrophoresis platform for 20 min. Neutralization

was performed in 0.4 M Tris buffer (pH 7.5) for 10 min, followed by 5 min in distilled water. Gels were fixed in methyl alcohol for 5 min, then stained with ethidium bromide. A fluorescence microscope (Eclipse 800, Nikon, Tokyo, Japan) was used to photograph fifty randomly selected nuclei per trial, which were examined using the Comet Assay IV image analysis software (Perceptive Instruments, Suffolk, UK).

2.2.4. Kidney histopathological examination

Kidney tissues were fixed in 10% formalin solution, dehydrated in varying alcohol concentrations, cleared with xylol, and embedded in paraffin wax. For histological examination, 5 μm sections were stained with Harris hematoxylin and eosin (H & E) [33].

2.2.5. Statistical analysis

One-way ANOVA was used with the SPSS 16 software to analyze all of the data. The significance of the variations between means was determined using Duncan's multiple range tests. P ≤ 0.05 was used as an indicator of significance, and all data were represented as mean ± standard error.

3. Results

3.1. Kidney weights

The pumpkin and linseed oils significantly increased (P ≤ 0.05) the weight of the kidney compared with control. BPA significantly diminished the weight of the kidney (P ≤ 0.05) compared to control. The administration of pumpkin and linseed oils prior to or with BPA significantly increased the kidney weights (P ≤ 0.05) but not to the level of the control. The increase in kidney weights of mice treated with pumpkin was more than that observed in mice treated with linseed, but the difference was not significant (Fig. 1).

3.2. Kidney function

Tables 1 and 2 illustrate the creatinine, uric acid, and urea levels in all treated groups. Pumpkin and linseed oils produced a significant decrease in the levels of creatinine and uric acid (P ≤ 0.05) compared to control, while the decrease in urea was not significant. BPA caused a significant elevation in the levels of creatinine, uric acid, and urea (P ≤ 0.05) compared with control. The two oils significantly

diminished the kidney function markers (P ≤ 0.05) compared to those in BPA-treated mice.
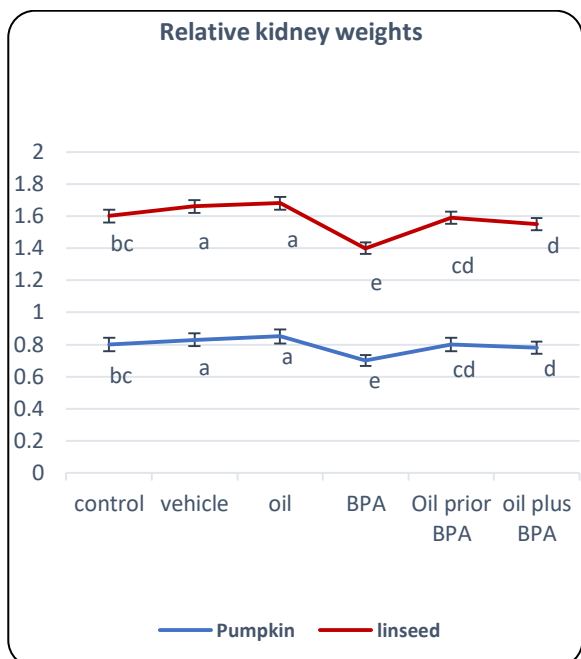

Fig. 1: Role of Pumpkin and Linseed Oils in Attenuating the Kidney Weight Loss Induced By Bisphenol-A (BPA). Different superscripts indicate significant differences (P ≤ 0.05).

Table 1: Effect of Pumpkin Oil on Kidney Functions of Bisphenol-A (BPA)-Treated Mice.

Experimental Groups	Creatinine (Mg/dl)	Uric Acid	Urea (Mg/dl)
Control	0.86 ± 0.07^c	3.03 ± 0.09^d	20.50 ± 1.04^c
Vehicle	0.70 ± 0.08^{cd}	2.98 ± 0.08^d	21.50 ± 1.04^c
Pumpkin oil	0.56 ± 0.05^d	2.70 ± 0.04^e	18.83 ± 0.64^c
BPA	2.70 ± 0.10^a	7.46 ± 0.12^a	44.75 ± 2.83^a
Pumpkin oil prior BPA	1.13 ± 0.07^b	3.90 ± 0.06^c	27.50 ± 1.04^b
Pumpkin oil plus BPA	1.36 ± 0.07^b	4.80 ± 0.06^b	31.75 ± 1.10^b

Values are represented as means ± SE. Identical superscripts indicate no significant difference ($P \le 0.05$).

3.3. DNA damage

DNA damage induced by BPA in mice kidney cells was evaluated using comet assays. BPA produced significant elevation of the level of DNA damage in kidney cells, as represented in the comet assay parameters, as seen in Tables 3 and 4. The administration of pumpkin oil prior to or together with BPA significantly lessened the amount of DNA damage induced by BPA in kidney cells. The administration of oil prior to the administration of BPA was more effective than administration of the two simultaneously. There was no significant difference between the effects of the two oils on the DNA damage parameters, except the pumpkin oil decreased the percent of tailed cells more significantly than linseed oil (Fig. 2).

Table 2: Impact of Linseed Oil on Kidney Functions of Bisphenol-A (BPA)-Treated Mice.

Experimental Groups	Creatinine (Mg/dl)	Uric Acid (Mg/dl)	Urea (Mg/dl)
Control	0.86 ± 0.15^d	3.03 ± 0.09^d	20.50 ± 1.04^c
Vehicle	0.70 ± 0.17^{de}	2.98 ± 0.08^d	21.50 ± 1.04^c
Linseed oil	0.53 ± 0.05^e	2.90 ± 0.07^e	20.25 ± 0.675^c
BPA	2.70 ± 0.10^a	7.46 ± 0.12^a	44.75 ± 2.83^a
Linseed oil prior BPA	1.30 ± 0.10^c	4.36 ± 0.19^c	28.75 ± 1.10^b
Linseed oil plus BPA	1.60 ± 0.17^b	5.15 ± 0.09^b	32.75 ± 0.85^b

Results are illustrated as means ± SE. The differences in means with different superscripts were significant ($P \le 0.05$).

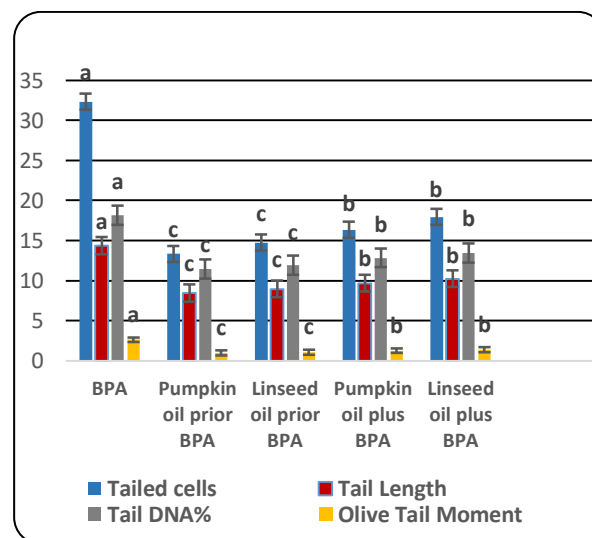**Table 3. Effect of Pumpkin Oil on DNA Damage in kidney Cells of Bisphenol-A (BPA)-Treated Male Mice.**

Experimental Groups	Tailed cells	Tail length	Tail DNA	Olive tail
Control	10.67 ± 0.33[d]	7.29 ± 0.20[d]	10.28 ± 0.21[d]	0.75 ± 0.04[d]
Vehicle	10.33 ± 0.88[d]	7.36 ± 0.26[d]	10.43 ± 0.37[d]	0.77 ± 0.05[d]
Pumpkin oil	9.33 ± 0.88[d]	6.70 ± 0.19[d]	9.95 ± 0.14[d]	0.66 ± 0.03[d]
BPA	32.33 ± 0.88[a]	14.37 ±0.30[a]	18.13 ± 0.26[a]	2.61 ± 0.09[a]
Pumpkin oil prior BPA	13.33 ± 0.39[c]	8.43 ± 0.24[c]	11.47 ± 0.28[c]	0.97 ± 0.05[c]
Pumpkin oil plus BPA	16.35 ± 0.26[b]	9.67 ± 0.17[b]	12.84 ± 0.55[b]	1.24 ± 0.05[b]

Data are shown as means ± SE. The means that have the same superscript do not differ significantly ($P \le 0.05$).

Table 4. Effect of Linseed Oil on DNA Damage in Kidney Cells of Bisphenol-A (BPA)-Exposed Male Mice.

Experimental Groups	Tailed Cells	Tail length (µm)	Tail DNA	Olive tail moment (µm)
Control	10.66 ± 0.33[d]	7.29 ± 0.20[d]	10.28± 0.21[d]	0.75 ± 0.04[d]
Vehicle	10.33 ± 0.88[d]	7.36 ± 0.26[d]	10.44± 0.37[d]	0.77 ± 0.05[d]
Linseed oil	9.67 ± 0.88[d]	6.80± 0.17[d]	9.97 ± 0.25[d]	0.67± 0.03[d]
BPA	32.33± 0.88[a]	14.37± 0.30[a]	18.13± 0.26[a]	2.61± 0.09[a]
Linseed oil prior BPA	14.74± 0.26[c]	8.96 ± 0.23[c]	11.89 ± 0.41[c]	1.06± 0.03[c]
Linseed oil plus BPA	17.95± 0.39[b]	10.22± 0.64[b]	13.42± 0.633[b]	1.37± 0.107[b]

Data are expressed as means ± SD. Different superscripts indicate significant differences ($P \le 0.05$).

Fig. 2: Comparison Between the Attenuating Effect of Pumpkin and Linseed Oil on Bisphenol-A (BPA) DNA Damage in Kidney Cells.* Significant difference ($P \le 0.05$).

3.4. Histopathological results

The histology of control mice kidney showing normal appearance of the glomeruli and a renal tubule (Fig. 3A). BPA administration for 28 successive days caused several histopathological changes in kidney tissues. Kidney sections of BPA-treated mice screening showed glomerular and interstitial blood vessel congestion, focal interstitial mononuclear inflammatory cell infiltration, and marked granular and vacuolar degeneration and necrosis of the renal tubular epithelium with nuclear pyknosis. Renal casts were observed in the lumen of most of the renal tubules (Fig. 3B). The cross-section in the kidneys of mice treated with pumpkin oil prior to BPA administration showed mild swelling and granular degeneration of the renal tubular epithelium, with some necrotic cells (Fig. 3C). The kidney sections of mice treated with pumpkin oil plus BPA gavage revealed crowding intratubular blood vessels and variable degrees of necrobiosis of the renal tubular epithelial lining (Fig. 3D). The kidney of the mice administered linseed oil prior to BPA showed many necrotic cells among the degenerated renal tubular epithelial cells (Fig. 3E). The kidney sections of mice treated with linseed oil plus BPA exhibited infiltration of focal interstitial inflammatory cells with necrobiotic changes of the renal tubular epithelium (**Fig. 3F**).
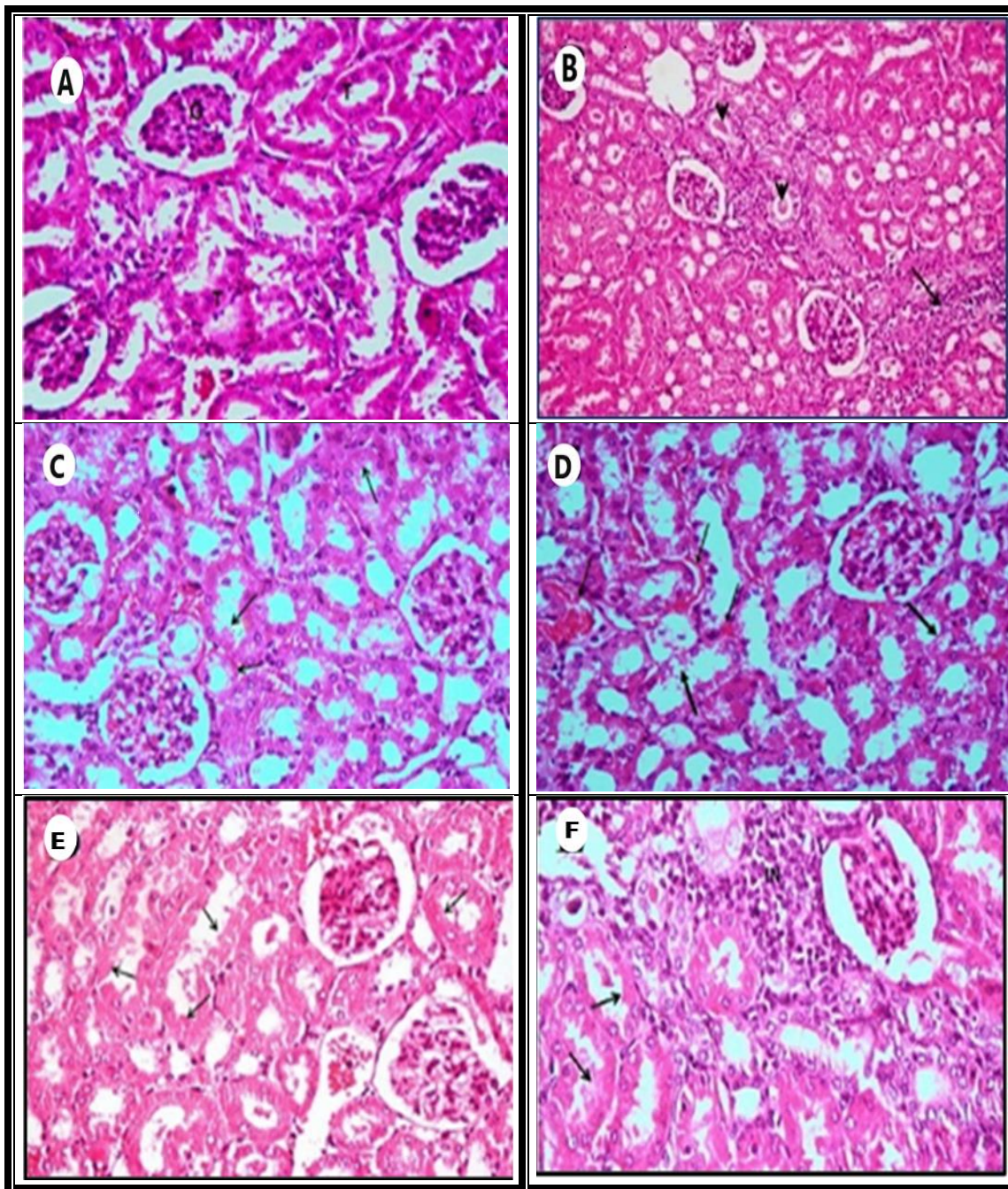
Fig. 3: Image of mouse kidney cells of A) control showing normal appearance of the glomeruli (G) and a renal tubule (T). B) BPA-treated mouse showing infiltration of focal interstitial mononuclear inflammatory cells (arrow), degeneration, and necrosis of a renal tubular epithelium with appearance of a renal cast in the lumen of the renal tubules (arrowhead). C) Pumpkin oil prior to BPA showing mild swelling and granular degeneration of the renal tubular epithelium with some necrotic cells (arrow). D) Pumpkin oil plus BPA showing congestion of the blood vessels (thin arrow) and variable degrees of necrobiosis changes of the renal tubular epithelial linings (thick arrow) E) Linseed oil prior to BPA showing many necrotic cells (arrow) among the degenerated renal tubular epithelial cells. F) Linseed oil plus BPA showing infiltration of focal interstitial inflammatory cells (IN) with necrobiosis (arrow) of the renal tubular epithelium (H&E X400).

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4. Discussion

Vegetable oils are a rich source of nutraceuticals, playing a pivotal role in human health and nutrition [34]. Pumpkin, as a dietary ingredient, has anti-diabetic, anti-carcinogenic, and antioxidant effects [17]. Linseed is an excellent source of fatty acids, lignan, and fiber that offers health benefits due to their anti-inflammatory, antioxidative, and lipid controlling properties [35]. The goal of the present work was to explore the role of pumpkin and linseed oils in lessening the deleterious effects of BPA on the kidney tissues of male mice. Our findings revealed the beneficial effects of pumpkin and linseed oils administered before or concurrently with BPA, on the nephrotoxicity caused by BPA in mice. The observed beneficial effects of pumpkin and linseed oils against BPA-induced renal damage could have caused the elevation of kidney weight, inhibition of DNA damage, enhancement of kidney function, and protection against glomerular damage and tubular necrosis.

Toxic substances disrupt the body's metabolic processes, affecting animal development and resulting in a decrease in organ weight. Therefore, body weight and the relative weight of potential target organs are important indicators of the toxicity of an agent [36]. A significant decrease in relative kidney weight was observed in BPA-treated male mice. Our data are in agreement with those of Shirani et al. [37], who found that BPA administration significantly decreased the kidney weight of rats. Asma et al. [38] reported that BPA exposure caused a reduction in body and organ weight of rats. While the result showed that the oral administration of pumpkin and linseed oils either prior to or plus to BPA treatment significantly elevated the relative kidney weight.

Biomarkers of renal toxicity include blood levels of creatinine, uric acid, and urea. Serum creatinine and urea are released into the bloodstream when the kidneys are impaired, and increased levels of these biomarkers indicate kidney cell damage [39]. The present investigation indicated that recurrent exposure to BPA resulted in increases in serum creatinine, uric acid, and urea levels. Similar to our results, numerous studies have observed that creatinine uric acid and urea levels are significantly elevated in BPA-intoxicated rats [40-42&37]. A deficiency in glomerular filtration and tubular activity has been found to cause a reduction in the ability to excrete waste materials [10].

Our results indicated that BPA produced significant elevation in the percentage of DNA damage, as measured by comet assay in kidney cells. In agreement with these results, several researchers have established that BPA at different doses enhanced the level of DNA damage, as estimated using comet assay in lymphocytes, spleen, kidneys, and lung, as well as in germ cells [43&44] in RWPE-1 cells [45] in human epithelial type 2 cells (Hep-2) [46] in gonadal cells of the fish Goodea atripinnis [47]. Also, BPA has a genotoxic impact on Chinese hamster ovary cells [48], and in rats [49]. Our data showed that pumpkin and linseed oils alleviated the percentage of DNA damage caused by BPA to kidney cells. These results followed numerous studies that confirmed the positive effect of pumpkin and linseed oils against DNA damage induced by different chemicals in kidney cells [50-53].

The histological study also displayed renal proximal tubular damage in BPA-treated animals. In harmony with these findings, Ahmed et al. [40] and Rahimi et al. [41] reported that BPA induces many histopathological alterations in the kidneys of male rats. Renal histopathological changes may be linked to BPA genotoxicity, since DNA damage can cause mutations and other types of cell injury [54].

In contrast, the two patterns of gavage of pumpkin and linseed oils attenuated the histopathological structure alterations induced by BPA. Several studies support our results, finding that the pumpkin oil treatments attenuated the biochemical disruption, DNA damage and alterations to the normal histology of the kidney cells. Pumpkin oil possesses free radical scavenging and total antioxidant activities, owing to the presence of phenolic and flavonoid compounds [13&53&55]. Numerous investigations have suggested that flaxseed oil protects rat kidneys against the biochemical and histopathological alterations induced by thioacetamide and pesticide residues [56&57]. These results indicate the effectiveness of flaxseed oil in the inhibition of nephrotoxicity, due to its antioxidant role. Phenolic compounds of flaxseed oil, such as ellagic acid, p-hydroxybenzoic acid, p-coumaric acid, ascorbic acid, and ferulic acid, have strong antioxidant properties due to their redox properties [58].

5. Conclusion

Alterations in biochemical markers, genotoxicity, and histopathological defects are some of the destructive effects produced in Kidney cells by exposure to BPA. Pumpkin and linseed oils through the two regimens of treatment with BPA showed strong antioxidant activity, ameliorating the biochemical, genetic, and pathological disorders induced in mouse kidney cells. Pumpkin oil appeared to be more effective than linseed, and the administration of pumpkin oil prior to BPA exposure was more effective than concomitant administration. According to the findings, pumpkin and linseed oils have promising activity against the undesirable effects caused by BPA.

Conflicts of Interest

The authors declare no conflicts of interest

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