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Protective effect Ethanol extract of *Nigella sativa. L* on hepatic damage induced by naphthalene in male rats

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Abstract:-

Oxidative stress with subsequent production of reactive oxygen species has been postulated as one of the mechanisms of naphthalene toxicity . In the present study ,the effect of oral administration of the natural antioxidant and free radical scavenger , alcoholic extract of *Nigella sativa* (300mg/kg),has been investigated in rats following the concomitant administration of naphthalene (1g/kg)and measured of selective parameters indicative of liver function .Serum alanine aminotransferase (ALT), alkaline phosphates (ALP), and total protein(TP) . Naphthalene hepatotoxicity was evident by the significant elevation of rats serum activities of ALT,ALP, and significant depression of TP .on the other hand ,concurrent administration of alcoholic extract of *Nigella sativa* significantly attenuated the naphthalene-induced disturbances in serum liver function enzymes , and significant elevation of TP. In conclusion ,that alcoholic extract of *Nigella sativa* appears to be a potent candidate to ameliorate the oxidative stress and hepatotoxicity associated with naphthalene in rats and change in some biological markers related to liver disease.

Introduction:-

The bioactivated xenobiotics naphthalene is a pervasive environmental contaminant . It is one of the volatile aromatic hydrocarbons that are widely used commercially(22). Humans are exposed to naphthalene from a number of different sources including workplace exposures as in the aluminum smelting industry or where naphthalene is a starting material in the production of phthalic anhydride(11). It is used in the synthesis of dyes, resins, plastics, plus many pharmaceuticals including veterinary medicine, insect repellents, mothballs and toilet bowel deodorants (22,11). In addition, naphthalene is a natural constituent of coal tar and crude oil and has been identified in cigarette smoke and emission from fossil fuel combustion(23).

Naphthalene toxicity is highly species, tissue and cell selective(23). Naphthalene exposure is associated with several toxic manifestations in humans and laboratory animals, it has been found to cause cataract, hemolytic anemia, and damage of bronchial epithelial (Clara) cells and proximal tubules of the kidney (19,15).Moreover, naphthalene was involved in hepatocyte injury and liver dysfunction (19,20,26). Toxic manifestations of naphthalene have been attributed to oxidative stress caused by generation of reactive oxygen species (ROS) (19,2,17).

Human liver is capable of metabolizing naphthalene rapidly and efficiently into stable protein-reactive and cytotoxic metabolites, but if these metabolites are not rapidly detoxified by microsomal proteins and damage cell membrane and tissue macromolecules including DNA, proteins and lipids . Moreover, intracellular reduced glutathione was proven to provide a major detoxification process for naphthalene metabolites (20).

The world health organization estimated that 80% of the earth's inhabitants rely on traditional medicine for their primary health care needs, and most of this therapy involves the use of plant

extracts or their active components. Those plants and their components are perceived as "natural" and "safe" by consumers. A large number of these plants and their extract have shown beneficial effects to human health and disease prevention .Among the promising medicinal plants , *Nigella sativa*(commonly known as black seed) is consider as a biological response modifier. Thymoquinone is the bioactive and the most abundant constituent of the volatile oil of this seed which has been shown to possess therapeutic effects, including anti inflammatory ,antimicrobial , anticancer, antihypertensive , anti diabetic , and anti-oxidants agent (3) , Thymoquinone were found to be highly bio available providing significantly greater protection against free radical-induced lipid per oxidation and DNA damage(9).

Thus, many recent studies suggest that in-vivo and in-vitro *Nigella sativa* (thymoquinone) exposure may protect multiple organs from a variety of toxic assaults(4,8).

The liver is the main detoxifying organ in the body, and as such it possesses a high metabolic rate and it is subjected to many insults potentially causative of oxidative stress . Consequently, a correct status of the hepatic antioxidant defense system is of major importance for the maintenance of health. Therefore, the present study was designed to investigate the effects of oral administration of the environmental xenobiotics toxicant ,naphthalene induced hepatotoxicity .Additionally , this study examined whether the concomitant administration of the natural antioxidant ,*Nigella sativa* extract would protect rats from naphthalene –evoked disturbance in the selected liver parameters .

Material and methods:-

Identification of plant

Nigella sativa seeds were purchased from commercial sources .The seeds were botanically authenticated and its identification was confirmed by a specialist of plant taxonomy at the National Herbarium of Iraq Botany Directorate In Abu-Ghraib , under scientific name *Nigella sativa* .L .

Preparation of *Nigella sativa* seeds extract

The extraction of *Nigella sativa* seeds was prepared according to the procedure of (6) with special modification .The seeds were cleaned ,dried and powdered in an electrical grinder and stored at 5 Ċ until further use. Seed powder was extracted with a sufficient volume of 96% ethanol using Soxhlet extraction .Ethanol was evaporated at 40-50 Ċ under reduced pressure and the yield of extract was dissolved in water before use.

Animals

Thirty adult male albino Wister rats with a body weight (180-200gm) were used in this investigation . Their ages were ranged between (2.5-3) months. The animals were handled under standard laboratory conditions of a 12-hour light /dark cycle . Food and water available *ad libitum* along the experimental period.

Treatment

The animals were randomly divided into three groups (10 rats/groups) and were treated daily for 42 days as follows:. Group (A) untreated and served as control group , Group (B) the rats in this groups were orally intubated (by gavages needle) naphthalene (1g/kg B.W) (19) .Group (C) rats in this group were administered orally naphthalene (1g/kg) plus a (300mg/kg) of *Nigella sativa* seeds extract. Fasting blood samples were collected at zero, 21,42 days of experiment . Blood were drawn via cardiac puncture technique from anesthetized rats and the serum was used for the assay of serum alanine aminotransferase (ALT) activities , alkaline phosphates (ALP) activities and serum total protein concentration.

Statistical analysis

Data were performed on the basis of two way analysis of variance (ANOVA) using significant level of ($p<0.05$).Specific group differences were determined using least significant differences(LSD)(18).

Results:-

No statistical differences were observed between groups during the zero time of the experiment. Serum alanine amino transferase activity(ALT) significantly increase ($P<0.05$) in naphthalene treated group(B) at day 21 and 42 of the experiment comparing to alcoholic extract of *Nigella sativa* treated group (C) and control . At the end of the experiment (day 42) significant reduction ($P<0.05$) in (ALT) was observed after orally administration of alcoholic extract of *Nigella sativa* concurrently with naphthalene in group (C) comparing to group (B) that was treated with naphthalene alone. Within the time , the value of ALT tended to increase significantly ($P<0.05$) after the periods of 42 days in (B) as compared to the pretreatment period table (1) shows the result.

Table :(1) serum alanine amino transferase , ALT (IU/L)activity in male rats orally administered by naphthalene and ethanol extract of *Nigella sativa* compared with control group

Groups / Time (Days)	Group(A) Control	Group (B) Naphthalene	Group (C) naphthalene+ *Nigella sativa*
Zero	59.00±1.67 A a	58.80±1.80 A a	59.00±0.83 A a
21	58.80±1.46 A a	94.20±2.31 B b	79.00±3.49 C b
42	60.60±2.11 A a	107.60±4.20 B c	75.80±1.52 C b

Capital letters denote between group differences , (P<0.05).
Small letters denote within group differences , (P<0.05) .

The result of table (2) showed a significant increase ($P<0.05$) in serum ALP activity in B and C treated group at the 21 and 42 days of experiment as compared to control . However naphthalene intubation (B) caused significant elevation in this parameter at the end of the experiment comparing to the control and *Nigella sativa* seed treated groups. From the data pertaining in the table, alcoholic extract of *Nigella sativa* suppressed the elevation serum ALP activity at group C

(452.60±12.77) comparing to naphthalene (631.80±13.54) treated group at day 42 of the experiment.

Table :(2) Serum alkaline phosphatase ALP (IU/L) in male rats orally administered by naphthalene and ethanol extract of *Nigella sativa* compared with control group

Groups / Time (Days)	Group (A) Control	Group (B) Naphthalene	Group(C) naphthalene+ *Nigella sativa*
Zero	247.80±11.36 A a	249.40± 0.74 A a	248.20±0.37 A a
21	247.00±11.88 A a	583.20±19.56 B b	462.40±13.98 C b
42	246.81±5.48 A a	631.80±13.54 B c	452.60±12.77 C b

Capital letters denote between group differences , ($P<0.05$).
Small letters denote within group differences , ($P<0.05$) .

The concentration of total serum protein TSP (gm/dl) in rats after daily oral administration of naphthalene and alcoholic extract of *Nigella sativa* seed were shown in table (3). Statistical differences were absent between groups during pretreatment period ($P>0.05$). There was a clear reduction ($P<0.05$) in TSP concentration after 21 days of treatment in two treated groups (B,C) compared with the control group(A). Moreover, TSP concentration in rats treated with extract showed a significantly increased concentration ($P<0.05$) in day 42 group (C) (6.56±0.10) as compared to group (B) (4.66±0.08). Within the time significant reduction ($P > 0.05$) in mean value of TSP were observed in (B) and (C)treated groups comparing to the pretreated period.

Table:(3) Total serum protein concentration(gm/dl) in male rats orally administered by naphthalene and ethanol extract of *Nigella sativa* compared with control group.

Groups / Time (Days)	Group (A) Control	Group (B) Naphthalene	Group (C) naphthalene+ *Nigella sativa*
Zero	8.12±0.09 A a	8.40±0.05 A a	8.30±0.08 A a
21	8.04±0.16 A a	3.88± 0.12 B b	5.96±0.16 C b
42	8.14±0.12 A a	4.66±0.08 B c	6.56±0.10 C c

Capital letters denote between group differences , (P<0.05).
Small letters denote within group differences , (P<0.05) .

Discussion:-

The result of the present study revealed that rats intoxicated with naphthalene (1g / kg B.W) for 42 days , showed significant of liver injury as observed from significant increase in activities of liver enzyme (ALT,ALP), beside significant depression of TP. The elevation of serum liver enzyme table (1,2) is a reflection of radical mediated lipid per oxidation of liver cell membrane (12) . The liver produces a large amount of ALT, ALP which are secreted to the circulation with injury or death , where leakage enzyme escapes from the cytosol leading to a rise in the serum level of these enzymes(24)Mechanism of increased activity of ALT& ALP in serum include enzyme release from damaged cells or induction of enzyme activity (increased enzyme synthesis) from drug administration (5) .Besides, release of liver enzyme from cytosol can occur secondary to cellular necrosis with membrane damage(21). Moreover , increase in ALP level may be due to increase in lysosome activity that represent one of the important changes before cell death or may be disruption of bile flow either inside or outside hepatocyte this may lead to increase in ALP activity concentration in serum (14).

On the other hand, the result of present study show significant depression of TP due to naphthalene induced oxidative stress table (23) .Several studies have demonstrated that the pathological effects of naphthalene are mediated by induction of oxidative stress (19). As mentioned earlier, naphthalene generates reactive oxygen species, with subsequent decrease in serum protein concentration (17).

While when rats kept on oral intubation of *Nigella sativa* extract concurrently with naphthalene exerted significant hepatoprotective against naphthalene induce oxidative damage. The present study showed that the administration of alcoholic extract of *Nigella sativa* effectively improve liver function by lowering the activities of ALT, ALP (tables 1,2) . This result is in agreement with that reported by (16) in which they shows that administration of *Nigella sativa* L extract greatly attenuated the CCL4-elecited changes in the levels of liver biomarkers (ALT and

ALP) and significantly reduced lipid per oxidation , DNA fragmentation and depletion of glutathione contentents in hepatic tissues . Generally thymoquinone have been demonstrated to inhibit oxidative stress through modulation of metabolic functions, enhancement of detoxification pathways, and / or prevention of the interaction of xenobiotics with biological molecules (10). Thymoquinone the main compound of the essential oil inhibit non enzymatic lipid peroxidation in liposomes (7), have appreciable free radical scavenging properties (3) .

Thus suggesting that the alcoholic extract of *Nigella sativa* that protects the hepatocytes from liver damage and subsequent leakage of enzymes into the circulation , and may have acurative effect (decrease levels of enzyme markers). It has been shown that, *Nigella sativa* possesses antioxidant activity by way of free radical scavenging capacity and thus lowering lipid peroxidation (25).

The present study demonstrated that rats kept on oral intubation of alcoholic extract of *Nigella sativa* cause elevation in TSP (table 3). *Nigella .Sativa* seeds contain 36%–38% fixed oils, proteins, alkaloids, saponin and 0.4%–2.5% essential oil has been found to increase the level of total serum protein through stimulation of protein synthesis , accelerates the regeneration process and the production of liver cells (13). Beside, *Nigella sativa* causes suppression of gluconeogensis and prevents catabolic protein and conversion to glucose (1), and this may lead to increase level of total serum protein concentration in serum.

Therefore, the *Nigella sativa* extract offers vast possibilities in the treatment of various liver disorders. This may be due to the high level of antioxidant, which was claimed to be the mechanism of hepatoprotection .In conclusion, it is plausible to suggest that naphthalene may trigger the production of ROS. Coupled with impaired oxidant/antioxidant balance leading to hepatotoxicity, and the disturbance in the level of hepatic enzymes. The extract of *Nigella sativa* exhibited a beneficial effect as a natural hepatoprotectant and attenuate the liver cells apoptosis.

References:-

1. AL-GABY, A. M. (1998). Amino acid composition biological effects of supplementing broad bean and corn proteins with *Nigella sativa* (Black cumin) cake protein. *Nahrung*; 42 : 290-294.

2. Bagchi,D.; Balmoori,J.; Bagchi,M.; Williams,C.B. and Stohs,S.J. (2005). Comparative effects of TCDD, endrine, naphthalene and chromium (VI) on oxidative stress and tissue damage in the liver and brain tissues of mice.Toxicol ;175: 73-82.

3. Burits, M. and Bucar,F.(2000). Antioxidant activity of *Nigella sativa* essential oil .Phytotherapy Research ; 14: 323-328.

4. Daba, M.H.and Abdul-Rahman .M.S.(1998). Hepatoprotective activity of thymoquinone in isolated rat hepatocytes.Toxicol Lett ;95:23-29.

5. Dufour, D.R. ; Lott,J.A,; Nolte,F.S.; Gretch,D.R. ; Koff, R.S. and Seeff, L.B. (2000). Diagnosis and monitoring of hepatic injury: II. Recommendations for use of labrotory tests Clin Chem ., 46 : 2050-68.

6. Hadjzadeh,M.A.R.; Khoei,A.; Hadjzadeh,Z. and Parizady,M.(2007). Ethanolic extract of *Nigella sativa*. L seed on ethylene glycol-induced kidney calculi in rats. Urol.J; 4(2):86-90.

7. Houghton,P.J.;Zarka,R.; Heras,B. and Hoult,R.S.(1995). Fixed oil of *Nigella sativa* and derived thymoquinone inhibit eicosanoid generation in leakocytes and membrane lipid peroxidation .Planta.Med;61:33-36.

8. Mansour ,M.A.; Ginawi, O.T.; El-Hadiyah ,T.; El-Khatib, A.S.; Al-Shabanah, O.A. and Al-Sawaf , H.A.(2001). Effects of volatile oil constituents of *Nigella sativa* on carbon tetrachloride-induced hepatotoxicity in mice: evidence for antioxidant effects of thymoquinone . Res Commun Mol Pathol Pharmacol ;110:239-251.

9. Mansour, M.A.; Nagi, M.N.; Al-Khatib,A.S. and Al-Bekairi,A.M.(2002).Effects of thymoquinone on antioxidant enzyme activities .lipid peroxidation and DT diaphorase in different tissues of mice: a possible mechanism of action cell. Biochem Funct;20; 143-151.

10. Nagi, N.K.; Alam, O.A.; Badary, O.A. ;al-Shabanah, H.A.; al-Sawaf and A.M. al-Bekairy.(1999). Thymoquinone protects against carbon tetracholide hepatotoxicity in mice via an antioxidant mechanism. Biochem. Mol. Biol. Int. 47(1): 153–159

11. Preuss,R.;Angerer,J.and Drexler,H.(2003). Naphthalene-an environmental and occupational toxicant . Int Arch Ocupp Environ Health ; 76(8): 556-576.

12. Ray, SD.;Kumar,MA.; and Bagchi, D.(1999).A novel proanthocyanidin IH636 grape seed extract increases in vivo Bcl- Xl expression and prevents ace taminophen-included programmed and unprogrammed cell death in mouse liver. Arch Biochem Biophys., 369:42-58.

13. SALEM, M. L. (2005). Immunomodulatroy and therapeutic properties of the *Nigella sativa* L. seed. *Int. Immunopharmacol*; 5 : 1749-1770.

14. Sastry, K.V. and Agrawal, M.K. (1997).Cadmium chloride induced enzymological changesbin kidney and overy of ateleost fish channa pimctatus,Bull. Environ. Contain.Toxicol.,48:11-20.

15. Schreiner,C.A.(2003). Genetic toxicity of naphthalene : a review .J Toxicol Environ Health ; 6: 161-183.

16. Seckin,D. and Ilhan,N.(2005).Protective effect of *Nigella sativa* seeds on CCL4- induced hepatotoxicity.F.U.Saglik Bil.Dergisi; 19(3): 175-179

17. Shi, H.;Yunxia,S.; Wange,X.; Luo,Y. and Ji,L.(2005).Hydroxyl radical production and oxidative damage induced by cadmium and naphthalene in liver of Carassius auratus. Comp Biochem Physiol C;140:115-121.

18. Steel,R.G. and Torries,J.H.(1980). Principles and Procedures of Stastisticas. A biometrical approach, 2nd edition. McGraw-Hill Book Co. New York, USA .

19. Stohs,S.J.; Ohia,S. and Bagchi,D.(2002). Naphthalene toxicity and antioxidant nutrients. Toxicol ; 180: 97-105.

20. Tingle,M.D.; Primohamed,M.; Templeton,E.; Wilson,A.S.; Madden,S.; Kitteringham,N.R. and Park ,B.K.(1993). An investigation of the formation of cytotoxic , genotoxic, protein-reactive and stable metabolites from naphthalene by human liver microsomes. Biochem Pharmacol ;46 : 1529-1538.

21. Valentine, B.A.; Blue, J.T.; Shelley, S.M.;and Cooper, B.J.(1990). Increase serum alanine amino transferase activity muscle necrosis in the dog. J Vet Intern Med.;4:140-143.

22. West,J.A.A.;Pakehham,G.;Morin,D.;Fleschner,C.A.;Buckpitt,A.R.and Plopper, C.G. (2001) .Inhaled naphthalene causes dose dependent clara cell cytotoxicity in mice but not in rats. Toxicol Appl pharmacol ;173: 114-119.

23. Willems,B.A.; Melnick,R.L.; Kohn,M.C. and Portier,C.J.(2001). A physiological based pharmacokinetic model for inhalation and intravenous administration of naphthalene in rats and mice. Toxicol Appl pharmacol ;176:81-91.

24. Wolf, PL.(1999). Biochemical diagnosis of liver disease. Indian J Clin Biochem .,100:59-90.

25. Yildiz,F.;Coban,S.;Terzi,A.;Ates,M.;Aksoy,N.;Cakir,H.;Ocak,A.;and Bitiren, M. (2008).*Nigella sativa* relieves the deleterious effects of ischemia reperfusion injury on liver.World J Gastroenterol;14(33):5204-5209.

26. Zhao,W. and Ramos,K.(1998). Cytotoxic response profiles of cultured rats hepatocytes to selected aromatic hydrocarbons.Toxicol In Vitro;12:175-182.

التأثير الوقائي لمستخلص الحبة السوداء على التلف الكبدي المستحدث بالنافثالين في ذكور الجرذان

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الخلاصة :-

إن الإجهاد ألتأكسدي الذي يعقبه إنتاج الأوكسجين النشط هو احد آليات سمية مادة النافثالين . وفي هذا البحث تم دراسة تأثير الإعطاء الفموي لمضاد الأكسدة الطبيعي وكاسح الجذور الحرة،المستخلص الكحولي للحبة السوداء (300ملغم/كغم) بعد الإعطاء المترافق لمادة النافثالين (1غ/كغم) للجرذان ثم بعد ذلك قياس المؤشرات الدالة على وظيفة الكبد . ولهذا تم قياس مستوى الأنزيم الناقل للامينALT , وتركيز أنزيم الفوسفاتيز القاعدي ALP ، وتركيز البروتين . لقد ثبت بصورة جلية تأثير مادة النافثالين السام للكبد متمثلا في الارتفاع المعنوي لمستويات أنزيمات الكبد المذكورة أعلاه والانخفاض المعنوي لتركيز البروتين . إن الإعطاء المترافق للمستخلص الكحولي للحبة السوداء اضعف بشكل كبير الاضطرابات التي أحدثها النافثالين في أنزيمات الكبد وأحدث ارتفاع معنوي في تركيز البروتين . يمكن أن نستنتج بآن هذه الدراسة قد أظهرت أن المستخلص الكحولي للحبة السوداء هو احد المواد المرشحة القوية لتحسين الإجهاد ألتأكسدي والتسمم الكبدي المصاحب النافثالين في الجرذان في بعض المعايير الكيموحيوية التي تعد مؤشرا لوظيفة الكبد.