Comparative hepatoprotective effect of *Nigella sativa* pre- and post-treatment to rabbits

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Abstract: Synthetic drugs are associated with adverse side-effects and rapid increase in resistance to most of them inspires to evaluate plants for their therapeutic values. We have been aimed to suggest the medicinal use of *Nigella sativa* seed aqueous extract to minimize the severity of liver damage via its antioxidant properties and its role in maintenance of cell ion-homeostasis. Annoyances in serum levels of some antioxidants and trace metals in human hepatitis C infected patients were compared with that from acetaminophen-induced hepatotoxic rabbits. Serum analysis of human patients and that of hepatotoxic rabbits have exhibited the same trend of incidence of liver marker enzymes, antioxidant levels, and trace metal concentrations, except for the serum levels of cobalt. Significance of pre-/ or post-treatment of *Nigella sativa* to acetaminophen induced-hepatotoxic rabbit has also evaluated. NS post-treatment to rabbits has been found effective in normalizing the levels ($P<0.001$) of serum liver markers; especially the ALP levels, and the antioxidants; with significant effect on the serum catalase levels. However, NS pre-treatment has shown its role ($P<0.001$) in maintaining the serum nickel and cobalt concentrations. Therefore, we suggest the use of *Nigella sativa* seeds as pre-/ or post-treatment therapy, and also as supplement to the normal medications of liver infection to normalize the status of cell antioxidants and trace metal concentrations.

Keywords: Black cumin, hepatotoxicity, hepatitis C, trace metals, antioxidants.

INTRODUCTION

Hepatotoxicity in its acute form causes liver cancer (Wolf *et al.*, 1999). Acute liver injury (ALI) leads to extensive damage of the hepatocellular tissue, with reduced cell mass and blood flow. It is associated with increased serum alanine aminotansferase (SALT), serum aspartate aminotansferase (SAST), total serum bilirubin (TSB) and serum alkalanine phosphatase (SALP) levels than normal (Sabate *et al*., 2007). However, immune system itself is not enough to remedy (Heydtmann *et al*., 2001). Attempts have been made evaluating the hepatoprotective effects of natural plant preparations to develop effective phytochemical agents. Despite the relatively little knowledge regarding their modes of action (Matthews, 1999), about 80% of the world's population use plant-based remedies as their primary form of healthcare (WHO, 2011).

Nigella sativa (NS) (Black cumin) is among the herbs with therapeutic potentials against liver damage. Several studies have confirmed that *N. sativa* extracts and the main constituent of seed oil, thymoquinone, possess antioxidant, anti-inflammatory and hepatoprotective properties with some specific mechanisms of action, which support the suggestion to consider it an emerging natural drug (Khader and Eckl, 2014). Although the underlying mechanism is not well defined, the antioxidant, anti-inflammatory and anti-angiogenesis properties of NS may play a role in this effect (Ali *et al*., 2003). The main cause of liver diseases is considered to be oxidative stress (Paradis *et al.*, 1997). Lipid peroxy radicals lead to increased cell membrane permeability, decreased cell membrane fluidity, inactivation of membrane proteins and loss of polarity of mitochondrial membranes. Among the cell's antioxidants are enzymatic and non enzymatic metabolites that scavenge the oxidants, produced during various cell metabolic activities, to protect cell membrane (Abdel-Daim, 2014). Recently, *N. sativa* and thymoquinone has been found entailed in reducing the oxidative stress through its antioxidant effects and free-radical scavenging activity against liver fibrosis in rats (Saricicek *et al*., 2014).

The nutritional significance of the trace metals has been widely recognized including their role as constituent components of many metal proteins and metalloenzymes. Stress (*e.g.* disease) conditions may cause annoyance in concentrations of metabolites present naturally. Therefore, the serum levels of trace metals have been frequently reported as good marker for diagnosis of various diseases (Ferner, 2001). In drug-induced hepatotoxicity each cell may be the target of drug toxicity so it may cause chronic

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hepatitis like symptoms (Zimmerman, 2000). Therefore, acetaminophen, commonly called paracetamol, was used to observe the disorder in the levels of antioxidants and trace metal concentrations in rabbits to evaluate the effect of *N. sativa* seed aqueous extract treatment on the status of some biochemical attributes to investigate the mechanism of its possible therapeutic role.

MATERIALS AND METHODS

Chemicals

All the chemicals used in the study were of analytical grade. The levels of serum enzymes were determined by Serum enzyme kits based on the manufacturer's instructions using autoanalyzer (Leica, Japan). The serum Alanine Aminotransferase (ALT) and serum Aspartate Transaminase (AST) assays were performed using commercially available kits by Analyticon Biotechnologies, Germany. The assay for serum Alkaline Phosphates (ALP) was performed using commercially available alkaline phosphatase-kit by Merck diagnostics, France. Serum Albumin was determined using commercially available albumin-kit by proDia international diagnostics UAE by modified Bromocresol Green Method. Chemicals for antioxidant enzymes and trace metal analysis were purchased from sigma, and working solutions were maintained at °C in a refrigerator.

Preparation of Nigella sativa seed crude extract

The crude aqueous extract of *N. sativa* seeds was prepared following method by Sheriff *et al.* (2015) with small modifications. *Nigella sativa* seeds were washed dried in an oven at a temperature of 40°C and ground with blender and finally a fine powder was obtained through mortar and pastel. The *N. sativa* seed powder (15.25 mg/Kg/bw; ~11 black cumin seeds) (Sahih al-Bukhari) was dissolved in sterilized distilled water (1 mL) and left for 24hours. Then the extracts were filtered using Whatman filter paper (125 mm). The extract was orally administered by gavage daily to experimental animals for 12 days.

Serum biochemical assays

Liver marker enzymes, the antioxidant and the metal ion status were analyzed both in human patients and in animal model, rabbits. The enzyme antioxidant; catalase activity was measured spectrophotometrically (SHIMADZU, Japan) in sera samples using method described by Goth (1991). The concentration of non enzymatic antioxidant ascorbic acid (vitamin C) was estimated through HPLC according to Cerhata *et al.* (1994) and total bilirubin levels were measured by Jendrassik and Grof analysis following method by Garber, 1981. For estimation of some trace; iron, nickel and cobalt metals, the sample and different standard preparations were carried out following method by Rashed *et al.* (2010). The concentrations of trace metals (ppm) were measured by Atomic Absorption Spectrophotometer (SHIMADZU, Japan).

Experimental procedure

Thirty human patients including 21 males and 9 females ranging between 20-70 years with chronic hepatitis C infections were screened from District Head Quarter Hospital, Sargodha, Pakistan, to be included in this study for their sera antioxidant and metal ion status. For animal model; four animal groups, comprising five rabbits each were selected to analyze the possible therapeutic effect of *N. sativa* seed aqueous extract pre-/ and post-treatments on altered antioxidant and metal ion status of all treatment groups. Eighteen male local domestic rabbits with an average weight of 900-1000 g were purchased from the market. The animals were housed in large spacious cages at the animal house of the Department of Pharmacy, University of Sargodha, Pakistan. The animals were kept and maintained on standard laboratory condition of room temperature, humidity and under twelve hours dark–light cycle. They were fed with standard pellet diet and water. The animals were allowed to acclimatize to their new condition for two weeks before the commencement of the experiment.

In-vivo studies

The experiment was carried out following the instructions by "Principles of Laboratory Animal Care" (NIH publication # 85-23, revised in 1985). The study was performed by allocating the animals randomly to four groups of five animals each as follows;

Group I served as control (fed on normal diet).

Group II received Paracetamol (acetaminophen) 300 mg/Kg/bw (IM, at 72-hour intervals for 12 days) (Makoshi *et al.*, 2013)

Group III (pre-treated) rabbits were fed orally the *Nigella sativa* seeds crude extract (15.25 mg/Kg/bw) for 12 days. The animals were then subjected to liver damage, induced by paracetamol (IM, at 72-hour intervals for 12 days).

Group IV (post-treated) rabbits were injected with paracetamol (IM, at 72-hour intervals, 12 days) to induce liver damage. The animals were then fed with *Nigella sativa* seeds crude extract (15.25mg/Kg/bw) for 12 days.

After two weeks, the blood was collected from the jugular vein of the animals under deep anesthesia with chloroform. The serum was separated at 2500 rpm for 15 minutes, for the biochemical analysis.

STATISTICAL ANALYSIS

Data are presented as mean ± standard error of means. For establishing significant differences between the groups, the data were analyzed by paired sample t-test. Values were considered statistically significant if P value is less than 0.05 ($p<0.05$), using SPSS (Statistical Package for Social Sciences) software, version 17.0.

RESULTS

Assessment of some serum biochemical parameters in hepatitis C affected patients

Serum liver enzymes have been considered important diagnostic tools for liver diseases. In present study, the mean serum liver enzyme levels in human patients were found to be significantly increased in comparison to the control group (table 1). Increase in mean serum ALT level was significant (P<0.001) in hepatitis C patients, however, the highly significant (P<0.001) increase was noted in the mean serum ALP level from serum of hepatitis C patients. Among the antioxidants; a highly significant (P<0.001) decrease in catalase activity was noted in serum from hepatitis C patients compared to the control group. And also noted the significantly (P<0.001) decreased values for serum bilirubin in patients, as compared to the control group. Accordingly, the mean serum ascorbic acid level was also found decreased significantly (P<0.001) in hepatitis C patients as compared to the control groups, as depicted in table 1.

Evaluations of some trace metals status in sera from hepatitis c patients

The highly significant (P<0.001) decrease in mean serum nickel concentration was observed in hepatitis C patients when compared to healthy (normal) persons, along with a comparatively less significant decrease in mean serum cobalt concentration (fig. 1). However, some increase in mean serum iron concentration was observed in hepatitis C patients (fig. 1).

Assessment of some biochemical parameters in rabbits with acetaminophen-induced hepatotoxicity

The study of any herbal drug becomes more significant when it ameliorates the disease condition or reduces the side effects related to therapeutic drug. In the present investigation, the hepatoprotective effect of *N. sativa* crude aqueous extract was studied following the observations based on acetaminophen-induced liver damage in rabbits.

Status of some serum liver markers and antioxidants

We have found the significant increase in serum ALP (P<0.001) and AST (P<0.01) levels of rabbits after acetaminophen administration to rabbits (table 2). Non significant (P>0.05) changes have been noted in serum ALT, total protein and albumin levels. Highly significant (P<0.001) alterations have been noted in serum levels of ALP after the drug administration, like the serum values for hepatitis C patients. *Nigella sativa* pre treatment to rabbits (*N. sativa*+acetaminophen) has not shown any positive effect in reducing the serum AST or ALP levels compared to that of the acetaminophen administered rabbits. However, a remarkable (P<0.001) decrease in the serum ALT, AST and ALP levels has been noted from serum of *N. sativa* post-treated (acetaminophen+*N. sativa*) rabbits, even lowered than the control values. Comparatively less or negligible effect of *N. sativa* pre- and post-treatment was observed on the serum total protein and serum albumin levels in studied groups (table 2).

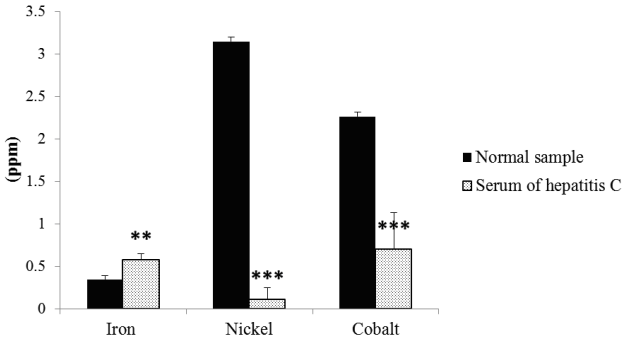

Fig. 1: Status of some trace metals in sera from hepatitis C patients: the mean serum iron concentration was increased in serum from hepatitis C patients as compared to the normal individuals. However, the mean serum nickel and cobalt concentrations were found decreased in hepatitis C patients, with significant reduction noted for mean serum nickel concentrations. (Hepatitis C patients vs normal significant at *=0.05, **=0.01, and***=0.001).

In present study the effect of *N. sativa* was investigated on serum antioxidants including; catalase, ascorbic acid and bilirubin. Serum catalase enzyme activity was found reduced following acetaminophen injection to diabetic group compared to the control group. However, the administration of crude aqueous extract of *N. sativa*, as pre- and post-treatment, has shown profound role in restoring the decreased activity of catalase induced by

Table1: Status of some serum liver markers and antioxidants in hepatitis C patients

Biochemical attributes	Normal sample	Hepatitis C patient
ALT(IU/L)	29.20±0.80	188.00±1.79***
AST(IU/L)	33.20±2.20	164.40±2.69***
ALP (IU/L)	95.80±8.65	744.20±14.74***
Bilirubin (mg/dL)	0.58±0.13	0.17±0.02***
Catalase (kU/L)	0.35±0.32	0.09±0.11***
Ascorbic acid (mg/dL)	0.27±0.12	0.07±0.05***

Hepatitis C vs Normal, Data is expressed as Mean ± SEM significant at (P<0.05-P<0.001; significant at *=0.05, **=0.01, and***=0.001).

Table 2: The effects of *N. sativa* pre- and post-treatments on status of serum liver markers and antioxidants of rabbits from different study groups

Biochemical attributes	G-I (Control)	G-II (acetaminophen)	G-III; N.S Pre treatment (*N. sativa*+acetaminophen)	G-IV; N.S Post treatment (acetaminophen+*N. sativa*)
ALT (IU/L)	78.00±1.15	77.50±1.31[a]	91.16±1.11[b,**]	46.00±0.93[c,***]
AST (IU/L)	63.83±2.39	75.33±2.92[a,**]	82.83±2.02[b,*]	39.66±3.29[c,***]
ALP (IU/L)	63.83±2.39	186.00±2.44[a,***]	144.00±3.39[b,***]	39.66±3.29[c,***]
Total protein (mg/dL)	6.00±0.00	6.16±0.17[a]	6.5±0.22[b]	7.16±0.17[c,*]
Albumin(mg/dL)	3.24±0.21	3.3±0.19[a]	2.82±0.16[b]	3.54±0.17[c]
Catalase (kU/L)	79.01±06.18	47.20±4.31[a,***]	152.20±12.77[b,***]	71.20±2.44[c,***]
Ascorbic acid (mg/dL)	10.0±63.0	0.29±0.01[a,**]	1.58±0.01[b,**]	1.58±0.01[c,**]
Bilirubin (mg/dL)	30.0±24.0	0.90±0.07[a,***]	1.56±0.09[b,**]	1.60±0.07[c]

Data is expressed as Mean ± SEM significant at (P<0.05-P<0.001), vs Normal, where *=0.05, **=0.01, and***=0.001.
[a] Group II is compared with Group I
[b] Group III is compared with Group II
[c] Group IV is compared with Group II

acetaminophen injection. Highly significant (P<0.001) changes in catalase levels were observed in *N. sativa* pre-treated group (G-III) that has shown even higher catalase levels than the normal group.

Among the non-enzymatic antioxidants; ascorbic acid and bilirubin, we have found the significant effect of *N. sativa* treatment on the ascorbic acid status. The acetaminophen treatment has resulted in a significant (P<0.01) decrease in ascorbic acid levels as exhibited by the CHC patients. Both the *N. sativa* pre- and post-treatments have increased the ascorbic acid levels at the same extent (P<0.01) in the toxic groups. And almost similar trend was noticed for the serum bilirubin levels.

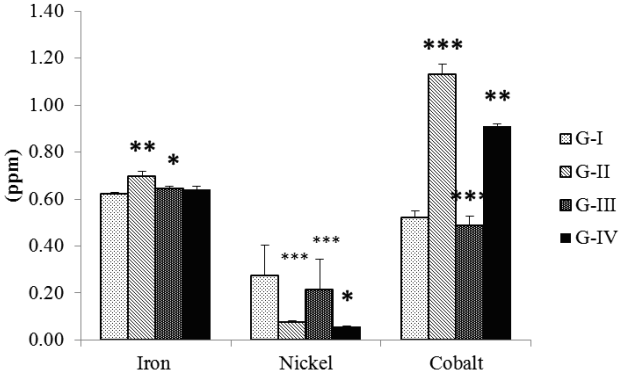

Fig. 2: Effects of *N. sativa* seed extract administration on status of some serum trace metals of rabbits from different study groups: Both the NS pre- and post-treatments (GIII and GIV, respectively) have noted equally effective in normalizing the elevated levels of serum iron concentrations of acetaminophen induced hepatotoxic rabbits (GII). However, the NS pre-treatment (GIII) has showed profound effect in retuning back to the normal values of increased serum cobalt and decreased serum nickel concentrations. (Hepatitis C patients vs Normal, significant at *=0.05, **=0.01, and***=0.001. Where Group II is compared with Group I, while Group III and IV are compared with Group II).

Status of some serum trace metals

Our findings have shown that acetaminophen has induced a significant (P<0.01) increase in mean serum iron concentration in rabbits compared to the normal group (fig. 2); as has been noted for the hepatitis C patients. Both the *N. sativa* pre- and post- treatments tend to normalize the iron concentrations, comparable to the normal values. Like iron, the mean serum cobalt concentration has also been found increased in acetaminophen-induced hepatotoxic rabbits, contrary to the levels found in serum of hepatitis C patients. However, this increase was very significant (P<0.001) as compared to that of iron. The *N. sativa* pre-treatment has found significant in reducing the elevated cobalt concentration, as compared to the NS post-treatment (fig. 2). The mean serum nickel concentration has been reduced significantly (P<0.001) upon administration of acetaminophen as compared to the normal animals, analogous to that found for hepatitis C patients. Again the *N. sativa* pre-treatment has been found very significant (P<0.001) in returning the nickel concentration, back to the normal values (fig. 2).

DISCUSSION

Annoyances in the levels of serum liver markers and the status of antioxidants and some trace metals

The exact mechanism of chronic viral hepatitis B and C pathology is not well known. We have been aimed to suggest medicinal use of seeds of *N. sativa* to overcome the consequences of the disease, primarily the oxidative (Mollazadeh and Hosseinzadeh, 2014), following analysis of its effects on status of some liver markers, antioxidants and the concentrations of some trace metals in the serum samples. The serum analysis of hepatitis C patients has shown the significant (P<0.001) increased levels of ALT and ALP levels in comparison to the values for control group. However, a highly significant (P<0.001) decrease in catalase activity, decreased values for serum bilirubin, and the decreased levels of ascorbic acid were noted in hepatitis C patients. However, El-Kannishy *et al*. (2012)

have found the increased serum bilirubin levels along with the elevation of the aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels in chronic hepatitis C patients as compared to the control group ($P<0.05$), due to the outflow of metabolites and proteins from cells to serum. They also demonstrated the significantly reduced levels of water-soluble ascorbic acid in CHC patients. It has been reported that ascorbic acid may stimulate α-tocopherol (Vitamin E) activities and the deficiency of vitamins C and/or E has enhanced the oxidative stress (Engin, 2009). Such vitamins act as antioxidant and maintain the eicosapentaenoic acid contents in mononuclear cells during antiviral therapy (Murakami *et al*., 2006). Due to the effective role of antioxidants in HCV pathogenesis, it has been proposed for treatment (Li *et al*., 2007).

The serum status of trace metals has also been studied in a large number of viral infections, for instance, the role of some trace elements iron (Fe), copper (Cu), cobalt (Co), manganese (Mn), zinc (Zn) and Pb has been studied in hepatitis virus infections (Rashed, 2011). Thus, the improvement of antioxidant defense (both metals and enzymes) of the body may attenuate oxidative damages to hepatocytes. Very few reports have been published for determining the concentration of cobalt (Co) and nickel (Ni) in hepatitis patients. However, it has been reported that their concentrations tend to decrease in patients suffering from the viral infection (Kajic *et al*., 2003). Serum from hepatitis C patients has showed the significant decrease in nickel and cobalt concentrations. It has been noted that cobalt chloride protects hamsters from acetaminophen-induced hepatotoxicity (Roberts *et al*., 1986). However, some increase in mean serum iron concentration was observed in the present study. Iron (Fe) is of utmost importance in cell environment, *e.g*. binding to hemoglobin. It induces lipid peroxidation as iron catalyzes the generation of active oxygen species, in hepatocytes (Younes and Siegers, 1985). Iron over load also affects the hepatitis C virus (HCV) replication (Shan *et al*., 2005). In patients affected with hepatitis B virus, the significantly high Fe concentration have been found in serum samples from the hepatitis B patients with high liver function test when compared to that of the normal liver function test and in healthy individuals (Hamid *et al*., 2013). Many of the earlier studies has shown the influence of iron overload on progression of hepatitis C viral disease, but we in the present study have found the very significant decrease in nickel concentration in hepatitis C disease, the reason yet not cleared. The variation in trace metal concentration may serve as the prognostic markers for the severity of the disease.

Restorative effects of Nigella sativa treatment to the serum liver markers and the antioxidants in hepatotoxic rabbits

Rabbits as the experimental animals have been used in present study because of the sensitive model for INH-induced hepatotoxicity (Sarich *et al*., 1995). The damage to the structural integrity of the liver has been ascribed by increase in serum levels of alanine aminotransferase (ALT) and serum aspartate aminotransferase (AST) (Chenoweth and Hake, 1962). *N. sativa* pre-treatment has not shown very impressive effects, however, the *N. sativa* post-treatment showed the significant decrease in the serum ALP levels of the acetaminophen administered rabbits. Therefore, in present study we have found the significance of *N. sativa* post-treatment in normalizing the status of liver marker enzymes. Contrary to our results, Yesmin *et al*. (2013) has reported the significance of pre-treatment of both, the aqueous and the n-hexane extract of *N. sativa*, evaluated in paracetamol treated rats. Likewise, Ghadlinge *et al*. (2014) has also demonstrated that usefulness of NS oil in prevention of hepatotoxicity. Administration of paracetamol (acetaminophen) was resulted a rise in the serum liver enzymes with a marked decline in serum total protein levels. However, the co-administration of NS oil with paracetamol has prevented significantly the fall in total serum protein levels and also reversed the elevated levels of serum AST, ALT, alkaline phosphatase and bilirubin. We propose, in absence of any drug comprising extracts from *N. sativa* seeds, supplementation of *N. sativa* seeds with prescribed medication to ameliorate the alterations in serum levels of liver marker enzymes.

Liver disease is associated with formation of oxygen derived free radicals (ROS) induced by several conditions such as viral hepatitis, alcohol abuse, liver cirrhosis and hepatocellular carcinoma (Ashour *et al*., 2014). Reactive oxygen species are responsible for nuclear DNA fragmentation and cell death. Phytochemicals have been found to stimulate the synthesis of antioxidant enzymes/ detoxification systems at the transcriptional level (Masella *et al*., 2005). The medicinal impact of seeds of *N. sativa* has been described by its active principle, thymoquinone (TQ), which acts as a scavenger of superoxide anion and also due to the presence of phenolic compounds in *N. sativa* which exhibit prominent antioxidant and hepatoprotective potential (Krishnan and Muthukrishnan, 2012). Administration of acetaminophen has reduced the serum catalase enzyme activity, both the *N. sativa* pre- and post-treatments showed affirmative effects in raising the activity of catalase enzyme, however, the *N. sativa* post treatment was effective ($P<0.001$) in returning back the normal catalase levels comparable to the control group. The observations have suggested the assisted role of *N. sativa* in maintaining the normal levels of catalase, despite acetaminophen injection. Krishnan and Muthukrishnan, (2012) has found the most significant effects of *N. sativa* seed extract on catalase levels in the *N. sativa* post-treated group, comparable to that of the control group.

Both the pre- and post-treatments of *N. sativa* have elevated the reduced ascorbic acid and serum bilirubin

levels in the hepatotoxic group, as exhibited by the CHC patients. The therapeutic value of ascorbic acid (AA) has be described recently by Abdel-Daim and Ghazy, (2015) who have evaluated the protective effect of *N. sativa* oil (NSO) and/or ascorbic acid against oxytetracycline-induced hepato-renal toxicity in rabbit model and found that the protective effect might was direct; inhibition of lipid peroxidation and scavenging free radicals, or indirect through the improvement of SOD and CAT activities. They suggested that NSO and AA could be used in combination for prevention and treatment of hepatic and renal diseases, especially those induced by oxidative damage. Ethanolic extract of *N. sativa* pre-treatment has been found very effective in preventing increments of liver enzymes and the total bilirubin levels when compared to paracetamol rat group (Kushwah *et al*., 2014). The findings support the suggestion that use of *N. sativa* may stimulate the enhanced production of AA to exhibit its antioxidant effect.

Serums trace metal stabilization by Nigella sativa treatment to hepatotoxic rabbits

Various trace metals have been associated with propagation of oxidative stress-related disorders in which serum/ erythrocyte metal concentrations may vary as compared to healthy individuals. The purpose of this study was to investigate the relation between oxidative stress (induced by acetaminophen) and levels of certain trace elements in the serum of acetaminophen induced hepatotoxic rabbits as well as the possible effect of *Nigella sativa* treatment in regularizing their concentrations.

Metal ions like iron and copper participate in redox cycling while cycling of oxidized and reduced forms of a toxicant leads to the formation of reactive oxygen free radicals which can deplete glutathione through oxidation or oxidize critical protein sulfhydryl groups involved in cellular or enzymatic regulation or can initiate lipid peroxidation (Saukkonen *et al*., 2006). Sakaida *et al*. (1995) have also reported an increase in mean serum iron concentration in rabbits injected with acetaminophen. They demonstrated that increase in iron might damage liver and an iron chelator deferoxamine (DFO) can protect against acetaminophen induced liver injury in vivo in rats. It has been found that chemically induced hepatotoxicity (drug-induced) may cause disturbances in ion-homeostasis due to the non-specific increases in plasma membrane permeability (Singh *et al*., 2011) and thymoquinone has been shown as having the ability to inhibit iron-dependent lipid peroxidation in a concentration-dependent manner (Nagi and Mansour, 2000). The *N. sativa* pre-treatment to the toxic group has been found effective in significantly lowering the elevated serum cobalt levels, and also in reverting back the nickel concentrations to the normal values. Variation in trace metal concentrations in situations where liver cells got damaged stipulates that the metal stabilization may assure the appropriate anti-hepatitis therapy.

Following is the graphical presentation of the findings of present study for comprehensive understanding of the therapeutic role of *N. sativa* in amelioration of liver disease (fig. 3).

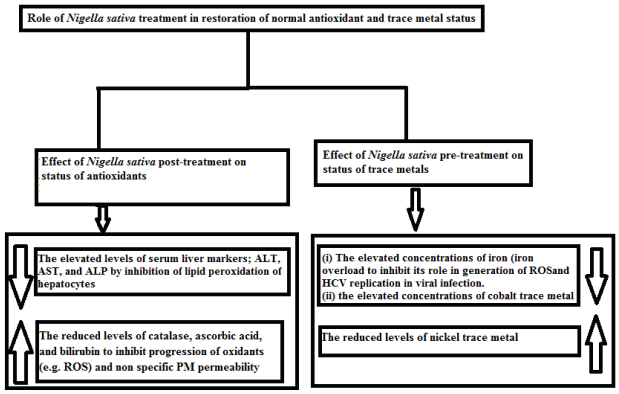

Fig. 3: Graphical presentation of the effects of NS seed aqueous extract treatment: *N. sativa* post-treatment was effective in normalizing the levels of serum liver markers and antioxidants, while *N. sativa* pre-treatment maintained the balance of serum trace metals.

CONCLUSION

The findings of the study has confirmed that pure aqueous extract of *Nigella sativa* seeds given orally has a hepatoprotective effect against acetaminophen-induced hepatotoxicity in rabbits via sustaining the antioxidant and the metal-ion homeostasis. Mostly, analogy was noted in status of biochemical parameters analyzed in sera from hepatitis C patients and that from the acetaminophen-induced hepatotoxic rabbits. Interestingly, we have found the *N. sativa* post-treatment effective in normalizing the levels of serum liver markers and antioxidants, while *N. sativa* pre-treatment has shown profound role in maintaining the balance of serum trace metals studied. Moreover, the results of the present study have suggested the supplementation of *N. sativa* seeds as an adjuvant therapy to normal medication during liver disease progression to overcome the metabolic disorders related to the antioxidant and trace metals disturbances.
In addition, the detailed study for absolute role of *N. sativa* seed aqueous extract in stabilization of other metal ions in cell's homeostasis is needed in future for complete understanding of its mechanism of action.

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