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A STUDY TO COMPARE THE HEPATOPROTECTIVE ROLE OF NIGELLA SATIVA AQUEOUS SOLUTION AND OIL SUSPENSION FOLLOWING ACETAMINOPHEN INDUCED HEPATOTOXICITY IN LAYER CHICKS

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ABSTRACT

This investigation is performed to compare the reported hepatoprotective activity of *Nigella sativa* (Black Seed) aqueous solution and oil suspension by measuring the mean serum Aspartate transaminase (AST) and Alanine transaminase (ALT) levels following the administration of single toxic dose of 300 mg acetaminophen / kg body weight in Layer chicks. Blood samples drawn after 72 hours of drug administration showed a non significant rise of AST and ALT in the chickens treated with *Nigella sativa* aqueous solution and unaltered levels of serum AST and non significant high levels of ALT in the chickens treated with *Nigella sativa* oil suspension for 15 days, in comparison to controls. Thus it is concluded that *Nigella sativa* oil effectively provides hepatoprotection against toxic agents due to its active ingredient thymoquinone with antioxidant property, and there is a need to explore the minimum effective dose of *Nigella sativa* oil.

KEYWORDS: Hepatoprotection, Layer chickens, Lliver enzymes, *Nigella sativa.*

INTRODUCTION

The seeds of *Nigella sativa* [(*N. sativa*) belonging to family Ranunculaceae], commonly known as Black Seed have long been used for the treatment of a wide range of diseases (Tariq 2008). The research conducted over the last five decades to investigate chemical and

pharmacological properties of *N. sativa* showed that Black Seeds contain fixed oils, proteins, alkaloids, saponin and essential oil (Ghosheh et al., 1999). The results of these extensive studies demonstrated the *N. sativa* to have anthelmintic (Akhtar and Riffat, 1991), histamine release inhibitor (Chakravarty, 1993), postcoital contraceptive (Keshri et al., 1995), antilipemic (Bashandy, 1996), antidiabetic (Hassanim and Hassan, 1996; Al-Lugmani and Zari, 2011), hepatoprotective (Daba and Abdel- Rahman, 1998; El- Dakhakhny et al., 2000), antimicrobial against a wide range of organisms (Sokmen et al., 1999; Justine and Yusuf, 2008), analgesic (Abdel- Fattah et al., 2000), diuretic and antihypertensive (Zaoui et al., 2000), bronchodilator and calcium antagonist (Gilani et al., 2001), antifungal (Khan et al., 2003), thus justifying the traditional therapeutic use of *N. sativa* (Randhawa, 2008).

Acetaminophen (Paracetamol) is a commonly used analgesic and antipyretic drug. It is usually safe when administered at therapeutic doses, however at overdoses through the cytochrome P-450 pathway, acetaminophen is converted to a highly toxic metabolite, N-acetyl-p- benzoquinone imine (NAPQI) that has a potential to cause hepatic necrosis and nephrotoxicity in both humans and animals (Madhuri and Bhandarkar, 2010; Yaman et al., 2011). In the recent years a lot of research in different experimental models treated with *N. sativa* showed a reduction in liver enzymes activities after experimentally induced liver injuries thus demonstrating the hepatoprotective role of *N. sativa* (Anwar et al., 2012; Gani and John, 2013; Hamed et al., 2013; Ragab et al., 2013). The objective of this study was to investigate and compare the hepatoprotective role of *N. sativa* aqueous solution and oil suspension by measuring the liver function marker enzymes Aspartate transaminase (AST) and Alanine transaminase (ALT) in layer chicks.

MATERIALS AND METHODS

60, one day old unvaccinated chicks, weighing approximately 44.4gm ± 2.5g, were brought from the local chicks hatchery in Karachi, Pakistan, and maintained at the Poultry Farm of the Department of Physiology, University of Karachi, Pakistan. Birds were fed on commercial diet (Table- 1) and water *ad libitum*.

Drug and Dosimetry

To induce hepatotoxicity in chickens acetaminophen (Panadol 500 mg tablet, Batch # AFP 17/1-07/2010), was purchased from local pharmacy. The oral dose calculated to administer the experimental birds was 300 mg / kg body weight. *N. sativa* aqueous solution and oil

suspension was orally administered daily in doses of 0.5mg and 0.2ml / bird to respective treatment groups.

Experimental Design

All chicks were equally divided into four groups, I, II, III and IV with 15 birds each. Groups I and II were kept as controls (C1, C2) fed on commercial diet. Whereas treated groups III and IV, along with diet were administrated orally *N. sativa* aqueous solution and oil suspension respectively from day 1 to day 15. On day 15 all chicks belonging to groups II, III and IV were given a single toxic dose of acetaminophen to induce liver injury.

Blood Sampling

Blood samples were drawn by cardiac puncture (Krista et al., 1988) on day 1, 15, 17 and 25.To obtain serum, whole blood samples were kept at room temperature (25°C), clear serum in the form of supernatant was then transferred to eppendrof tubes to be used immediately for biochemical analysis of enzymes AST and ALT.

Biochemical Analysis

Biochemical analysis of serum AST and ALT was done by using commercially available biochemical kits (Global biochemical kits, UK). Absorbance was read on spectrophotometer (Model NV201). Data was analyzed by t-test and statistical significance was considered at $p<0.05$.

RESULTS

AST: The consideration of Table-2 indicates a non significant rise ($p = 0.0640$) in the mean value of serum AST concentration of untreated group i.e. 6.009±3.21 U/L to 11.160±0.25 U/L from day 1 to day 25 respectively. Whereas the mean AST value of treated group II on day 15 was 9.114±1.31 U/L, after the administration of acetaminophen a significant increase ($p <0.05$) of 12.568±2.11 U/L and 13.2±1.79 U/L were observed on day 17 and day 25 respectively.

The chicks of group III, treated with *N. sativa* aqueous solution showed a non significant increase ($p = 0.8305$) in mean serum AST level from day 1 to day 15, and with a single dose of acetaminophen a non significant increase ($p = 0.4790$) from 6.533±2.34 to 7.754±1.37 U/L was observed from day 15 to day 17, which further non significantly decreased ($p = 0.1354$) to 3.674±3.53 U/L on day 25.

Group IV birds treated with *N. sativa* oil, from day 1 to day 15 also showed a non significant rise (p=0.9129) in mean serum AST level of 6.009±3.21 U/L to 6.241±1.27 U/L respectively. After the administration of acetaminophen mean serum AST value remained unaffected, which further reduced significantly ($p<0.05$) to 3.122±0.67 U/L on day 25.

ALT: The mean serum ALT values of control group I given in Table-3 showed a non significant increase ($p = 0.0663$) from 3.277±1.22 U/L to 5.327±0.72 U/L on day 1 to day 25 respectively. The chicks of group II also showed a non significant increase ($p = 0.5986$) in ALT levels from day 1 to day 15 i.e. 3.277±1.22 U/L to 4.848±1.23 U/L respectively. After the administration of acetaminophen, a significant rise ($p<0.05$) in ALT concentration of 7.073±2.10 U/L and 7.636±3.49 U/L were observed on day 17 and day 25 respectively.

The chicks of group III treated with *N. sativa* aqueous solution, after the administration of acetaminophen, also showed a non significant rise ($p = 0.5537$) of mean serum ALT levels from day 15 to day 17 i.e. 4.643±2.86 U/L to 6.196±3.03 U/L respectively, however on day 25 the ALT level non significantly reduced ($p = 0.8444$) to 5.69±2.89 U/L.

In group IV chicks treated with *N. sativa* oil, a non significant increase ($p = 0.8276$) of mean serum ALT level was observed from day 1 to day 15. On day 17 following the administration of acetaminophen the values of ALT level further increased non significantly ($p = 0.6866$) from 4.662±0.97 U/L to 5.093±2.32 U/L. On day 25 the mean ALT level was 3.777±1.82 U/L, indicating a non significant reduction ($p = 0.1291$).

Table 1: Composition of feed for layer chicks.

Nutrient	Units	Starter
Metabolizable Energy	Kcal / Kg	3000 – 3100
Crude Protein (min)	%	23
Crude Fat	%	5.6
Linoleic Acid	%	1.2
Salt	%	0.25 – 0.40
Calcium	%	0.95 – 1.00
Phosphorus available	%	0.40 – 0.45
Sodium	%	0.18
Chloride	%	0.20
Magnesium	%	0.06
Potassium	%	0.70

Note: All nutrients except Metabolizable Energy are % of total feed contents

Table 2: Comparison of mean serum AST concentrations (U/L) of control and treated chicks groups.

Days	Groups			
	I	II	III	IV
1	6.009±3.21	6.009±3.21	6.009±3.21	6.009±3.21
15	10.751 ± 0.15^{b1}	9.114 ± 1.31^{b1}	6.533 ± 2.34^{b1}	6.241 ± 1.27^{b1}
17	11.123 ± 4.6^{b1}	12.568 ± 2.11^{a1}	7.754 ± 1.37^{b1}	6.241±2.04
25	11.160 ± 0.25^{b1}	13.2 ± 1.79^{a1}	3.674 ± 3.53^{b2}	3.122 ± 0.67^{a2}

Each value is the mean ± SD of 15 observations. Subscripts ^{a1} and ^{a2} indicate significant increase and decrease respectively ($p< 0.05$); ^{b1} and ^{b2} indicate non significant increase and decrease respectively.

I = control 1 **II** = control 2 (Acetaminophen treated)

III = *N. sativa* (aqueous solution) treated **IV** = *N. sativa* (oil suspension) treated

Table 3: Comparison of mean serum ALT concentrations (U/L) of control and treated chicks groups.

Days		Groups		
	I	II	III	IV
1	3.277 ± 1.22^{b1}	3.277±1.22	3.277±1.22	3.277±1.22
15	4.743 ± 0.87^{b1}	4.848 ± 1.23^{b1}	4.643 ± 2.86^{b1}	4.662 ± 0.97^{b1}
17	4.950 ± 0.66^{b1}	7.073 ± 2.10^{a1}	6.196 ± 3.03^{b1}	5.093 ± 2.32^{b1}
25	5.327 ± 0.72^{b1}	7.636 ± 3.49^{a1}	5.69 ± 2.89^{b2}	3.777 ± 1.82^{b2}

Each value is the mean ± SD of 15 observations. Subscripts ^{a1} and ^{a2} indicate significant increase and decrease respectively ($p< 0.05$); ^{b1}and ^{b2} indicate non significant increase and decrease respectively.

I = control 1 **II** = control 2 (Acetaminophen treated)

III = *N. sativa* (aqueous solution) treated **IV** = *N. sativa* (oil suspension) treated

DISCUSSION

Liver is susceptible to metabolites that could produce direct toxicity or there may be possibility of immunological reaction by drug itself or else by its active metabolite (Kaplowitz, 2002), thus hepatic necrosis is the result of failure of glutathione pathway to detoxify the highly reactive metabolite of P-450 (Kwan et al., 1995; Michael et al., 1999; Han et al 2006; Malhi et al 2006; Ghosh et al., 2010; Hinson et al., 2010). With the advancement of new methods to detect the liver pathology, still the accurate assessment of serum liver enzymes can provide useful information about the extent and severity of liver damage (Ramaiah, 2007).On the whole serum AST and ALT levels are low, but these enzymes are

released into circulation following cellular damage and elevate because they are cytoplasmic in location (Al-Kubaisy and Al-Noaemi, 2007). As far as the enzymatic activities in birds are concerned relatively little information is available and the normal physiological values for enzymes had to be obtained (Mc Daniel et al., 1964; Hochleithner, 1994).

In contrast to the findings of Mc Daniel and Chute (1961), suggesting unaffected enzyme levels in growing birds and the work by El- Toukhy et al., 1989, indicating a reduction in AST and ALT concentrations at first 2 weeks of age; the present study showed a non significant rise of mean serum AST and ALT levels from day 1 to day 15 in chickens of all untreated and treated groups. However the increased AST levels during the early period of experiment are in agreement with the observations of Woodard et al. (1983) and Kudair and Al-Hussary (2010), where non-vaccinated growing birds showed a rise in serum AST levels. Age dependent elevated levels of ALT in birds had also been reported by Gylstorff and Grimm (1987). The significant high levels of serum transaminases followed by the administration of acetaminophen of group II chickens are indicative of impaired liver function. It could be explained by the work of Dixon et al. (1975), who in rats demonstrated a graded correlation between acetaminophen induced hepatic necrosis and serum transaminases within 24 hours to 72 hours. However, group III chickens, after the administration of acetaminophen showed a non significant high mean serum AST and ALT concentrations which later decreased non significantly. Whereas, unaltered AST concentration and non significant high level of ALT were observed in group IV chickens.

Many Studies suggested the antioxidant role of *N. sativa* seeds and its constituents (Nagi et al., 1999; Burits and Bucar, 2000; Al-Naqeeb et al., 2009; Ashraf et al., 2011; Leong et al., 2013). *N. sativa* oil contains thymoquinone as the main constituent of essential oil with strong antioxidant property. A significant reduction in serum liver enzymes activities has been demonstrated in experimental models, previously treated with *N. sativa* oil and followed by experimentally induced hepatic injuries, indicating a hepatoprotective role of thymoquinone through its antioxidant property (El- Dakhakhny et al., 2000; Mahmoud et al., 2002; Alenzi et al., 2010). However higher doses of thymoquinone were found to be lethal resulting in hepatic necrosis via oxidative stress (Mansour et al., 2001).

In the present study statistical analysis showed that *N. sativa* oil effectively maintained the concentrations of serum AST and ALT in chickens after acetaminophen administration thus providing hepatoprotection against expected injury. These findings are supported by similar

studies where *N. sativa* oil in chicks showed positive effects on serum urea and uric acid concentrations indicating hepato-renal protection (Khan et al., 2013). However, there is a need to work out the minimum effective dose of *N. sativa* oil to achieve hepatoprotection in chickens against various toxic agents.

REFERENCES

1. Abdel-Fattah AM, Marsumoto K and Watanabe H. Antinociceptive effects of *Nigella sativa* oil and its major component, thymoquinone, in mice. European Journal of Pharmacology, 2000; 400: 89-97.
2. Akhtar MS and Riffat S. Field trial of Saussurea lappa roots against nematodes and *Nigella sativa* seeds against cestodes in children. Journal of Pakistan Medical Association, 1991; 41: 185-187.
3. Alenzi FQ, El-Bolkiny YS and Salem ML. Protective effects of *Nigella sativa* oil and thymoquinone against toxicity induced by the anticancer drug cyclophosphamide. British Journal of Biomedical Sciences, 2010; 67(1): 20-28.
4. Al-Kubaisy K and Al-Noaemi M. A protective role of *Nigella sativa* oil against the harmful effect of CCl4 on the liver cells. The International Journal of Nutrition and Wellness, 2007; 3(1): 1-6.
5. Al-Lugmani A and Zari T. Long-term effects of *Nigella sativa L.* oil on some physiological parameters in normal and streptozotocin-induced diabetic rats. Journal of Diabetes Mellitus, 2011; 1: 46-53.
6. Al-Naqeeb G, Ismail M and Al-Zubairi AS. Fatty acid profile, α-Tocopherol content and antioxidant activity of oil extract from *Nigella sativa* seeds. International Journal of Pharmacology, 2009; 5(4): 244-250.
7. Anwar SH, Ahmed JH and Sawsan AS. A study of the effect of *Nigella sativa* (Black seeds) in isoniazid (INH) - induced hepatotoxicity in rabbits. Indian Journal of Pharmacology, 2012; 44(6): 678-682.
8. Ashraf SS, Rao MV, Kaneez FS, Qadri S, Al-Marzouqi AH, Chandranath IS and Adem A. *Nigella sativa* extract as a potent antioxidant for petrochemical-induced oxidative stress. Journal of Chromatographic Science, 2011; 49: 321-326.
9. Bashandy SA. Effects of *Nigella sativa* oil on liver and kidney functions of adult and senile rats. Egyptian Journal of Pharmaceutical Sciences, 1996; 37: 313-327.
10. Burits M and Bucar F. Antioxidant activity of *Nigella sativa* essential oil. Phytotherapy Research, 2000; 14: 323-328.

11. Chakravarty N. Inhibition of histamine release from mast cells by nigellone. Annals of Allergy, 1993; 70: 237-242.
12. Daba MH and Abdel-Rahman MS. Hepatoprotective activity of thymoquinone in isolated rat hepatocytes. Toxicology Letters, 1998; 95: 23-29.
13. Dixon MF, Fulker MJ, Walker BE, Kelleher J and Losowsky MS. Serum transaminase levels after experimental paracetamol-induced hepatic necrosis. Gut, 1975; 16(10): 800-807.
14. El-Dakhakhny M, Mady NI and Halim MA. *Nigella sativa L*. oil protects against induced hepatotoxicity and improves serum lipid profile in rats. Arzeimittelforschung, 2000; 50(9): 832-836.
15. El-Toukhy N, Aly SA and Soliman MK. Physiological studies on the level of some electrolytes and enzymes in normal and Newcastle vaccinated chicks. Assiut Veterinary Medical Journal, 1989; 21(42): 7-15.
16. Gani AMS and John SA. Evaluation of hepatoprotective effect of *Nigella sativa L*. International Journal of Pharmacy and Pharmaceutical Sciences, 2013; 5(4): 428-430.
17. Ghosh J, Das J, Manna P and Sil PC. Acetaminophen induced renal injury via oxidative stress and TNF-alpha production: therapeutic potential of arjunolic acid. Toxicology, 2010; 268: 8-18.
18. Ghosheh OA, Houdi AA and Crooks PA. High performance liquid chromatographic analysis of the pharmacologically active quinones and related compounds in the oil of the black seed (*Nigella sativa L.*). Journal of Pharmaceutical and Biomedical Analysis, 1999; 19(5): 757-762.
19. Gilani AH, Aziz N, Khurram IM, Chaudhary KS and Iqbal A. Bronchodilator, spasmolytic and calcium antagonist activities of *Nigella sativa* seeds (Kalonji): A traditional herbal product with multiple medicinal uses. Journal of Pakistan Medical Association, 2001; 51: 115-120.
20. Gylstorff I and Grimm F. Vogelkrank-heiten. Stuttgart: Verlag. Eugen, Ulmer, 1987; 133-146.
21. Hamed MA, El-Rigal NS and Ali SA. Effects of black seed oil on resolution of hepato - renal toxicity induced by bromobenzene in rats. European Review for Medical and Pharmacological Sciences, 2013; 17(5): 569-581.
22. Han D, Hanawa N, Saberi B and Kaplowitz N. Mechanism of liver injury.III. Role of glutathione redox status in liver injury. American Journal of Physiology. Gastrointestinal and Liver Physiology, 2006; 291: 1-7.

23. Hassanim NI and Hassan FM. A preliminary study on the effect of *Nigella sativa* seeds on hypoglycemia. Veterinary Medical Journal Giza, 1996; 44: 699-708.
24. Hinson JA, Roberts DW and James LP. Mechanism of acetaminophen-induced liver necrosis. Handbook of Experimental Pharmacology, 2010; 196: 369-405.
25. Hochleithner M. Biochemistries. In: Avian medicine: Principles and Application (Ritchie BW, Harrison GJ and Harrison LR eds.) Wingers Publishing, Inc. Florida, USA. 1994; 223-245.
26. Justine TE and Yusuf OK. Some biochemical and haematological effects of black seed (*Nigella sativa*) oil on *T. brucei*-infected rats. African Journal of Biomedical Research, 2008; 11: 79-85.
27. Kaplowitz N. Biochemical and cellular mechanism of toxic liver injury. Liver Diseases, 2002; 22: 137-144.
28. Keshri G, Singh MM, Lakshmi V and. Kamboj VP. Post-coital contraceptive efficacy of the seeds of *Nigella sativa* in rats. Indian Journal of Physiology and Pharmacology, 1995; 39: 59-62.
29. Khan MA, Ashfaq MK, Zuberi HS, Mahmood MS and Gilani AH. The *in vivo* antifungal activity of the aqueous extract from *Nigella sativa* seeds. Phytotherapy Research, 2003; 17: 183-186.
30. Khan TA, Khan MN, Hasan R, Fatima H and Kousar E. Effects of *Nigella sativa* (Black Seed) on serum levels of urea and uric acid in acetaminophen induced hepatotoxicity of commercial layer chickens. Journal of World's Poultry Research, 2013; 3(4): 89- 92.
31. Krista LM, Pedersoli WM, Spano J and Hebert R. Techniques for blood sampling in avian species. Veterinary and human toxicology. 1988; 30(1): 14- 20.
32. Kudair IM and Al-Hussary NAJ. Effect of vaccination on some biochemical parameters in broiler chickens. Iraqi Journal of Veterinary Sciences, 2010; 24(2): 59-64.
33. Kwan D, Bartle WR and Walker SE. Abnormal serum transaminases following therapeutic doses of acetaminophen in the absence of known risk factors. Digestive Diseases Sciences, 1995; 40(9): 1951-1955.
34. Leong FX, Mustafa MR and Jaarin K. *Nigella sativa* and its protective role in oxidative stress and hypertension. Evidence-Based Complementary and Alternative Medicine, 2013; (Article ID 120732).
35. Madhuri H and Bhandarkar AG. Histopathological changes in experimental paracetamol toxicity in poultry. Royal Veterinary Journal of India, 2010; 6(1): 23-25.

36. Mahmoud MR, El-Abhar HS and Saleh S. The effect of *Nigella sativa* oil against the liver damage induced by Schistosoma mansoni infection in mice. Journal of Ethnopharma cology, 2002; 79(1): 1-11.
37. Malhi H, Gores GJ and Lemasters JJ. Apoptosis and necrosis in the liver: A tale of two deaths? Hepatology, 2006; 4: 31-44.
38. Mansour MA, Ginawi OT, El-Hadiyah T, El-Khatib AS, Al-Shabanah OA and Al Sawaf HA. Effects of volatile oil constituents of *Nigella sativa* on carbon tetrachloride induced hepatotoxicity in mice: evidence for antioxidant effects of thymoquinone. Research Communications in Molecular Pathology and Pharmacology, 2001; 110(3-4): 239-251.
39. Mc Daniel LS and Chute HL. Enzyme activity levels in chicken plasma. American Journal of Veterinary Research, 1961; 22: 99-103.
40. Mc Daniel LS, Dempsey HA and Chute HL. Enzyme levels in birds. Bulletin T8, Technical Series. The Maine Agricultural Experiment Station, University of Maine, Orono, Maine. 1964.
41. Michael SL, Pumford NR, Mayeux PR. Niesman MR and Hinson JA. Pretreatment of mice with macrophage inactivators decreases acetaminophen hepatotoxicity and the formation of reactive oxygen and nitrogen species. Hepatology, 1999; 30(1): 186-195.
42. Nagi MN, Alam K, Badary OA, Al-Shababah OA, Al-Sawaf HA and Al-Bekairy AM. Thymoquinone protects against carbon tetrachloride hepatotoxicity in mice via an antioxidant mechanism. Biochemistry and Molecular Biology International, 1999; 47(1): 153-159.
43. Ragab AA, El-Reidy KFA and Gaafar HMA. Effect of pumpkin (*Cucurbita moschata*) and black seeds (*Nigella sativa*) oils on performance of rabbits: growth performance, blood hematology and carcass traits of growing rabbits. American Journal of Research Communication, 2013; 1-12.
44. Ramaiah SK. A toxicological guide to the diagnostic interpretation of hepatic biochemical parameters. Food and Chemical Toxicology, 2007; 45(9): 1551-1557.
45. Randhawa MA. Black seed, *Nigella sativa*, deserves more attention. Journal of Ayub Medical College Abbottabad, 2008; 20(2): 1-2.
46. Sokmen A, Jones BM and Erturk M. The *in vitro* antibacterial activity of Turkish medicinal plants. Journal of Ethnopharmacology, 1999; 67: 79-86.
47. Tariq M. *Nigella sativa* seeds: Folklore treatment in modern day medicine. Saudi Journal of Gastroenterology, 2008; 14(3): 105-106.

48. Woodard AE, Bohra P and Mayeda B. Blood parameters of 1year old and seven years old partridges (*Alectoris chukar*). Poultry Science, 1983; 2492-2496.
49. Yaman H, Isbilir S, Cakir E and Uysal B. Current issues with paracetamol induced toxicity. Journal of Experimental and Integrative Medicine, 2011; 1(3): 165-166.
50. Zaoui A, Cherrah Y, Dubois MAL, Settaf A, Amarouch H and Hassar M. Diuretic and hypotensive effects of *Nigella sativa* in the spontaneously hypertensive rat. Therapie, 2000; 55:379-382.