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

Ruqaiya Hasan1,3*, Taseer A. Khan2, Muhammad N. Khan2, and Aiman Kanwal3

1Department of Physiology, Faculty of Medicine, Umm Al-Qura University, Makkah, KSA.
2Poultry Research Laboratory, Department of Physiology, University of Karachi -75270. Pakistan.
3Hematology Unit, Department of Physiology, University of Karachi -75270. Pakistan.

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, Liver 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 eppendorf 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.**

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

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.

<table>
<thead>
<tr>
<th>Days</th>
<th>Groups</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>6.009±3.21</td>
<td>6.009±3.21</td>
<td>6.009±3.21</td>
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</tr>
<tr>
<td>15</td>
<td></td>
<td>10.751±0.15₁</td>
<td>9.114±1.31₁</td>
<td>6.533±2.34₁</td>
<td>6.241±1.27₁</td>
</tr>
<tr>
<td>25</td>
<td></td>
<td>11.160±0.25₁</td>
<td>13.2±1.79₁</td>
<td>3.674±3.53₂</td>
<td>3.122±0.67₂</td>
</tr>
</tbody>
</table>

Each value is the mean ± SD of 15 observations. Subscripts ₁ and ₂ indicate significant increase and decrease respectively (p< 0.05); ₁ and ₂ 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.

<table>
<thead>
<tr>
<th>Days</th>
<th>Groups</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>3.277±1.22₁</td>
<td>3.277±1.22₁</td>
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<tr>
<td>15</td>
<td></td>
<td>4.743±0.87₁</td>
<td>4.848±1.23₁</td>
<td>4.643±2.86₁</td>
<td>4.662±0.97₁</td>
</tr>
<tr>
<td>17</td>
<td></td>
<td>4.950±0.66₁</td>
<td>7.073±2.10₁</td>
<td>6.196±3.03₁</td>
<td>5.093±2.32₁</td>
</tr>
<tr>
<td>25</td>
<td></td>
<td>5.327±0.72₁</td>
<td>7.636±3.49₁</td>
<td>5.69±2.89₂</td>
<td>3.777±1.82₂</td>
</tr>
</tbody>
</table>

Each value is the mean ± SD of 15 observations. Subscripts ₁ and ₂ indicate significant increase and decrease respectively (p< 0.05); ₁ and ₂ 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 (McDaniel et al., 1964; Hochleithner, 1994).

In contrast to the findings of McDaniel 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.

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