



Effects of *Nigella sativa* (Black Seed) on Serum Levels of Urea and Uric Acid in Acetaminophen Induced Hepatotoxicity of Commercial Layer Chickens

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ABSTRACT

Acetaminophen is an analgesic and antipyretic agent administered in high doses causes kidney and liver necrosis both in humans and in animals. *Nigella sativa* (Black seed) has been reported to have hepatoprotective and nephroprotective properties. Present investigation is performed to find out the effects of aqueous solution of *Nigella sativa* and its oil extract on serum urea and uric acid levels of layer chicks after oral administration of a single dose of 300 mg acetaminophen/kg body weight. Observations indicated that oral administration of acetaminophen to layer chicks caused significant ($p < 0.05$) decrease in mean serum urea while non-significant decrease in mean serum uric acid concentration. The results of this study indicated that *Nigella sativa* has positive effects on urea and uric acid concentrations during the administration of acetaminophen overdose. However, *Nigella sativa* oil extract is more effective than its aqueous solution.

Key words: Acetaminophen, Hepatoprotective, Layer Chicks, *N. sativa* (Black seed).

INTRODUCTION

Herbal supplements use has been increased greatly over the past three decades because conventional medicinal plants are locally available, cheaper and easy to consume (Amin and Nagy, 2009). Nowadays use of medicinal herbs is the best solution to cure diseases in a 100% natural way as compare to other toxicants and unhealthy products (Al-Attar and Wafa'a, 2010).

Nigella sativa (Black seed) plant belongs to the family Ranunculaceae and is most extensively investigated for therapeutic purposes (Aggarwal et al., 2008). Pharmacological studies on *N. sativa* found analgesic, bronchodilator, hypolipidemic, anti-tumor, diuretic and immunopotentiator, hypotensive, calcium antagonist, antidiabetic, histamine release inhibitor, antioxidant, hepatoprotective, anthelmintic, antifungal, antibacterial, anticancer, and anti-inflammatory activities (Al-Logmani and Zari, 2011). The active constituents of black seed include nigellidine, thymoquinone, nigellone, nigellidine, thymoquinone, nigellimine-N-oxide, dithymoquinone, thymol, arvacrol, oxy-coumarin, alpha-hedrin, steryl-glucoside as well as large amounts of flavinoids,

tannins, essential fatty acids, 15 essential amino acids, ascorbic acid, iron and calcium (Gad et al., 1963; Atta et al., 1985, 1985a; Atta et al., 1995; Kumara and Huat, 2001). *N. sativa* seeds aqueous extract has been studied both in vitro and in vivo. An oral administration to rats (2g/kg) of the aqueous extract has shown a significant protection against increase gastric juice secretion induced by aspirin, acid output and gastric ulcers (Akhtar et al., 1996). A significant increase in insulin release was observed following the exposure of the cells from isolated rat pancreatic islets of Langerhan to *N. sativa* aqueous extracts or their basic aqueous sub fractions in the concentrations up to 5 mg/ml (Rchid et al., 2004). It has also been reported that birds fed on *N. sativa* tend to have a higher body mass than others (Amr et al., 2005).

Paracetamol (Acetaminophen) is an analgesic and antipyretic drug and is commonly used in headaches, muscles aches, arthritis, toothaches, cold and fever (Granberg and Rasmuson, 1999). Acetaminophen toxicity appeared when stored form of cellular glutathione decreases and large amount of acetaminophen is metabolized by cytochrome p-450

system which produces more N Acetyl-p-benzo-quinone imine (NAPQI). This NAPQI reacts with the cellular protein and membrane thus damaging the cell membrane and leads to cell death especially of hepatocytes. Acetaminophen toxicity may occur either due to single large toxic dose or repeated doses of acetaminophen e.g. 7.5 – 10g daily for 1 - 2 days (McEvoy, 2007). Acetaminophen also produced hepatocytes toxicity in chicks (Lindenthal et al., 1993). Acetaminophen overdose was the primary cause of acute liver failure in Western countries (Stravitz and Kramer, 2009). The chronic use of acetaminophen resulted in an increased chance of developing papillary necrosis, chronic renal failure or end-stage renal disease (O'Grady, 2007).

The purpose of this study was to observe the effects of aqueous solution and oil extract of *N. sativa* on serum urea and uric acid levels of acetaminophen induced hepatotoxicity in layer chicks as chickens have been widely used to investigate several important biomedical problems in non-mammalian vertebrate systems.

MATERIALS AND METHODS

One day old, sixty chicks, weighing approximately $44.4g \pm 2.5g$, unvaccinated were brought from local chicken hatchery in Karachi, Pakistan; maintained at the Poultry Farm of the Department of Physiology, University of Karachi, Pakistan and provided with feed (Table 1) and water ad libitum.

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

* All nutrients except Metabolizable Energy are the percentage of total feed contents.

All chicks were divided randomly into 4 groups A, B, C and D, each containing 15 birds. Prior to treatment, on first day blood was drawn from all chicks; serum was separated and immediately stored at -86°C for biochemical investigations. Group A and B were kept as controls (C1; C2) while birds in group C and D were orally administered daily with *N. sativa* aqueous solution and its oil extract in doses of 0.5 mg and 0.2 ml /bird respectively, from first day till the end of experiment. On day 15, blood samples were again drawn from all groups' chicks. Then birds of groups B, C and D were orally administered with a single dose of 300 mg / Kg body weight acetaminophen (Panadol 500 mg tablet, Batch # AFP 17/1-07/2010, purchased from local pharmacy) to induce acute hepatotoxicity. After 48 hours of drug administration i.e. on day 17 blood

was drawn from all 4 groups for biochemical estimations of serum urea and uric acid. Final blood sampling was done on day 25 to obtain serum for biochemical estimations.

Commercially available biochemical kits (Global biochemical kits, UK) were used to measure serum urea and serum uric acid concentrations. Absorbances were read through spectrophotometer (Model NV202, Madell Technology Co, USA). Data was subjected to statistical analysis by student's t-test and the level of statistical significance was set at $p < 0.05$.

RESULTS

Urea

The mean values of serum urea of both controls and experimental groups are presented in Table 2. It was observed that chicks in group A showed a significant ($p < 0.05$) rise of urea concentration from first day to day 25 with mean values of 29.73 ± 1.63 and 47.30 ± 0.46 mg/dl respectively. However, chicks in group B and C showed a significant ($p < 0.05$) rise of mean serum urea concentration from first day to day 15, whereas a non-significant ($p = 0.4471$) decrease in urea concentration was observed in test group D during this period.

Following the oral administration of single toxic dose of acetaminophen to the chicks of groups B, C and D at day 15, blood samples of B and C groups exhibited a significant ($p < 0.05$) decrease of mean serum urea concentration on day 17. However a non-significant ($p = 0.0534$) rise of urea level was observed in group D chicks. The chicks of group B and group C on day 25 showed a significant ($p < 0.05$) and non-significant ($p = 0.0611$) rise of mean serum urea concentrations respectively, whereas in group D chicks a non-significant ($p = 0.3855$) fall in mean serum urea level was observed.

Uric Acid

The mean values of serum uric acid of both controls and experimental groups are presented in Table 3. The serum uric acid concentration of birds in group A showed a non-significant decrease from first day to day 25, with mean values of 6.72 ± 0.09 and 6.53 ± 0.30 mg/dl respectively. However, the chicks of groups B and C had a non-significant ($p = 0.1804$; $p = 0.5560$) reduction in the mean serum uric acid concentration from first day to day 15 but the chicks of group D during this period had a non-significant ($p = 0.4182$) rise in mean serum uric acid concentration.

On day 17 after the oral administration of a single toxic dose of acetaminophen to groups B, C and D, a non-significant ($p = 0.4509$; $p = 0.0905$) reduction in mean serum uric acid levels of groups B and D chicks was observed, however chicks of group C showed a non-significant ($p = 0.0865$) increase for the same parameter.

The results on mean serum uric acid concentration obtained on day 25 from the chicks of group B showed a non-significant ($p = 0.7395$) rise while a significant ($p < 0.05$) reduction in mean serum uric acid was observed in groups C and D chicks.

Table 2. Comparison of mean serum urea concentrations (mg/dl) of control and treated chicks groups

Days	Groups			
	A	B	C	D
1	29.73±1.63	29.73±1.63	29.73±1.63	29.73±1.63
15	44.07±4.60 ^a	44.07±4.60 ^a	44.43±2.50 ^a	28.67±1.45
17	46.66±0.15 ^a	22.83±1.83 ^b	27.44±1.05 ^b	30.79±0.78
25	47.30±0.46 ^a	30.36±1.27 ^a	31.30±2.37	29.11±3.53

Each value is the mean ± SD of 15 observations. A: control 1; B: control 2 (Acetaminophen treated); C: *N. sativa* (aqueous solution) treated; D: *N. sativa* (oil extract) treated. ^a significant increase (p<0.05) within groups. ^b significant decrease (p<0.05) within groups.

Table 3. Comparison of mean serum uric acid concentrations (mg/dl) of control and treated chicks groups

Days	Groups			
	A	B	C	D
1	6.72±0.09	6.72±0.09	6.72±0.09	6.72±0.09
15	6.26±0.48	6.25±0.48	6.36±0.96	6.91±0.36
17	6.30±0.36	5.79±0.83	7.94±1.22	6.05±0.63
25	6.53±0.30	6.07±1.13	5.88±0.25 ^b	4.50±0.54 ^b

Each value is the mean ± SD of 15 observations. A: control 1; B: control 2 (Acetaminophen treated); C: *N. sativa* (aqueous solution) treated; D: *N. sativa* (oil extract) treated. ^b significant decrease (p<0.05) within groups.

DISCUSSION

The present investigation indicates that oral administration of acetaminophen to layer chicks caused a significant increase in serum urea (Table 2) and a non-significant decrease in serum uric acid (Table 3) concentrations. These results are in agreement with various previous researches which indicated that the exposure to acetaminophen leads to nephrotoxicity (Newton et al., 1986; Richie et al., 1992).

Acetaminophen is a commonly used analgesic and antipyretic agent which, at high doses, causes liver and kidney necrosis in man and animals (Palani et al., 2011). The highly significant (p<0.05) decrease in serum urea concentration of acetaminophen treated chicks may be due to the increase of serum protein, decrease in the rate of circulating amino acids and deamination takes place that consequently leads to the formation of less amount of ammonia which is eventually converted to urea (Al-Logmani and Zari, 2011). Uric acid in birds is the main end-product of nitrogen metabolism. Its presence in tissues reflects the extent of dietary protein, nutritional state or direction of protein metabolism (Bell et al., 1959). It has also been seen that the plasma uric acid of chickens was influenced by sex, age, nutrition and reproductive status. Biochemical studies show that there is reduction in the levels of uric acid in acetaminophen induced groups (Palani et al., 2011).

Pharmacologically *N. sativa* seeds (and some of its active constituents, e.g. volatile oil and thymoquinone) have been reported to provide protection against nephrotoxicity and hepatotoxicity induced by some toxins or anti-cancer drugs (Ali and Blunden, 2003). In the present study, the *N. sativa* aqueous solution and *N. sativa* oil extract administration shows hepatoprotective and nephroprotective effects on acetaminophen induced liver and renal injuries to chicks. *N. sativa* aqueous solution administered chicks showed significantly less liver and kidney necrosis as compare to control animals while *N. sativa* oil extract treated group showed comparatively greater resistance against hepatic and

renal injuries which is evident by almost normal concentrations of serum urea and uric acid.

It is concluded that *N. sativa* (Black seed) has positive effects on urea and uric acid concentrations during administration of acetaminophen over dosage, (Amr et al., 2005); however, its oil extract is more effective than aqueous solution.

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