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Administration of pickled olive fruits treated with acid black nigrosine dye induced hepato-renal dysfunction in mice

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ABSTRACT

Olive fruits (*Olea europaea* L) have several health and nutritional benefits due to their high contents of antioxidant compounds. This study evaluated the hepato-renal toxic effects of pickled olive fruits that were treated with acid black nigrosine dye (ABND) in mice. Twenty male Swiss albino mice (CD-1) were used to determine the median lethal dose (LD_{50}) of ABND. Then other forty male mice were equally divided into 4 groups (N = 10) as follows: Group 1 (Gp1) was served as a control group. Gp2 was administered orally with a normal olive fruits extract (NOFE) as 150 mg/kg b.wt. Gp3 was administered with pickled olive fruits extract treated with KOH and ABND (POFE/B/KOH/ABND) as 150 mg/kg b.wt. The last group (Gp4) was administered with ABND alone as 60 mg/kg b.wt. The treatment of all the experimental groups was daily for 2 consecutive months. At the end of the experiment, percentages of body weight (% b.wt.) changes were calculated. Blood and sera samples were collected for determination the hematological and biochemical parameters Liver and kidney tissues were collected for histopathological investigations. The results showed that the group of mice that were treated with POFE/B/KOH/ABND showed significant hepato-renal dysfunctions as evidenced by altering the some of hematological, biochemical and histopathological parameters.

Keywords: Olive, Pickling, Nigrosine dye, Hepato-renal functions.

1. Introduction

Olive (*Olea europaea L*) belongs to the family Oleaceae of the genus *Olea* (Aparicio-Soto et al., 2015). Olive fruits contain 115–145 calories per 100g. Each 100 g of ripe olive fruits contains 0.8 g protein, 6.3 g carbohydrates, 3.2 g fiber, 10.7 g fat (Maestri et al., 2019). Olive fruits contain high contents of vitamin E and antioxidants, which are linked to several health benefits, including a reduced risk of heart disease, decreased inflammation, and fight cancer (Ghanbari et al., 2012). Olive fruits are rich in iron, which is important for red blood cells to transport oxygen. Furthermore, they are good sources of copper, calcium, and sodium (Abbaspour et al., 2014).

The *in vitro* and *in vivo* studies reported that the isolated components of olive materials have shown several pharmacological activities including anticonvulsant, antiviral, antioxidant, immunomodulatory, antihypertensive, antidiabetic, antimicrobial, antihyperglycemic, gastroprotective, and wound healing activities (Ghanbari et al., 2012; Larussa et al., 2019). Olive fruits are rich in antioxidant compounds including oleuropein which is linked to many health benefits. A previous study reported that olive fruit extract improved the antioxidant capacity through altering colonic microbiota

composition in mice (Wang et al., 2021). Because olive fruits are very bitter, they are not usually eaten fresh, instead, olive fruits are pickled. This process of pickling ensures the remove of bitter compounds (Omar, 2010). Processing of olive fruits may take anywhere from few days up to few months depending on the followed method. Processing methods often rely on local traditions, which affect the fruit's taste, color, and texture. Previous studies reported whether fermented olive fruits have probiotic effects, this could lead to improved digestive health (Hurtado et al., 2012; Bautista-Gallego et al., 2013).

Different pickling processes of olive fruits promote quantitative and qualitative alterations in the phenolic compounds of olive fruits, thereby changing their nutritional and health properties (Marsilio et al., 2001). Pickling with the natural style has been confirmed as the best preparation for maintaining the highest content of polyphenols and triglycerides (Conte et al., 2020). Nutritional losses from the fresh olives result both from the alkali treatment and from leakage of soluble constituents from olive fruits into the brine. Previously, it has been reported that olive fruits processing altered the contents of α-tocopherol and fatty acids contents (Sakouhi et al., 2008).

Pickled items are those whose preparation and preservation involve a mix of salting, fermenting, and/or acidity. Table olives are included in this category. Processing can remove or at least lessen the fruit's inherent bitterness, making the finished product suitable for ingestion by humans. The majority of black olives found in Egypt's impoverished public markets are grown locally and are darkened by the application of synthetic black dyes like hematine and nigrosine. In an effort to cut processing costs, some small-scale olive darkening processors employ inexpensive commercial alkalis, such as Ca(OH)2, KOH, and NaOH, which are commonly used in industrial settings. However, according to Yousef et al. (1997), using such impure chemicals in food processing is prohibited (El-Makhzangy and Sulieman, 2008).

This study aimed to evaluate the possible toxic effect of the pickled olive fruits that were treated with acid black nigrosine dye (ABND) administration on the hepatorenal functionality in mice.

2. Materials and Methods

Chemicals

The acid black nigrosine dye (ABND) was purchased from the local market in Tanta city, Egypt. Potassium hydroxide was purchased from Al-Gomhoriya Company. Biochemical kits were purchased from Bio-diagnostic Company, Cairo, Egypt.

Collection of olive fruits

Oleaeuropae L were collected from the farms that located in Ismailia city, then they were transferred into the nutrition laboratory, Faculty of Specific Education, Tanta University. Olive fruits were identified and authenticated with relevant institutional and national guidelines by botanist in the Botany Department, Faculty of Science, Tanta University, Egypt.

Pickling process and olive fruits treatment conditions

Pickled olive fruits were prepared by first blanching and then packing ripe olive fruits directly with acidified brine. Fruits were blanched at 90 °C water for 5 min and immediately packed in dark glass jars containing brine solution, then the jars were closed tightly. The brine contained 5% acetic acid and 15% sodium chloride dissolved in water. Pickled products were stored away from light at room temperature for 4 weeks (Fayeket al., 2021).

Preparation of different olive flesh extracts

Pre- and post-pickling, olive fruits were separated into flesh parts and olive seeds. The flesh of olives in different conditions was cut into very fine pieces and left to dry in the shade. These compartments were ground in a mechanical mortar, and 50 g of each condition were added to 500 ml of 70% ethanol and left for 3 days at room temperature. Finally, the supernatants were filtered and left to dry to get the hydro-alcohol extract of each condition.

Animals

Sixty male Swiss albino mice (20 ± 4 g) allowed to acclimate for 1 week in the animal house conditions of the Faculty of Science, Tanta

University, before being divided into groups. The institutional animal care committee at Zoology Department, Faculty of Science, Tanta University- Egypt, approved the experimental design. Target values for temperature and relative humidity were about 22 ± 1ºC and 55 ± 5%, respectively 12 hrs. light- dark (day/night) cycle was achieved. Mice were given drinking tap water and normal experimental pelleted animal food *ad libitium*.

Determination of the LD_{50} of ABND

A total number of 20 male Swiss albino mice (20 ± 2 g) were divided into five groups of four mice each. These groups were injected with a single dose of 325,750, 1500, 3000 and 5000 mg/kg of ABND. Mice were noticed for 24 h to assess the acute toxicity of the stain. LD50 value of the stain was calculated using the arithmetic method of Karber as modified by (Angalabiri-Owei and Isirima, 2014).

Experimental protocols

After 1 week of acclimation period in the Animal Facility, forty mice were divided into 4 groups (10 mice /group) according to body weights to minimize the standard errors between groups as follows: Group 1 (Gp1) was the normal control group that was administered orally with dist. H_2O daily (150 µl/mouse). Gp2 was administered orally with normal olive fruits extract (NOFE) (150 mg/kg). Gp3 was administered orally with pickled olive fruits extract (POFE/B/KOH/ABND) (150 mg/kg) (Almatroodi et al., 2020). Gp4 was administered orally with ABND (60 mg/kg). Treatment of all groups was daily for 2 months.

At the end of the experiment, all animals were sacrificed; blood samples were collected from all groups for determination of hematological parameters. The sera were separated and frozen at -20°C until used for determination of liver and kidney functions. Liver and kidney tissues were collected for biochemical and histopathological investigations. Percentages of body weight (% b. wt) changes of all mice were calculated.

Determination of hematological and biochemical parameters

Blood samples were collected from arterial blood vessels and heart chambers then sera were separated by centrifugation for biochemical analysis. Platelets, hemoglobin content (Hb g/dL), red blood cells (RBCs), white blood cell (WBCs) and differential counts were determined in fresh blood samples by the electronic blood counter. Liver and kidney tissues were collected, and homogenates were prepared in ice-cold phosphate buffer saline. Serum alanine aminotransaminase, (ALT), serum aspartate aminotransaminase (AST), urea, and creatinine levels were determined according to the manufacturer's protocols.

Histological investigation

Specimens from livers and kidneys were preserved in 10% phosphate-buffered formalin at 4-5 mm3 thickness, dehydrated in graded alcohol series, cleared in xylene, and embedded in paraffin blocks. 4-5 µm sections of the collected sections were stained with hematoxylin and eosin for histopathological examination (Bancroft and Gamble, 2002).

Statistical analysis

One–way analysis of variance (ANOVA) was used to assess the significant differences. The criterion for statistical significance was set at $P \leq 0.05$. All data are presented as mean ± SD.

3. Results

Acute LD_{50} of ABND and body weight changes in experimental animals.

The results showed that there was no mortality or toxic signs up to 5000 mg/kg. Initial (I.B.wt) and final (F.B.wt) body weights for the different groups were determined at the beginning of experiment day zero (D-0) and 30 days after injection. The results showed that the % body weight change for the control group was 3.8%, while the % body weight (b. wt) change of NOFE was 8.3%. The % body weight change in POFE/B/KOH/ABND and the group of mice that were treated with ABND was 9.5% and – 0.15%, respectively (Table 1).

Hematological parameters of different groups.

The results showed that treatment of groups with NOFE, POFE/B/KOH/ABND, and ABND did not change the total number of red blood cells (R.B.Cs), hemoglobin (Hb) and hematocrit (Hct). The total platelets count was increased in NOFE when compared to the normal control and NOFE control groups. While the total number of

platelets did not change in the groups of mice that were treated with POFE/B/KOH/ABND, and ABND when compared to the normal control and NOFE control groups (Table 2). The results showed that treatment of different groups with NOFE, POFE/B/KOH/ABND, and ABND did not change the total count of white blood cells (W.B.Cs) or their differential percentages (Table 3).

Effects on liver and kidney functions

The results showed that the activity of ALT was not affected in NOFE compared to the control group. ALT activity was increased in the group of mice that treated with POFE/B/KOH/ABND, and ABND when compared to the normal control and NOFE control groups. Similarly, AST activity did not show significant changes after treatment of mice with NOFE. However, the activity AST was increased in the group of mice that were treated with POFE/B/KOH/ABND, or ABND when compared to the control group. Similar profile was reported in the kidney biomarkers after different treatment protocols (Table 4). Similarly, treatment with ABND negatively affected kidney functions (urea and creatinine) as indicated in groups POFE/B/KOH/ABND, or ABND when compared to the normal control and NOFE control groups.

Administration of ABND induced liver histopathological alterations.

Liver sections of control mice showed a normal hepatic structure in which polygonal hepatocytes with prominent nuclei are arranged in a radial pattern. The hepatic strands alternate with narrow blood sinusoids lined by an endothelial cell layer containing Kupffer cells (Fig. 1a). NOFE administered mice showed normal hepatocytes with normal architecture. Slight cellular infiltration surrounding the central vein may appear (Fig. 1b). POFE/B/KOH/ABND administered mice showed marked disorganization, congested central veins with intensive degeneration of its epithelial lining, most of the hepatocytes were highly degenerated with vacuolated cytoplasm, others with pyknotic nuclei, also karyolitic nuclei were seen, area around central vein associated with cellular infiltration (Fig. 1c). ABND administered mice showed hepatic degeneration with irregular congested central vein, widening of blood sinusoids, pyknotic nuclei, Kupffer cells, and area of infiltration around central vein (Fig. 1d).

Administration of ABND induced kidney histopathological changes.

The renal cortex of control mice appeared normal, with a normal glomerulus, proximal and distal convoluted tubules, and Bowman's capsule, there were no signs of inflammation (Fig. 2a). The kidney sections of NOFE administered mice showed more or less normal like architecture of glomeruli, most of the renal tubules were elongated and widened (Fig. 2b). POFE/B/KOH/ABND administered mice showed destructed, shrunken, and congested glomeruli with irregular Bowman's space, damaged renal tubules and lost their characteristic appearance with distinct vacuolated and highly degenerated lining epithelium; cellular infiltration and hemorrhage at the intratubular spaces were observed (Fig. 2c). ABND administered mice showed disorganized glomeruli with irregular Bowman's space, widening of some renal tubules, occluded with hyaline casts and degeneration of some epithelial lining, others with vacuolated cytoplasm, damaged and their contents intermixed with each other, also intratubular hemorrhage was observed (Fig. 2d).

Table 1. Initial, final body weights and %b.wt change in different groups.

Groups	I. B. wt. (g.)	F. B. wt. (g.)	% Change in body wt.
Control	24.05±1.25	24.96± 0.98	3.8% [a]
NOFE	24.76±2.58	26.82 ± 3.15	8.3% [c]
POFE/B/KOH/ABND	26.04±1.70	28.52 ± 4.19	9.5% [c]
ABND	26.18±1.45	26.14 ± 3.44	- 0.15% [d]

Data represented as means ± S.D.; I.B.wt: Initial body weight; F.B.wt: Final body weight; NOFE: Normal olive flesh extract; POFE/B/KOH/ABND: Pickled olive flesh extract boiled for 5 minutes and treated with KOH and acid black nigrosine dye; ABND: Acid black nigrosine dye. Means that do not share a letter are significantly different (Tukey's test, $p < 0.05$).

Table 2. RBCs, Hb, Hct% and platelets count in different groups.

Groups	RBCs ($x10^6$ /µL)	Hb (g/dL)	Hct (%)	Platelets ($x10^3$ /µL)
Control	9.35 ± 0.51 [a,b]	14.53 ± 1.53 [a]	44.50 ± 4.85 [a]	741.0 ± 46.13 [a]
NOFE	7.81± 0.99 [a,b]	10.66 ± 1.52 [a,b]	33.96 ± 5.60 [a,b]	927.66 ± 40.21 [c]
POFE/B/KOH/ABND	9.20 ± 0.31 [a,b]	12.96 ± 1.05 [a,b]	40.06 ± 3.28 [a,b]	806.0 ± 284.75 [b]
ABND	9.57 ± 1.37 [a,b]	13.80 ± 1.93 [a,b]	42.06± 5.27 [a,b]	831.0 ± 149.4 [b]

Data represented as means ± S.D.; NOFE: Normal olive flesh extract; POFE/B/KOH/ABND**:** Pickled olive flesh extract boiled for 5 minutes and treated with KOH and acid black nigrosine dye; ABND**:** Acid black nigrosine dye; RBCs: Red blood cells, Hb**:** Hemoglobin, Hct**:** Hematocrit. Means that do not share a letter are significantly different (Tukey's test, $p < 0.05$).

Table 3. Total leucocyte counts and their differential percentages in different groups.

Groups	W.B.Cs ($\times10^3$/ul)	Differential count		
		Mono. (%)	Lymph. (%)	Neut. (%)
Control	10.13 ± 2.35	2.0 ± 0.0[a]	89.00 ± 1.0	9.0 ± 1.0[a]
NOFE	10.81 ± 4.19	2.66 ± 1.15 [a]	83.33 ± 2.51	11.33 ± 1.08[a]
POFE/B/KOH/ABND	10.76 ± 1.51	3.00 ± 1.0 [a]	85.66 ± 1.15	11.33 ± 0.57[a]
ABND	9.93 ± 2.36	5.00 ± 1.73[b]	78.66 ± 4.93	16.33 ±1.21[b]

Data represented as means ± S.D.; NOFE**:** Normal olive flesh extract; POFE/B/KOH/ABND**:** Pickled olive flesh extract boiled for 5 minutes and treated with KOH and acid black nigrosine dye; ABND**:** Acid black nigrosine dye; W.B.Cs: White blood cells; Mono**:** Monocytes**;** Lymph**:** Lymphocytes; Neut**:** Neutrophils**.** Means that do not share a letter are significantly different (Tukey's test, $p < 0.05$).

Table 4. ALT, AST activities, urea, and creatinine levels in different groups.

Groups	ALT (U/L)	AST (U/L)	Urea (mg/dL)	Creatinine (mg/dL)
Control	59.66 ± 7.76 [a]	91.6 ± 24.54 [a]	27.0 ± 17.08 [b]	0.8 ± 0.34[a]
NOFE	55.67 ± 4.51 [a]	99.5 ±111.1 [a]	23.0 ± 4.35 [b]	0.6 ± 0.2[a]
POFE/B/KOH/ABND	144.6 ± 26.8[b]	258.3± 38.8 [b]	54.33±5.50 [a]	1.2± 0.23[b]
ABND	79.0 ± 4.35 [b]	202.6 ± 28.0 [a,b]	48.33± 2.3 [a]	1.23 ± 0.11[b]

Data are represented as means ± S.D. NOFE**:** Normal olive flesh extract; POFE/B/KOH/ABND: Pickled olive flesh extract boiled for 5 minutes and treated with KOH and acid black nigrosine dye; ABND**:** Acid black nigrosine dye ; ALT: Alanine aminotransaminase; AST**:** Aspartate aminotransferase. Means that do not share a letter are significantly different (Tukey's test, $p < 0.05$).

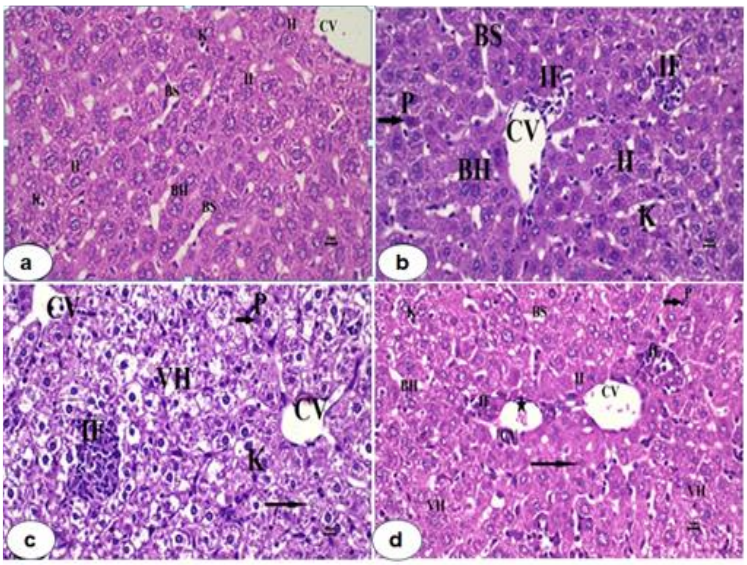

Fig. 1 (a-d): Photomicrographs of liver sections of: **(a)** Control group showing normal hepatic structure with radial arrangement of polygonal hepatocyte containing pronounced nuclei (H), central vein (CV), blood sinusoids (BS) lined by endothelial cells and distinct phagocytic Kupffer cells (K). **(b)** NOFE administered mice showing normal hepatocytes with normal architecture. Slight cellular infiltration surrounding the central vein may appear (IF). **(c)** POFE/B/KOH/ABND administered mice showing marked disorganization, congested central veins (CV) with intensive degeneration of its epithelial lining, most of hepatocytes are highly degenerated with vacuolated cytoplasm (VH), others with pyknotic nuclei (thick arrows), also karyolitic nuclei were seen (thin arrow), area around central vein associated with cellular infiltration (IF). (**d)** ABND administered mice showing hepatic degeneration with irregular congested central vein (CV), widening of blood sinusoids (BS), pyknotic nuclei (thick arrows), and Kupffer cells (K), area of infiltration around central vein (IF). (high magnification; magnification power = 400) stained with hematoxylin and eosin).

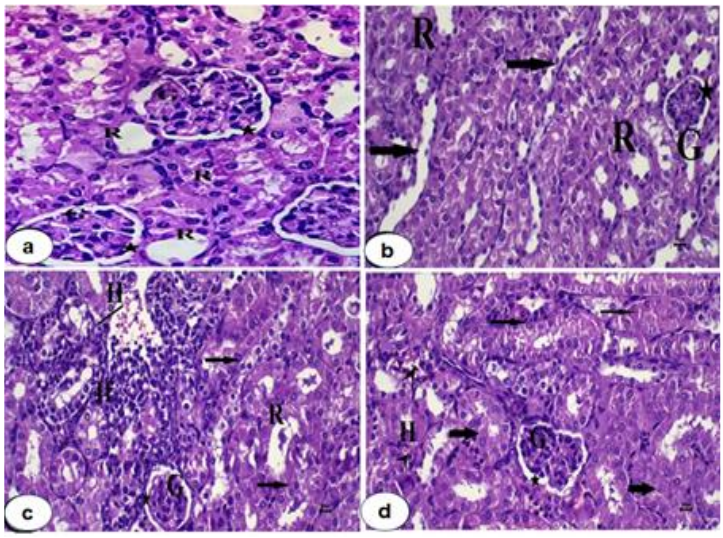

Fig. 2 (a-d): Photomicrographs of kidney sections of: **(a)** control group showing normal structure of the cortex, normal glomeruli (G) with normal Bowman's space (*), and normal renal tubules (R) lined by cuboidal epithelium. **(b)** NOFE administered mice showing more or less normal like architecture of glomeruli, most of the renal tubules (R) are elongated and widened. **(c)** POFE/B/KOH/ABND administered mice showing destructed, shrunken and congested glomeruli (G) with irregular Bowman's space (*), damaged renal tubules (R) and lost their characteristic appearance with distinct vacuolated and highly degenerated lining epithelium; cellular infiltration (IF) and hemorrhage (H) at the intertubular spaces were observed **(d)** ABND administered mice showing disorganized glomeruli (G) with irregular Bowman's space (*), widening of some renal tubules (R), occluded with hyaline casts (thick arrows) and degeneration of some epithelial lining, others with vacuolated cytoplasm (V), damaged and their contents intermixed with each other (thin arrows), also intratubular hemorrhage are observed (arrow head). (high magnification; magnification power = 400) stained with hematoxylin and eosin).

4. Discussion

Olive fruits have been reported to provide several biomedical potentials, including anticonvulsant, antioxidant, antihypertensive, analgesic, antidiabetic, antimicrobial, antihyperglycemic, antinociceptive, gastroprotective, and wound healing activities (Ghanbari et al., 2012; Larussa et al., 2019). This study aimed to evaluate the hepato-renal toxic effect of pickled olive fruits treated with acid black nigrosine dye (ABND) in mice. The results showed that there was no mortality or toxic signs up to 5000 mg/kg and the % body weight change in POFE/B/KOH/ABND and the group of mice that were treated with ABND were 9.5% and – 0.15%, respectively.

The results of our study agree with the results of studies by Viola and Viola (2009) and Fathy et al. (2018). The results showed that the activities of ALT and AST were increased in the group of mice that were treated with POFE/B/KOH/ABND, and ABND when compared to the normal control and NOFE control groups. Recently, a previous study indicated a protective effect of olive and thyme phenols supplemented in the diet, resulting in a higher concentration of endogenous α-tocopherol in the rat liver (El-Masry et al., 2022). Also, Hassanen and Ahmed (2015) showed that treatment with olive oil exhibited improvement in hepatorenal functions and reduced the severity of the liver injury. Antioxidant protection by phenolics from olive and thyme against oxidative stress occurs primarily through a direct antioxidant effect and may be related to the phenolic plasmatic metabolites (Rubió et al., 2014). The results showed that urea and creatinine levels were increased in the groups of mice that were injected with POFE/B/KOH/ABND, and ABND compared to the normal control and NOFE control groups. Olive oil can be used as a protective compound for coping with kidney disease (renal toxicity, renal ischemia-reperfusion, kidney stones, kidney damage caused by lupus erythematosus and urinary tract infections) and reduce kidney damage parameters (urea and creatinine) (Babaeenezhad et al., 2019).

Conclusion

Collectively, pickling olive fruits in the presence of ABND led to some hepato-renal injuries by inducing biochemical and histopathological alterations.

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Conflict of interest

All authors declared that there was no conflict of interest.

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Author contributions

SR conceptualized the study, performed the experiments, analysis date and wrote the draft. The author read and approved the final manuscript.

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