Protective effect of *Nigella sativa* seed extracts against oxytetracycline induced liver and kidney injuries in albino rats

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ABSTRACT

This research aimed to determine the antioxidant activity and protective effects of *Nigella sativa* seed extracts against liver and kidney injuries induced by oxytetracycline (OTC) in albino rats. Oil extract (NSO) was prepared by cold pressing that contains fixed and essential oil. Essential oil (EO) was also prepared by hydro-distillation; its contents were identified as twenty-six terpenoids. Ethanolic extract (NSET) was also prepared and its contents of phenols and flavonoids were quantified. Antioxidant activity of both NSO and NSET were measured using DPPH and ABTS. The protective effect of each of NSO and NSET against oxidative stress was determined in Albino rats. Results showed that the liver and kidney weight gain at the end of experiment were decreased in the both extracts while increased in the OTC-treated group. Enzymatic antioxidants, SOD, CAT, and G-px, showed a substantial inhibition in OTC-treated group compared with extract-treated groups. Liver function and kidney function parameters as well as malondialdehyde (MDA) level increased significantly in OTC-treated group while they decreased in extract-treated groups. The preventive properties of the extracts were validated in histological investigation of liver and kidney.

Keywords: Kidney function, Liver function, Oxidative stress.

INTRODUCTION

Nigella sativa, known as the black bean, grows in various regions around the world, including Eastern and Southern Europe, Pakistan, and India. It has been used since ancient times. (Tanbek et al 2017) presented the scientific concept of medicinal plants including *N. sativa* oil against anti-thioacetamide that causes liver damage in rats. *N. sativa* oil, and various extracts have been widely used in the preventive role in many countries. *N. sativa* is one of the natural medications lists and are used in Tibb-e-Nabawi and Indian traditional medicine, as well as used as spice or food additives (Ahmad et al 2021).

Ansari and Satish 2013 reported that *N. sativa* is a liver tonic, diuretic, digestive, antidiarrheal, appetite stimulant, antibacterial, and diaphoretic. Besides, it is used as antidiabetic, anticancer, antimicrobial, anti-inflammatory, antispasmodic, powerful antioxidant, immunomodulatory, and analgesic properties. It can also be used as a bronchodilator, anti-obesity, antimigraine, anti-back pain, and anti-high pressure. "It has many pharmacological properties, so it can protect the liver, kidneys, and stomach due to its anthelmintic and carminative properties. Abscesses, nasal ulcers, orchitis, eczema, and swollen joints can all be treated with the black seed (Ali and Blunden 2003).

At the same time, the effectiveness of the seeds is compatible with their extracts that protect cells with its biological components, led by thymoquinone, which inhibits lipid peroxidation and then oxidative stress (Al-Seeni et al 2018).

Several assays have been determined in this field by Burits and Bucar 2000. They showed that the essential oil contains ϱ-cymene, thymoquinone, α-thujene, β-pinene, α-pinene, and carvacrol as the major constituents.

According to Hassanien et al 2015, *Nigella sativa* biologically influenced hepatic and renal toxicity, and is used to combat oxidative stress by increasing antioxidant enzymes levels and decreasing oxidative enzymes activity".

Rasouli et al 2017 conducted study on *N.sativa* oil to show high lipid profile, especially unsaturated fatty acids, and the pharmacological tests proved its powerful role in improving antioxidant properties by scavenging free radicals.

Many studies have been conducted on the pharmacological characteristics of *N. sativa*, including mitochondrial dysfunction caused by free radicals. Current study demonstrates the ability to combat oxidative stress by using two extracts of *N. sativa* (NSO and NSET) against liver and kidney injuries induced by OTC in albino rats.

MATERIALS AND METHODS

Plant and chemical substances

N. sativa seeds were obtained from commercial marketplaces to obtain the ethanolic extract (NSET). Cold pressed oil (NSO) was purchased from the National Research Center in Dokki, Cairo, Egypt. Oxytetracycline (OTC) was provided by Al-Gomhouria Pharmaceutical Company, Cairo, Egypt. Other chemicals are also classified as analytically pure.

NSET extract preparation

N. sativa seeds were cleaned, dried, ground, weighted and extracted using *n*-hexane, ethyl acetate, and 70% ethanol subsequently. The ethanol extract was concentrated in a rotary evaporator (Akinwumi et al 2020).

Fatty acid composition in NSO extract

The fatty acid composition of the oil was determined following the modified method of Zahran and Tawfeuk, 2019. One mL of *n*-hexane was added to 15 mg of oil samples and vortexed for 30 sec. followed by 1 mL of sodium methoxide (0.4 mol). The mixtures were vortexed for 30 seconds and were allowed to settle for 15 minutes. The upper phase contained the fatty acids methyl esters (FAMEs) was separated with an HP 6890 plus gas chromatography (Hewlett Packard, USA), using a capillary column Supelco™ SP- 2380 capillary column (30 m×0.25 mm×0.20 μm) (Sigma-Aldrich, USA), 30 m length, diameter 0.25 mm and film thickness 0.25 μm. The detector (FID) and the injection temperatures were 260 °C. Column temperature was 50 °C (3 min) to 225 °C (17.5 min) at 10 °C/min. The carrier gas was helium at a flow rate of 1.2 mL/ min. The FAMEs were identified by comparing their relative and absolute retention times to those of authentic standards of FAMEs (from C4:0 to C24:0). The fatty acid composition was reported as a relative percentage of the total peak area.

Determination of chemical components of essential oil in seeds

The hydro-distillation was used to extract the essential oil from seeds (Erdoğan et al 2020). The biological components were estimated using GC analysis. It was performed using a Perkin Elmer Auto System XL fitted with a flame ionization detector (FID). The capillary column utilized was a ZB-5 fused silica capillary column (60 m × 0.32 mm). Oven temperature was initially set at 50°C and then gradually increased to 240°C at a rate of 3°C/min. Helium was used as the carrier gas at a flow rate of 1.1 mL/min. The temperature of the injector and detector was set to 270°C. The EO contents were identified by matching retention time with standards.

Estimation of total phenolic contents (TPC) and total flavonoids (TF) in NSET extract

The total phenolic contents were determined according to Khan et al 2018, and total flavonoids according to Parthasarathi and Park 2015.

Measuring antioxidant activity

DPPH and ABTS scavenging activity of NSO and NSET extracts

The DPPH scavenging activity of both extracts is determined according to Kadam and Lele 2017. 1ml of DPPH solution was added to 1 ml of different concentrations (5-25μg/mL of extract and standard), then incubated in dark room for 30 min at 28°C. The yellow color was measured at 517 nm.

To determine ABTS, potassium persulfate was stimulated scavenging activity in two steps, and the green color was measured at 734 nm (Dong et al 2015).

The following equation was used to calculate the antioxidant activity to each parameter as a percentage of inhibition to convert in to IC50 (concentration required to inhibit 50% of the radicals) by linear equation.

$$I\,[\%] = (A_0 - A / A_0) \times 100$$

Where I [%] is percent inhibition, A_0 is the value of blank absorption i.e. methanol, and A is the absorbance value of the analyzed sample.

Experimental design and animals

Animal House Colony, National Research Center, Dokki, Giza, Egypt, provided 3-month-old male Sprague–Dawley rats (120–150 g/ animal). All animals were treated humanely in accordance with the Animal Care and Use Committee's requirements. Forty- two rats were fed for 28 days and divided into six groups each of 7.

The first group control (C) was fed on healthy food without any treatments. Positive control (+) was injected intraperitoneally with OTC 200 mg/kg bw (Jayanthi and Subash, 2010) in the second group. The third group received NSET (800 mg/Kg bw orally as supported by Ílhan and Seçein 2005) in the first two weeks then animals were injected intraperitoneally by OTC in the following two

weeks. The fourth group received NSO (2ml/Kg of bw orally as mentioned by Saleem et al 2012) in the first two weeks then animals were injected intraperitoneally by OTC in the following two weeks. The fifth group; animals were fed orally by NSET only. Finally, the sixth groupwere fed orally by NSO only.

Biochemical analysis

Hepatic functions i.e., alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were determined (Gella et al 1985). Renal functions i.e. urea and creatinine assessed according to Tietz (1995); uses Arab-Lab-Egypt kits. MDA levels were determined by the mode of interaction of MDA with acid (Bayrak et al 2008) and expressed as nmol/mL in serum samples. superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (G-PX) were evaluated in plasma (Sultan et al 2014) where heparin was used to stop blood-clotting in blood.

Histopathological Examination for liver and kidney

The organs have been washed in saline, and the preparation of paraffin sections were done as follows: 10% formalin to make fixation. The dehydration has been done through ascending grades of alcohol (70% alcohol at 1.5 hours, 90% alcohol at 1.5 hours, and absolute alcohol at 3 hours), then the specimens were cleared in xylene for 4 hours. The cleared specimens were impregnated in soft pure paraffin through three different grades (each one for one hour) at 56 °C to make infiltration. Finally, the specimens were embedded in paraffin wax at 58 °C and oriented in blocks. Paraffin sections of 3-5 micron thickness were cut to histological study. On the other hand, the Staining by Hematoxylin and Eosin (H & E) were mounted in DPX and covered. This method was close to what Al-Azzawi and Parraj (2016) mentioned.

Statistical analysis

Data were statistically analyzed using the SPSS version 20 program. Following ANOVA, Tukey's post hoc test was performed to determine the lowest significant difference. Means and S.E. were calculated using descriptive statistics.

RESULTS AND DISCUSSION

Identification of fatty acids in NSO extract

The Fatty acids composition of black seed oil was reported in Table (1). The analysis revealed a small amount of myristic, arachidic, and stearic acids as saturated fatty acids (SFA). Palmitic acid was (13.05%), while the small amount of unsaturated fatty acids (UFA) was palmitoleic, linolenic n-3. On the other hand, the major fatty acids were presence in long-chain fatty acids, such as linoleic and oleic acid (58.31and 24.39%, respectively). The obtained data as shown in Table (1) and Fig. 1 were close to the illustrated results of cold-pressed black cumin seed oil obtained by (Lutterodta et al 2010). It can be enhanced by the preventive treatments for patients exposed to oxidative stress by NSO. The results of this study showed that the NSO has the necessary chemical properties that increase the antioxidant capacity. Thus, lipid peroxidation (oxidative stress) has been reduced as well as the harmful effects of stress on body tissues (Rasouli et al 2017).

Composition of EO in seeds

The chemical components of *N. sativa* essential oil (Table 2), and Fig. 2 showed the results of the GC chromatogram. GC analyses using FID revealed that 26 components were identified, including *p*-cymene (41.08%), α-thujene (12.69%), *trans-p*-mentha-2,8-dien-1-ol (10.83%), ß-pinene (6.57%), α-pinene (5.50%), β-caryophyllene (3.69%), γ-terpinene (3.36%), thymoquinone (0.19%), sabinene (2.72%), *trans*-sabinene hydrate (1.86%), thymol (1.36%), carvacrol (1.16%), dihydrocarvone (0.78%), α-terpinene (0.75), *p*-cymene-8-ol (0.71%), and cyclosativene (0.69%). These data were almost identical with (Burits and Bucar 2000), which states that using *N. sativa* seeds from sex samples and a commercial fixed oil had a qualitative composition of volatile compounds. However, they added that thymoquinone and its components, carvacrol, t-anethole, and 4-terpineol exhibited a respectable radical scavenging property. They also found that the essential oil had varying antioxidant efficacy in non-enzymatic lipid peroxidation in liposomes and deoxyribose degradation experiment using efficient OH radical scavenging agents.

TPC and TF contents

The antioxidant components present in NSO and NSET were estimated respectively to evaluate the antioxidant properties. NSO recorded 4.95±1.50 mg GAE/g dw of total phenolic content and 4.03±0.07 mg QAE/g dw of total flavonoids, while total phenolic content recorded 33.24±0.76 mg GAE/g dw and 18.67±1.35 mg QAE /g dw of total flavonoids in NSET. Mariod et al (2009) showed the same trend in the phenolic content test of phenolic rich fractions was obtained from black cumin.

Antioxidant activity

Table 4 shows the scavenging activity of DPPH and ABTS to assess NSO and NSET. The DPPH for those ranged from 177.66 ± 2.78 to 534 ± 5.23 μgTE/mL, and 40.06 ± 7.75 to 60 ± 14.73 IC50 μg/mL, respectively. The ratio is similar to (Guergouri et al 2017) in their study on Algerian total oil of *N.sativa*. Additionally, ABTS ranged from 1,021 ± 40.02 to 435 ± 5.23 μgTE/mL and 35.84 ± 7.71 to 6.45 ± 3.37 IC50 μg/mL for NSO and NSET respectively. Moreover, the coefficient, which refers to sample concentration that inhibited 50% of the radicals, as- IC50. And the lower IC50, the better antioxidant activity as shown in ABTS parameter.

Tables 3 and 4 are closed to the findings of Dorman et al 2003, who noted a good relationship between TPC and antioxidant activity in fruits, vegetables, and medicinal plants. In the current study, the polar fissure in phenols and flavonoids related to the antioxidant capacity is approved by Kadam and Lele 2017 in the extraction, characterization, and estimation the bioactive properties of *N. sativa seed* cake extracts. In addition to the EO components that vary according to geographical and environmental conditions, (Bourgou et al., 2010) showed a high antioxidant capacity (Nagi and Mansour 2000).

Effect of treatments on liver and kidney weights

(Table 5); it is concerned with the effect of the treated groups that lead to significant values approached to the control, while positive control significantly increased at ($p \leq 0.05$). (Mousavi 2015) described that renal reperfusion leads to the possibility of interstitial edema that resulted in an increase in the kidney weights for the body, and the treatment of the liver and kidneys can be done by thymoquinone or *N.sativa* in general, as documented by Ates and Ortatath 2020.

Biochemical determinations of liver and kidney functions in blood serum

The significance of the treatments included the use of NSO, NSET converges to the control ($p \leq 0.05$). The AST, ALT, urea, and creatinine indicators of the positive control were considerably increased (Tables 6 and 7). The occurrence of lipid peroxidation can lead to the destruction of cells and consequently the excretion of liver enzymes and their rise from the cytosol to the blood serum (Hamad, 2012). Creatinine is a measure of glomerular function. Therefore, any damage to the kidneys will increase in the levels of urea and creatinine in the blood (Dollah et al 2013). (Tables 6 and 7) show the significance of the treatments included the use of NSO, NSET converges to the control ($p \leq 0.05$), but The AST, ALT, urea, and creatinine indicators of the positive control were considerably increased.

Determination of antioxidant activity and lipid peroxidation

(Table 8) displays MDA, SOD, CAT, and GSH-PX averages. Where MDA significantly increased as a result of OTC-treated group and significantly decreased with NSO and NSET treated groups; however, in contrast to the significance of antioxidant enzymes such as SOD, CAT, and GSH-PX, the findings of this study are consistent with those of (Mohebbati et al 2017), and (Abou Zaid et al 2015).

Histopathological Examination

The histopathological examination of the liver in control animals exhibited average portal tracts, average central veins, and average hepatocytes (Fig. 3a), but the positive control group treated with OTC showed markedly edematous portal tract with the markedly dilated congested portal vein (PV), average bile ducts, and hepatocytes with vacuolated cytoplasm in the peri-portal area (Fig. 3b), and the possibility of many vacuolated hepatocytes injuring the liver is due to the adaptation of cells to resist more insults rather than the common hydropic of the cells (Nayak et al 1996) . The combination of OTC and NSET displayed a mildly edematous portal tract with the markedly dilated congested portal vein (PV), and average bile ducts (Fig. 3c), and the combination of OTC and NSO; portal tract with the markedly dilated congested portal vein (PV), average bile ducts, and average central vein (Fig. 3d). The group of Animals which received NSO only showed average portal tract, average central veins (CV), and average hepatocytes (Fig. 3e), but the group that received NSET only exhibited portal tract with the markedly dilated congested portal vein (PV), average bile ducts, average central veins (CV), and average hepatocytes (Fig. 3f). The histopathological examination of the kidney was showed in the control group; a typical renal capsule, glomeruli of typical size, and tubules of typical size (Fig. 4a), and in the positive control; kidney with a typical renal capsule, glomeruli of tiny size, and tubules that are significantly dilated (Fig. 4b) , and occurs anywhere along the nephron or

collecting duct system . It may occur in focal areas or tracts that extend along the entire length of the kidney sections. Renal tubule dilation may occur from xenobiotic administration, secondary mechanisms, or unknown etiology (Greaves 2012), while the combination of OTC+NSET group (Fig. 4c), OTC+NSO group (Fig. 4d), and a group treated with NSO only (Fig. 4e) showed a typical renal capsule, glomeruli of typical size, and tubules of typical size, while the group treated with NSET only clarified a typical renal capsule, glomeruli of typical size, tubules of typical size, and interstitial blood veins that are moderately dilated and congested (Fig. 4f).

CONCLUSION

The mechanism of responding to the anti-oxidative property of *N. sativa* extracts can be enhanced by ethanolic and oil extracts. Other studies mentioned also almost parallel effects close to the quality of extracts to current study. These extracts containing exogenous antioxidants which have a strong and powerful influence to stimulate the internal antioxidant mechanism led to positive scavenging radical effects. However, the protective role included in the study was revealed by distributing extract doses before induction with toxic substances. Consequently, positive results were obtained in response to the preventive role. Therefore, the study recommended that *N. sativa* seed extracts could be used as a natural antioxidant and protection against free radicals.

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REFERENCES

Abou Zaid, O.A.R., Badwi, A.F.M., El Sayed, N.A.E. 2015: Ameliorative effect of novel nanocomposite: Basic curcumin nanoparticles modified with basic Nano black seeds (Nigella sativa) and calcium ascorbate on metabolic changes in experimentally induced tumor in female mice. Benha Veterinary Medical Journal 29(2), 235-244. DOI: 10.21608/bvmj.2015.31719.

Ahmad, M.D.F., Ahmed, F.A., Ashraf, S.A., Saad, H., Whab, Sh., Khan, M.I., Mohan, S., Hakeem, K.H.R., Athar, M.D.T. 2021: An updated knowledge of black seed (Nigella sativa Linn.): Review of phytochemical constituents and pharmacological properties. Journal of Herbal Medicine 25, 1-37. DOI: 10.1016/j.hermed.2020.100404.

Akinwumi, K.A., Jubril, A.J., Olaniyan, O.O., Umar, Y.Y., 2020: Ethanol extract of Nigella sativa has antioxidant and ameliorative effect against nickel chloride-induced hepato-renal injury in rats. Clinical Phytoscience 6, 1-12. DOI: 10.1186/s40816-020-00205-9.

Al-Azzawi, A.F.S., Barraaj, A.H., 2016: Histological and Biochemical Study of Nigella sativa Seeds Effects on Kidneys of Male Albino Rats Treated with Rifampicin. World Journal of Experimental Biosciences 4,176-180.

Ali, B.H., Blunden, G., 2003: Pharmacological and Toxicological Properties of Nigella sativa. Phytotherapy Research 17, 299-305. DOI: 10.1002/ptr.1309.

Al-Seeni, M.N., El Rabey, H.A., Al-Hamed, A.M., Zamazamia, M.A, 2018: Nigella sativa oil protects against tartrazine toxicity in male rats. Toxicol Rep. 5,146–155.DOI: 10.1016/j.toxrep.2017.12.022.

Ansari, Z, Satish, T. 2013: Traditional uses of Nigella sativa, in Malegaon region of Nashik – A review. Indian Journal of Pure & Applied Biosciences 1, 19-23. DOI: 10.1016/S2221-1691(13)60075-1.

Ates, M.B., Ortatath, M., 2020: Protective effect of nigella sativa and thymoquinone on relative liver weight increase caused by aflatoxin in broilers. Eurasian journal of veterinary. Sciences 36(2), 107-114. DOI: 10.15312/EurasianJVetSci.2020.267

Bayrak, O., Bavbek, N., Karatas, O.F., Bayrak, R., Catal, F., Cimentepe, E., Akbas, A., Yildirim, E., Unal, D., Akcay, A., 2008: Nigella sativa protects against ischaemia/reperfusion injury in rat kidneys. Nephrology, Dialysis, Transplantation 23, 2206-2212. DOI: 10.1093/ndt/gfm953.

Bourgou, S., Pichette, A., Marzouk, B., Legault, J. 2010: Bioactivities of black cumin essential oil and its main terpenes from Tunisia. South African Journal of Botany 76, 210-216.DOI: 10.1016/j.sajb.2009.10.009

Burits, M., Bucar, F. 2000: Antioxidant activity of Nigella sativa essential oil. Phytotherapy Research 14(5), 323-328. DOI: 10.1002/1099-1573(200008)14:5<323::aid-ptr621>3.0.co;2-q.

Dollah, M.A., Parhizkar, S., Izwan, M. 2013: Effect of Nigella Sativa on the kidney function in rats. Avicenna Journal of Phytomedicine 3,152-158.

Dong, J.W., Cai, L., Xing, Y., Yu, J., Ding, Z.T. 2015: Re-evaluation of ABTS*+ assay for total antioxidant capacity of natural products.

Natural Product Communications 10, 2169-2172. DOI: 10.1177/1934578X1501001239

Dorman, H.J.D., Koşar, M., Kahlos, K., Holm, Y., Hiltunen, R. 2003: Antioxidant properties and composition of aqueous extracts from Mentha species, hybrids, varieties, and cultivars. Journal of Agricultural and Food Chemistry 51, 4563-4569. DOI: 10.1021/jf034108k.

Erdoğan, Ü., Yilmazer, M., Erbaş, S. 2020: Hydrodistillation of Nigella sativa Seed and analysis of thymoquinone with HPLC and GC-MS. Bilge International Journal of Science and Technology Research 4(1), 27-30. DOI: 10.30516/bilgesci.688845.

Gella, F.J., Olivella, T., Cruz Pastor, M., Arenas, J., Moreno, R., Durban, R., Gómez, J.A. 1985: A simple procedure for routine determination of aspartate aminotransferase and alanine aminotransferase with pyridoxal phosphate. Clinica Chimica Acta 153, 241-247. DOI: 10.1016/0009-8981(85)90358-4.

Greaves, P. 2012: Histopathology of Preclinical Toxicity Studies, 4th ed. Elsevier, Amsterdam, 560.

Guergouri, F.Z., Sobhi, W., Benboubetra, M., 2017: Antioxidant activity of Algerian Nigella sativa oil and its unsaponifiable fraction. The Journal of Phytopharmacology 6(4), 234-238. ISSN: 2320-480X. Online at: http://www.phytopharmajournal.com.

Hamad, Z.M. 2012: Protective effect Ethanol extract of Nigella L. on hepatic damage induced by naphthalene in male rats. AL-Qadisiya Journal for Science 17, 1-8. ISSN-1997-4290.

Hassanien, M.F., Assiri, A.M., Alzohairy, A.M., Oraby, H.F. 2015: Health-Promoting value and food applications of black cumin essential oil: On Overview. Journal of food science and technology 52(10), 6136-6142. DOI: 10.1007/s13197-015-1785-4. https://www.researchgate.net/publication/325811202.

Ílhan, N., Seçein, D. 2005: Protective effect of Nigella sativa aides on CCl4-induced hepatotoxicity. Fırat University Medical Journal of Health Sciences 19(3), 175-179.

Jayanthi, R., Subash, P. 2010: Antioxidant effect of caffeic acid on oxytetracycline induced lipid peroxidation in albino rats. Indian Journal of Clinical Biochemistry 25(4), 371-375. DOI: 10.1007/s12291-010-0052-8.

Kadam, D., Lele, S.S. 2017: Extraction, Characterization, and bioactive properties of Nigella sativa Seed Cake. Journal of Food Science and Technology 54, 3936-3947. DOI: 10.1007/s13197-017-2853-8.

Khan, M.S., Yusufzai, S.K., Rafatullah, M., Sarijadi, M.S. 2018: Determination of Total phenolic Content, Total Flavonoid Content and Antioxidant activity of Various Organic Crude Extracts of Licuala spinosa Leaves from Sabah, Malaysia. ASM Science Journal 11(3), 53-58.

Lutterodta, H., Luthera, M., Slavina, M., Yinb, J.J., Parry, G., Gaod, J.M., Yu, L.L. 2010: Fatty acid profile, thymoquinone content, oxidative stability, and antioxidant properties of cold-pressed black cumin seed oils. LWT - Food Science and Technology, 43, 1409 -1413. Doi:10.1016/j.lwt.2010.04.009.

Mariod, A.A., Ibrahim, R.M., Ismail, M., Ismail, N. 2009: Antioxidant activity and phenolic content of phenolic rich fractions obtained from black cumin (Nigella sativa) seedcake. Food Chemistry 116, 306–312. DOI: 10.1016/j.foodchem.2009.02.051

Mohebbati, R., Hosseni, M., Haghshenas, M., Nazaribor, A., Beheshti, F. 2017: The effects of Nigella sativa extract on renal tissue oxidative damage during neonatal and juvenile growth in propylthiouracil-induced hypothyroid rats. Endocrine Regulations 51(2), 105-113. DOI: 10.1515/enr-2017-0010.

Mousavi, G. 2015: Study on the effect of black cumin (Nigella sativa Linn.) on experimental renal ischemia – Reperfusion injury in rats. Acta Cirurgica Brasileira 30(8), 542-550. DOI: 10.1590/S0102-865020150080000005.

Nagi, M.N., Mansour, M.A. 2000: Protective effect of thymoquinone against doxorubicininduced cardiotoxicity in rats: A possible mechanism of protection. Pharmacological Research 41, 283–289. DOI: 10.1006/phrs.1999.0585.

Nayak, N.C., Sathar, S.A., Mughal, S., Duttagupta, S., Mathur, M., Chopra, P. 1996: The nature and significance of liver cell vacuolation following hepatocellular injury--an analysis based on observations on rats rendered tolerant to hepatotoxic damage. Virchows Arch 428(6), 353-65. DOI: 10.1007/BF00202202.

Parthasarathi, S., Park, Y.K. 2015: Determination of total phenolic flavonoid contents and antioxidant activity of different m BHT fractions. Apoly Herbal Medicine 28(6), 2161-2166.

Rasouli, H., Farzaei, M.H., Khodarahmi, R. 2017: Polyphenols and their benefits: A review. 20, 1700–1741. DOI: 10.1080/10942912.2017.1354017.

Ratz-Łyko, A., Herman, A., Arct, J., Pytkowska, K. 2014: Evaluation of Antioxidant and antimicrobial Activities of Oenothera biennis, Borago officinals, and Nigella sativa Seed Cake Extracts. Food Science and Biotechnology 23(4), 1029-1036. DOI: 10.1007/s10068-014-0140-2.

Saleem, U., Ahmad, B., Rehman, K., Mahmood, S., Alam, M, Erum, A. 2012: Nephroprotective

effect of vitamin C and Nigella sativa oil on gentamicin associated nephrotoxicity in rabbits. Pakistan Journal of Pharmaceutical Sciences 25(4), 727-730. PMID: 23009987

Sultan, M.T., Butt, M.S., Karim, R., Iqbal, S.Z., Ahmad, S., Zia-Ul-Haq, M., Aliberti, L., Ahmad, A.N., De Feo, V. 2014: Effect of Nigella sativa fixed and essential oils on antioxidant status, hepatic enzymes, and immunity in streptozotocin-induced diabetes mellitus. BMC Complementary and Alternative Medicine 14(193), 193. DOI: 10.1186/1472-6882-14-193

Tanbek, K., Ozerol, E., Bilgiç, S., Iraz, M., Sahin, N., Colak, C. 2017: Protective effect of Nigella sativa oil against thioacetamide-induced liver injury in rats. Medicine Science | International Medical Journal 6(1), 96-103. DOI: 10.5455/medscience.2016.05.8531.

Tietz, N.W. 1995: Clinical Guide to Laboratory Tests, 3rd Edition. W.B. Saunders Co. Philadelphia, PA.

Zahran, H.A., Tawfeuk, H.Z. 2019: physicochemical properties of new peanut (Arachis hypogaea L.) varieties. OCL 26, 19. DOI: 10.1051/ocl/2019018.

Table 1: GC-FID analysis of fatty acid composition of cold-press oil (NSO) of *Nigella Sativa L.*

Fatty acids		
Common name	Abbreviation	Area %
Myristic acid	C14:0	0.17
Palmitic acid	C16:0	13.05
Palmitoleic acid	C16:1	0.2
Stearic acid	C18:0	3.39
Oleic acid	C18:1	24.39
Linoleic acid	C18:2	58.31
Linolenic acid n3	C18:3 n3	0.2
Arachidic acid	C20:0	0.35

Table 2: Chemical components of EO

No	Volatile compounds	Relative area (%)
1	α-Thujene	14.99
2	α-Pinene	5.50
3	Camphene	0.13
4	Sabinene	2.72
5	ß- Pinene	6.57
6	Myrcene	0.36
7	3-carene	0.28
8	α-Terpinene	0.75
9	*p*-Cymene	41.08
10	γ-Terpinene	3.36
11	Terpinolene	0.12
12	*trans*-Sabinene hydrate	1.86
13	Linalool	0.23
14	*trans-p*-Mentha-2,8-dien-1-ol	10.83
15	Borneol	0.15
16	Terpinen-4-ol	0.36
17	*p*-Cymen-8-ol	0.71
18	*trans*-Dihydrocarvone	0.21
19	Dihydrocarvone	0.78
20	Thymoquinone	0.19
21	Thymol	1.36
22	Carvacrol	1.16
23	Cyclosativene	0.69
24	*(z)*-Caryophyllene	0.24
25	β-Caryophyllene	3.69
26	Aromadendrene	0.68

Table 3: TPC and TF content in NSO and NSET

Parameters Extracts	TPC (mg GAE/g) mean± S.E	TF (mg QAE /g) mean± S.E
Nigella sativa oil extract (NSO)	4.95±1.5	4.03±0.07
Nigella sativa ethanolic extract (NSET)	33.24± 0.76	18.67±1.35

Total phenolic content TPC and total flavonoids TF NSET by descriptive statistics (mean and standard error ± S.E.)

Table 4. TE equivalent and IC50 for the scavenging activity of DPPH and ABTS radicals

Parameters Extracts	DPPH		ABTS	
	(μgTE/mL) Mean± S.E	IC50(μg/mL) Mean± S.E	(μgTE/mL) Mean± S.E	IC50(μg/mL) Mean± S.E
Nigella sativa oil extract (NSO)	177.66 ± 2.78	40.06 ± 7.75	1,021 ± 40.02	35.84 ± 7.71
Nigella sativa ethanolic extract (NSET)	534 ± 10.65	60 ± 14.73	435 ± 5.23	6.45 ± 3.37

Each mean followed by standard error S.E., (DPPH) = 1,1-diphenyl-2-picryl-hydrazyl and (ABTS) radical = 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid). IC50 = concentration required to inhibit 50% of DPPH and ABTS. Equivalent amounts of the standard substance Trolox (TE), and the resulting quantity: μg/mL.

Table 5: Effect of treatments on liver and kidney weight gain

Organ		Liver		Kidney	
Groups		Mean	S.E.±	Mean	S.E.±
1.00	control (C)	5.75	0.16	1.23 *	0.06
2.00	(+) control	7.04****	0.22	2.31****	0.21
3.00	OTC + NSET	5.41*	0.28	1.26*	0.06
4.00	OTC + NSO	5.39*	0.26	1.32*	0.07
5.00	NSO	5.48*	0.34	1.12*	0.03
6.00	NSET	5.01*	0.19	1.25*	0.06

Six groups 1:6 (n = 7). Average values were calculated as mean and standard error as S.E. ANOVA with Tukey's post hoc test was used to determine significant differences in liver and kidney weights affected by treatments., *: **** indicate a significant differences ($p \leq 0.05$). The same symbol in different cells indicate a non-significant ($p \leq 0.05$).

Table 6: Impact of treatments on liver function enzymes (ALT and AST)

Function Groups		ALT (U/L) Statistics		AST (U/L) Statistics	
		Mean	S.E.±	Mean	S.E.±
1.00	control (C)	35.66*	3.28	138.20*	4.02
2.00	(+) control	88.66****	1.66	228.33***	10.92
3.00	OTC + NSET	28.50*	1.32	127.50*	7.79
4.00	OTC + NSO	32.50**	3.09	132.20*	14.37
5.00	NSO	28.80*	2.78	143.25*	15.12
6.00	NSET	17.66***	0.88	129.80*	7.68

Six groups 1:6 (n = 7). Average values were evaluated as mean and standard error as S.E. ANOVA with Tukey's post hoc test was used to determine significant differences in liver functions (AST and ALT) affected by treatments., *: **** indicate a significant difference ($p \leq 0.05$). The same symbol in different cells indicate a non-significant ($p \leq 0.05$).

Table 7: Effect of treatments on kidney functions (Urea and Creatinine)

Function Groups		Urea (mg/dL)		Creatinine (mg/dL)	
		Statistics		Statistics	
		Mean	S.E.±	Mean	S.E.±
1.00	control (C)	46.33*	3.48	0.64*	0.065
2.00	(+) control	78.00****	6.67	11.04****	1.86
3.00	OTC + NSET	49.50*	0.50	0.73*	0.14
4.00	OTC + NSO	33.60*	2.44	0.73*	0.10
5.00	NSO	44.60*	2.76	0.80*	0.05
6.00	NSET	47*	3.72	0.62*	0.07

Six groups 1:6 (n = 7). Average values were evaluated as mean and standard error as S.E. ANOVA with Tukey's post hoc test was used to determine significant differences in kidney functions (Urea and Creatinine) affected by treatments. *: **** indicate a significant difference ($p \leq 0.05$). The same symbol in different cells indicate a non-significant ($p \leq 0.05$).

Table 8: Lipid peroxidation (MDA) and antioxidant enzyme activities

Parameter Groups	MDA		SOD		CAT		GSH-PX	
	Mean	S.E.±	Mean	S.E.±	Mean	S.E.±	Mean	S.E.±
1.00 control (C)	109.56 *	1.79	27.78***	1.53	5.38*	.500	33.54**	0.55
2.00 (+) control	376.32****	58.49	10.43****	1.01	1.58****	0.46	12.76****	0.83
3.00 OTC + NSET	126.81*	2.85	18.37***	0.99	3.82	0.42	29.78 *	1.15
4.00 OTC + NSO	119.47 *	5.13	15.96***	1.97	4.48 *	0. 68	27.75**	0.95
5.00 NSO	130.58 *	10.10	24.62**	1.56	5.49 *	0.50	31.39*	0.90
6.00 NSET	114.40 *	3.61	28.67***	1.14	4.83 *	0.27	30.86*	0.60

Six groups 1:6 (n = 7). Average values were evaluated as mean and standard error as S.E. ANOVA with Tukey's post hoc test was used to determine significant differences in MDA, SOD, CAT, and GSH-PX affected by treatments. *: **** indicate a significant difference ($p \leq 0.05$). The same symbol in different cells indicates a non-significant ($p \leq 0.05$).

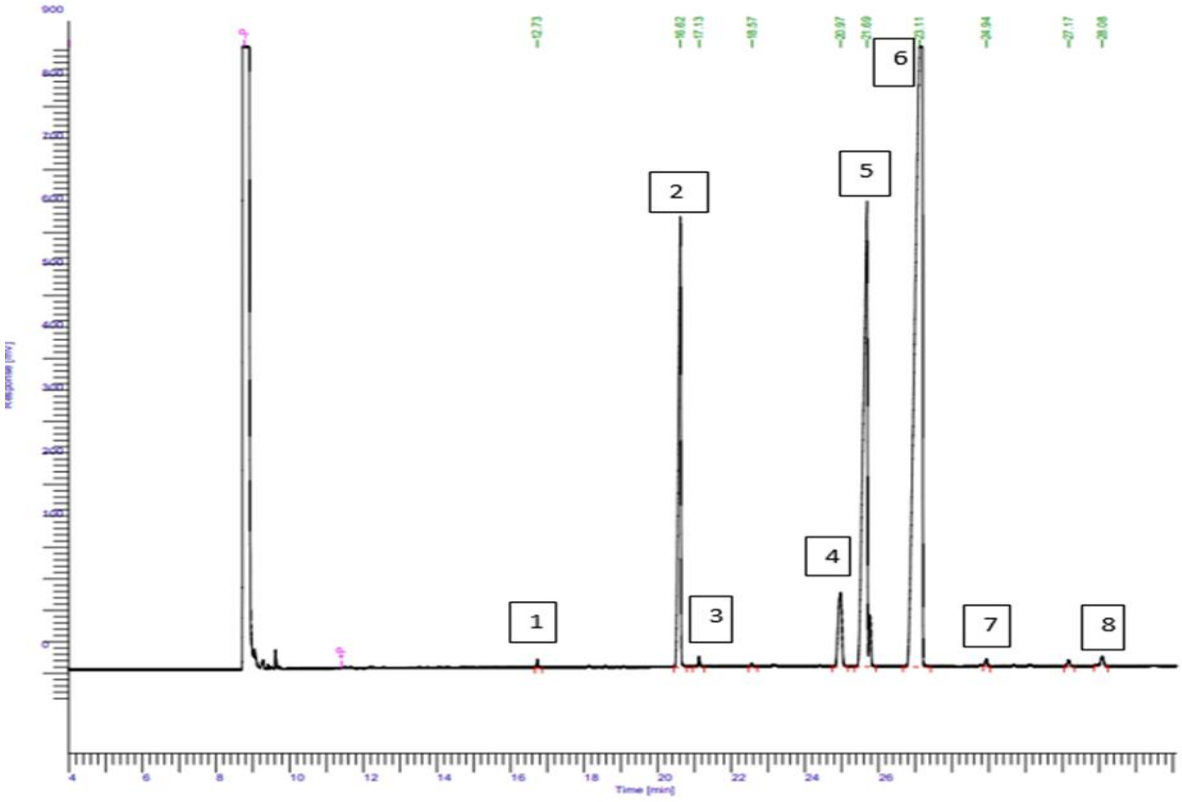

Figure 1: Chromatogram of fatty acid composition of NSO extract.

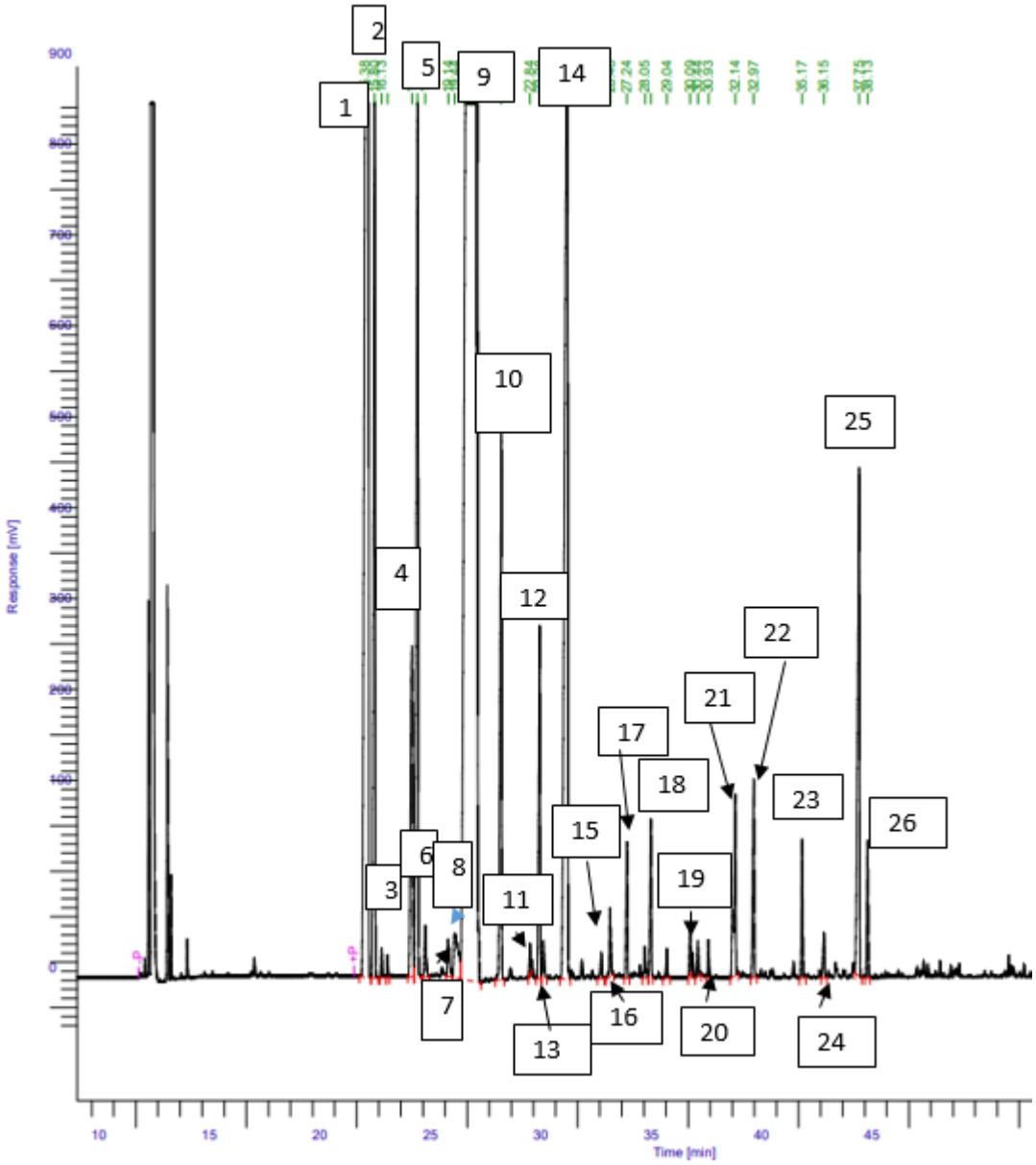

Figure 2: Chromatogram of essential oil (EO) contents.

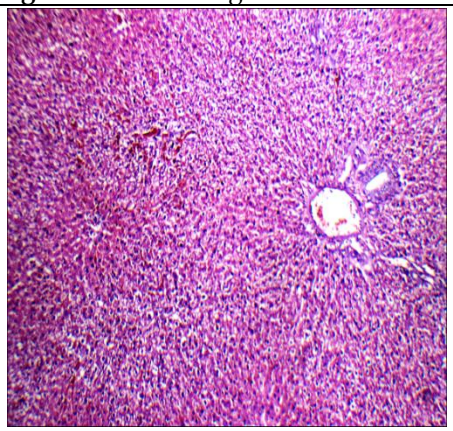

Figure 3a: (Group 1; Control): Slide C: liver showing average portal tracts, average central veins and average hepatocytes (H&E X 200).

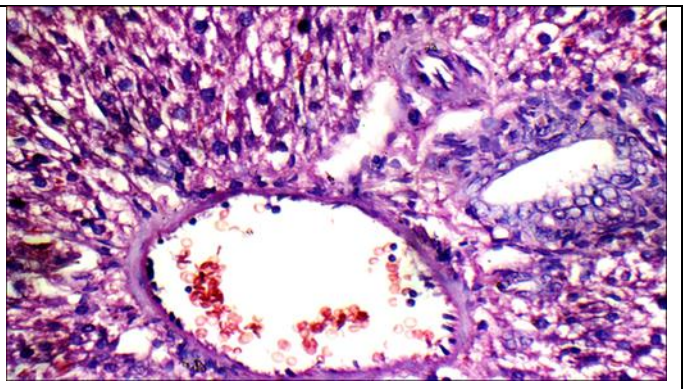

Figure 3b: Slide OX: high power view showing markedly edematous portal tract with markedly dilated congested (PV), average bile ducts, and hepatocytes with vacuolated cytoplasm in peri-portal area (H&E X 200).

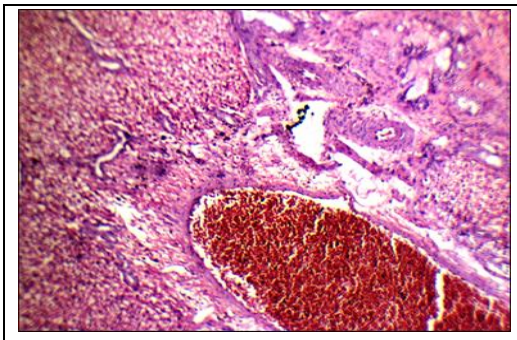

Figure 3c: (Group 6; OTC+NSET): Slide OE: showing mildly edematous portal tract with markedly dilated congested (PV), and average bile ducts (H&E X 200).

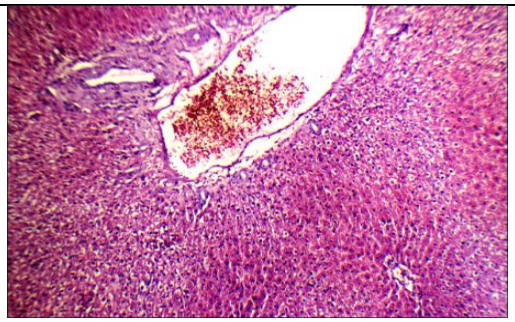

Figure 3d: (Group 5; OTC + NSO): Slide OO: liver showing portal tract with markedly dilated congested (PV), average bile ducts, and average central vein (H&E X 200).

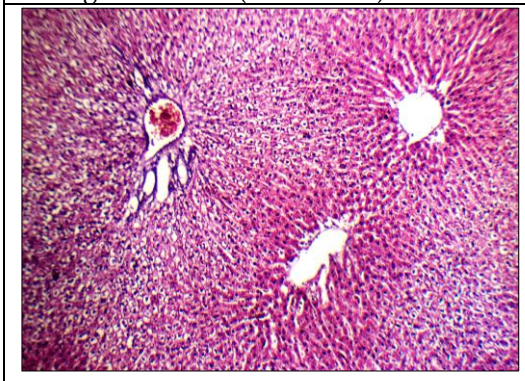

Figure 3e: (Group 3; NSO): Slide OI: liver showing average portal tract, average (CV) and average hepatocytes (H&E X 200).

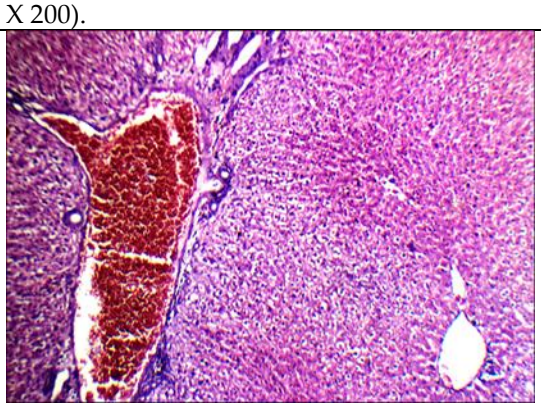

Figure 3f: (Group 2; NSET): Slide EX: liver showing portal tract with markedly dilated congested (PV), average bile ducts, average (CV), and average hepatocytes (H&E X 200).

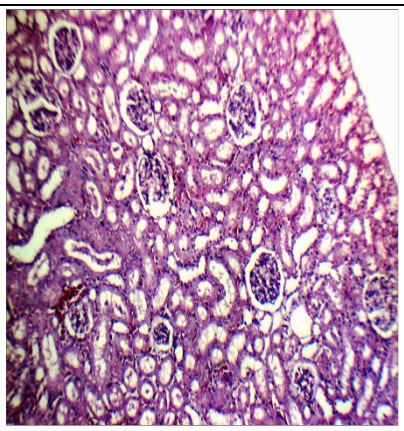

Figure 4a: (Group 1; Control): a kidney with a typical renal capsule, glomeruli of typical size, and tubules of typical size (H&E × 200).

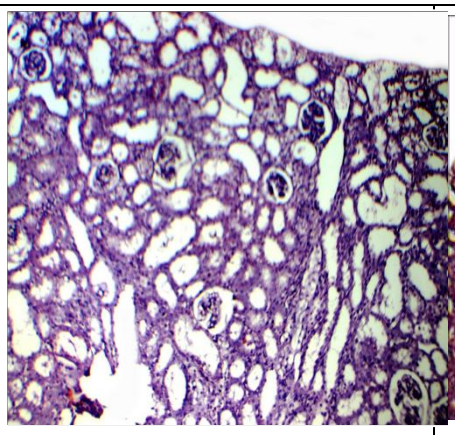

Figure 4b: (Group 2; OTC): Slide OX: kidney with a typical renal capsule, glomeruli of tiny size, and tubules that are significantly dilated (H&E × 200).

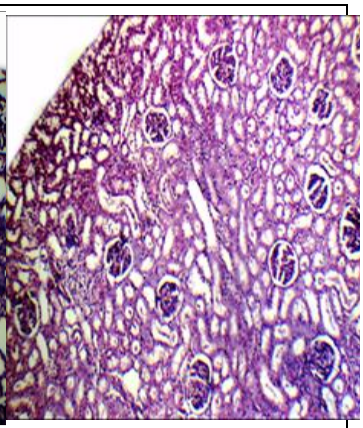

Figure 4c: (Group 3; OTC + NSET): Slide OE: a kidney with a typical renal capsule, glomeruli of typical size, and tubules of typical size (H&E × 200).

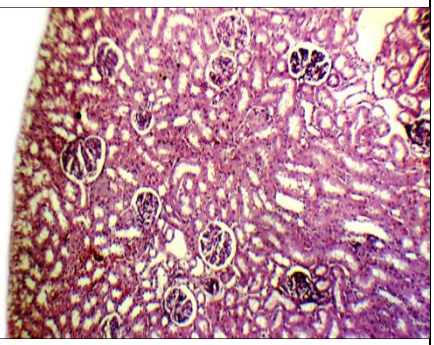

Figure 4d: (Group 4; OTC + NSO): Slide OO: a kidney with a typical renal capsule, glomeruli of typical size, and tubules of typical size (H&E × 200).

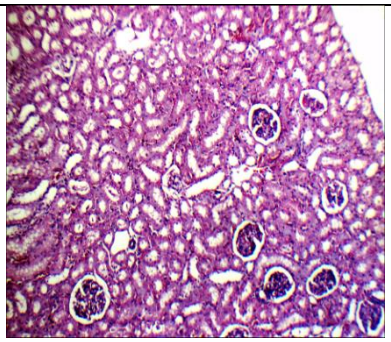

Figure 4e: (Group 5; NSO) Slide OI: a kidney with a typical renal capsule, glomeruli of typical size, and tubules of typical size (H&E × 200).

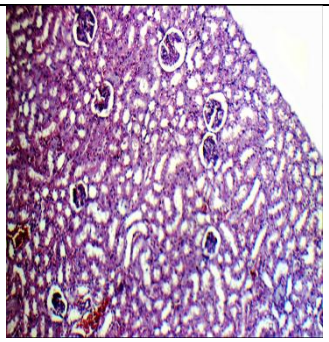

Figure 4f: (Group 6; NSET): Slide EX: a kidney with a typical renal capsule, glomeruli of typical size, tubules of typical size, and interstitial blood veins that are moderately dilated and congested (H&E ×200).

التأثير الوقائي لمستخلصات بذور حبة البركة ضد إصابات الكبد والكلى التي يسببها OCT في الفئران

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تهدف الدراسة للتعرف على التأثير الوقائي لمستخلصات بذور حبة البركة على الآثار الضارة الناتجة عن إصابات الفئران البيضاء نتيجة الحقن بمادة OTC. أظهرت نتائج الأحماض الدهنية باستخدام GLCوجود أحماض دهنية مشبعة و غير مشبعة وأن نسبة حمض اللينولييك كانت 58.31% الكاسح لظاهرة بيروكسيد الدهون. كما أظهر كروماتوجرام للزيت الطيار احتواءه على ست وعشرين مركباً من Terpenoids منها *p*-Cymene و α-Thujene و *trans-p*-Mentha-2,8-dien-1-ol بنسبة عالية. كما أظهر تقدير مكونات كل من المستخلص الإيثانولي والزيت من الفينولات الكلية والفلافونويدات الكلية وجودها بكميات معنوية وجميعها مركبات لها تأثير كاسح للتخلص من الشوارد الحرة كمضادات أكسدة، قُدرا أيضاً نسب تثبيط فحص النشاط المضاد للأكسدة على مستوى قياسات DPPH• , ABTS• مع حساب النسبة المئوية لتثبيط 50% من الشقوق الحرة التي أظهرت نتيجة معنوية لكلا المستخلصين في تجربة بيولوجية مدتها 28 يوماً لتقييم أثر المستخلصات على ذكور الفئران البيضاء في 6 مجموعات : مجموعة كونترول تغذية صحية فقط، مجموعة ثانية كنترول+ محقونة الغشاء البريتوني بمركب سام، مجموعة ثالثة ورابعة محقونة مع تجريعها مستخلص إيثانولي، وأخرى بمستخلص الزيت، المجموعة الخامسة والسادسة تناولتا المستخلصات فقط طول مدة التجربة حيث المستخلص الإيثانولي NSET ومستخلص الزيت NSO. أظهرت أوزان كل من كبد وكلى الفئران في نهاية التجربة مستويات مقاربة للكنترول للمجموعات المعاملة بالمستخلصات وزيادة في المجموعات المعاملة بالمركب السام مقارنة بالكنترول عند مستوى معنوية.($p\leq0.05$) كما أظهرت نتائج تحاليل وظائف كل من الكبد ALT, AST والكلى Urea, Creatinine زيادة معنوية للمجموعات المعاملة بالمركب السام أما مضادات الأكسدة الإنزيمية SOD,CAT,G-px أظهرت انخفاضاً معنوياً للمجموعة المعاملة بالمادة السامة على نقيض تلك المعاملة بالمستخلصات عند نفس مستوى المعنوية مقارنة بالكنترول.

الكلمات الاسترشادية: وظائف الكبد, وظائف الكلى, الاجهاد التأكسدى.