# Potential of *Nigella sativa* seed aqueous extract in ameliorating quinine-induced thrombocytopenia in rats

**Mubshara Saadia[1]*, Saba Rehman[1], Sehrish Robin[1], Tahira Ruby[2], Muhammad Sher[1], Waseeq Ahmad Siddiqui[1]and Mahmood Ahmad Khan[3]**

[1]Department of Chemistry, University of Sargodha, Sargodha, Pakistan
[2]Department of Life Sciences, The Islamia University of Bahawalpur, Pakistan
[3]Department of Statistics, University of Agriculture, Faisalabad, Pakistan

**Abstract**: Dengue infection is rapidly spreading in most of the countries of south Asia. It is of utmost importance to explore the plants with "anti-thrombocytopenic activity" the dreadful response of dengue fever. The present study was conducted to investigate the potential of aqueous extract of *Nigella sativa* (black cumin) seeds in alleviating the severity of dengue disease by raising the platelet count (PLT). Serum samples of thirty patients with dengue hemorrhagic fever (DHF) were analysed for different biochemical parameters. When compared with control groups, the patients were found with very low PLT count (7.62 fold), reduced antioxidant levels; catalase (1.4 fold), ascorbic acid (1.1 fold), bilirubin (1.06 fold), and severe deficiency of micronutrient concentrations; cobalt (2.27 fold), iron (2.35 fold) and nickel (71.46 fold). Similar parameters were studied in albino rats to observe the changes in serum levels of biochemical markers, after administration of single dose of choloroquine phosphate (IM, 1.5 mL saline). The drug successfully induced thrombocytopenia along with significant decrease in levels of antioxidants and trace metals. Administration of *N. sativa* aqueous seed extract (15.25 mg/kg/bw) for 12 days resulted in an increase in PLT count (1.59 fold) as compared to control group. *N. sativa* post-treatment was found effective in elevating the serum levels of catalase, ascorbic acid, and bilirubin (1.06, 1.58 and 0.4 folds respectively). However, the *N. sativa* pre-treatment was useful in increasing the levels of micronutrients; iron, nickel and cobalt when compared to quinine-induced group. From the above findings it was suggested that *N. sativa* seed aqueous extract supplementation would be a promising solution for declined PLT count and associated consequences.

**Keywords**: *Nigella sativa*, Dengue, Thrombocytopenia, Platelet Count, Antioxidants, Micronutrients.

## INTRODUCTION

Dengue fever (DF) is caused by the viruses, mosquito-borne members of family Flaviviridae (Murthy and Rani, 2009). Dengue virus has four distinct but antigenically related serotypes. However, infection with one serotype is useless to provide immunity against the other serotypes (WHO, 2009). The dengue problem is endemic in 100 countries with 25 million people at risk in the tropical and sub tropical regions (WHO, 2008). South East Asia, Bangladesh, India and Pakistan often suffer the outbreaks of dengue fever (Akram and Ahmad, 2005).

Currently there is no vaccine available, for the prevention of dengue infection (Beaute and Vong, 2010). Only supportive care is given to patient i.e., nursing care, fluid balance, electrolytes and blood clotting parameters (NaTHNaC, 2009) and if signs of dehydration or bleeding occurs, the patients are usually hospitalized (Goel *et al.*, 2004). Acetaminophen might be used to treat the patient, however; aspirin, antibiotics, nonsteroidal anti-inflammatory drugs (NSAIDs), and corticosteroids are avoided (Akram *et al.*, 2010) as the drugs most often associated with Drug-Induced Immunologic Thrombocytopenia (DITP) (Van den Bemt *et al.*, 2004) .

**Corresponding author:* e-mail: mubs04@yahoo.com

Progress to development of a specific vaccine for dengue has been unsuccessful due to its single-stranded RNA genome and the prevalence of four closely related, but antigenically distinct serotypes of the disease causing virus (Muhamad *et al.*, 2010). Immunity developed against one serotype does not ensure protection from the other three serotypes (Qi *et al.*, 2008). Thus, the development of vaccine for only one or two serotypes would increase the risk of more serious illness (e.g. DHF) by the other serotypes (Rees *et al.*, 2008). The immunogenic, safe tetravalent vaccines are now undergoing clinical trials (Ahmad *et al.*, 2011).

Due to side effects related to synthesized drugs, more than 80% population of the world, according to World Health Organization (WHO), relies on traditional plant based medicine for health needs. Several medicinal plants have been explored for its pharmacologically active constituents found in different plant parts e.g. seeds, roots, leaves, bark etc. to prepare decoctions, syrups, extracts or tinctures for medicinal purposes (Rahmani *et al.*, 2014). However, there have been very few phyto-medicines yet found with relative activities such as *Nigella sativa* L. seeds (Al-Tayib *et al.*, 2009). Recent reports have exhibited the promising activities against various diseases; especially the antidiabetic effects (Desai *et al.*,

2015; Nehar *et al*., 2015); hypolipidemic, hemostatic, fibrinolytic and anticoagulant effects (Al-Snafi, 2015); antimicrobial, immunomodulatory and anti-inflammatory activities (Tembhurne *et al*., 2014). Rahmani and Salah (2015) have summarized in their review the therapeutic effects of black seed in the diseases treatment and prevention through regulation of various biological activities including antioxidant, anti-inflammatory, anti-tumor, hepato-protective and other genetic activities. Alkharfy *et al*. (2010) published a patent describing the application of thymoquinone, the most extensively studied component of *N. sativa* oil, in treatment of sepsis. Previous studies have reported no toxic effects of *N. Sativa* treatment to animal models (Ahmad *et al*., 2013) and similarly, no serious side effects were found during the experimental trials (Paarakh, 2010).

With the growing environmental distress, increasing epidemics and unavailability of a definite drug treatment, the present study was conducted to find out a solution to reduce the DF symptoms especially the abrupt decline in the thrombocyte counts using *Nigella sativa* (black cumin) seed aqueous extract because of its widespread approval for disease remedy. Moreover, we have not found yet any other report describing the use of *N. sativa* seed aqueous extract to treat thrombocytopenia.

## MATERIALS AND METHODS

The research study was conducted at the Department of Chemistry, and Department of Pharmacy, University of Sargodha, Pakistan. The experimental work was carried out into two Phases; Phase-I comprised of biochemical evaluations including Platelet count (PLT) from blood, and antioxidants and trace metals from serum of dengue patients. In Phase-II, the same biochemical markers were evaluated from blood and serum samples of quinine-induced thrombocytopenic rats, and the consequences of therapeutic application of *Nigella sativa* seed aqueous extract to animals were also analysed. The institutional ethical committee approved the study protocol with the ethical guidelines.

### *Selection criterion for DHF patients*

Human blood samples were collected from District Head Quarter (DHQ) Hospital Sargodha, Pakistan. Patients of dengue with a positive serology were selected for sample collection. Blood samples of 30 patients with dengue hemorrhagic fever (DHF) including 21 males and 9 females ranging between 16-45 years and not suffering from any chronic or infectious disease were collected. The same selection criterion was adopted for the normal individuals.

### *Animals*

Albino male rats with an average weight of 250-300 g were purchased from Department of Pharmacy, University of Sargodha, Pakistan. The animals were housed in large spacious cages at the animal house, Department of Pharmacy, according to guideline suggested by the institutional ethical committee. The animals were fed with standard pellet diet and water ad libitum and maintained on standard laboratory conditions. Animals were acclimatized for two weeks before the start of experiment.

### *Quinine-induced thrombocytopenia*

Albino rats were subjected to induced thrombocytopenia, a severe complication observed in dengue patients, by administering choloroquine phosphate diluted in saline water (5mL in 100mL saline). Experimental animals were subjected to an intramuscular injection of 1M, 1.5 mL dilution, an optimized drug dose obtained after several trials.

### *Extract preparation*

Seeds of *Nigella sativa* (black cumin) were purchased from local herbalist in Pakistan and authenticated by a specialist of Plant Taxonomy, Department of Biological Sciences, Faculty of Science, University of Sargodha, Pakistan. 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 body weight) was dissolved in sterilized distilled water (1 mL) and left for 24 hours. Then the extract was filtered using Whatman filter paper (125 mm). The extract was orally administered by gavage daily to experimental animals for 12 days.

### *Experimental procedure*

The therapeutic studies were performed by allocating twenty animals randomly to four groups of five animals each.

- Group I served as control (fed on normal diet).
- Group II received choloroquine phosphate (IM, 1.5 mL saline dilution, one dose).
- Group III (pre-treated) choloroquine phosphate rats were fed orally the *N. sativa* seeds crude aqueous extract (15.25 mg/kg/bw (11 black cumin seeds) for 12 days. The rats were then subjected to quinine (one dose) to induce thrombocytopenia.
- Group IV (post-treated) rats were first injected with choloroquine phosphate (one dose) to low platelet count; the animals were then fed with *N. sativa* seeds aqueous extract (15.25 mg/kg/bw (11 black cumin seeds) for 12 days.

After two weeks, all animals were humanely sacrificed. Blood samples from each study group were collected after two days of drug administration, and serum was separated by centrifugation at 4,500 rpm for 10 minutes to study the biochemical parameters.

### *Biochemical assays*

Blood and serum samples from dengue patients, as well as from experimental animals were analysed for changes in

platelet (PLT) count, levels of some of the antioxidants and trace elements. To evaluate the potential therapeutic effect of *N. sativa* seed water extract, following biochemical parameters were studied. Total platelet (PLT) count of the blood samples was determined at the University Medical and Diagnostic Centre Sargodha (UMDC), Pakistan. Oxidative parameters were analyzed through alterations in serum levels of enzymatic and non-enzymatic antioxidants. Activity of catalase enzyme was measured spectrophotometrically (SHIMADZU, Japan) using method described by Goth (1991). Ascorbic acid (vitamin C) as non enzymatic antioxidant, was measured through HPLC following method by Cerhata *et al.* (1994). Total bilirubin concentration was measured by Jendrassik and Grof analysis (Garber, 1981). Serum trace metals were analysed as complementary to oxidative parameters. Samples and different standards were prepared following method by Rashed *et al.* (2010). Concentrations of iron, nickel and cobalt (ppm) were measured by atomic absorption spectrophotometer (SHIMADZU, Japan) (Burtis and Ashwood, 1970).

## STATISTICAL ANALYSIS

Data was presented as mean ± standard deviation of means. For establishing significant differences between groups, paired sample t-test was employed. 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

The experiment was conducted in two phases;
**Phase-I**: Assessment of Some Serum Biochemical Parameters from Dengue Effected Patients.

***Total platelet count***
Haematological assessment has revealed a highly significant decrease in platelets count in blood samples from the dengue patients as compared to the normal (control) blood samples; a 7.62 fold decrease was noted (fig.1).

***Catalase activity***
The catalase activity was determined to infer the activities of endogenous antioxidant enzymes. The serum of dengue patient has shown 1.4 fold decreases in catalase activity as compared to normal values (fig. 2a).

***Ascorbic acid***
The status of vitamin C in patients was investigated to assess the levels of non-enzyme antioxidants. We have found a slight decrease (1.1 fold) in ascorbic acid contents from serum of dengue patient as compared to normal individual (fig. 2b).

***Total bilirubin***
Total bilirubin (T-bil) serves both as an antioxidant and an important indicator of dengue fever severity. Similar to vitamin C contents, we also found a very slight difference (1.06 fold decrease) for bilirubin values in patients as compared to the normal individuals (fig. 2c).

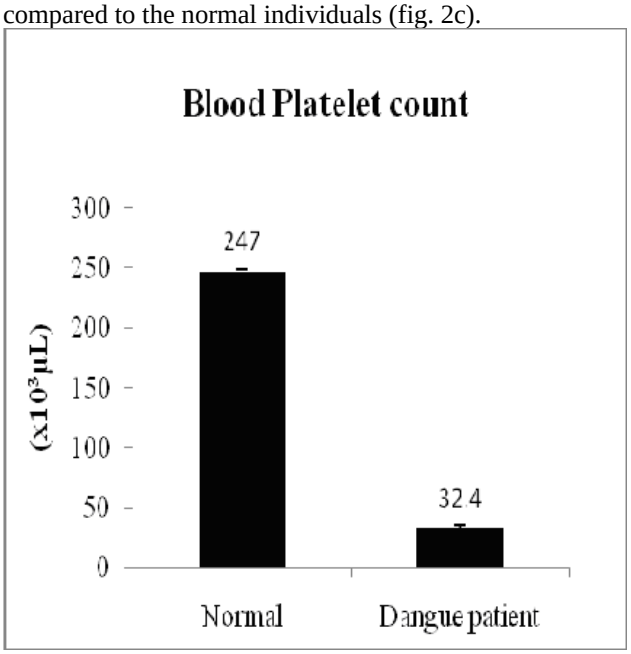


**Fig. 1**: Blood platelet count of dengue patients: A significant decrease in total platelet count was found in blood from dengue patients. Bars represent the standard deviation. Means were compared at level $p<0.05$.

***Trace elements***
In the following study we discussed the impact of dengue infection on normal status of three of the important essential trace metals, iron, nickel, and cobalt.
***Cobalt***: Cobalt is a constituent of vitamin B12, necessary for a normal bone marrow function for production of erythrocytes. We have found a significant decrease (2.27 folds) in the mean values for cobalt concentration from serum of dengue infected patient (fig.3).
***Iron***: In the present study, the serum iron levels in dengue virus infected patients were investigated in conjunction with the serum cobalt levels. Figure 3 represents a slight decrease (2.35 folds) in iron trace metal concentrations found in serum from dengue infected patients as compared to the normal values.
***Nickel***: A significant decrease (71.46 folds) in mean value of nickel in serum of dengue patients was observed as compared to normal individual (fig. 3).

**Phase-II**: Role of *Nigella sativa* in Restoration of Some Biochemical Parameters in Rats with Quinine-Induced Thrombocytopenia

***Total platelet count in animal model***
Quinine salt (choloroquine phosphate) was used in the present study to induce thrombocytopenia in rats.

Administration of quinine has induces severe thrombocytopenia in rats, as observed in dengue infected humans. Therapeutic effects of *N. sativa* herbal treatment was investigated in present study especially to augment thrombocytes in blood, by evaluating the same biochemical parameters as discussed in phase-I.

The oral administration of *Nigella sativa* seed aqueous extract (15.25mg/kg/bw) for two weeks showed very affirmative results. The study also evaluated the effectiveness of *N. sativa* pre- or post-treatment. It has been found that *N. sativa* post-treatment was very effective in elevating the platelet (PLT) count (1.59 fold) as compared to control (fig. 4).

***Oxidative parameters***

*Catalase*

While investigating the role of *N. sativa* treatment in the present study, *N. sativa* post treatment was found useful in elevating the level of catalase (1.06 fold).

***Ascorbic acid***

To suggest the effectiveness between the pre- and post- *N. sativa* treatment, we have found better role of *N. sativa* post-treatment in regularizing the ascorbic acid contents (1.589 fold) to normal levels (fig.5b).

***Bilirubin***

We have found the beneficial effects of *N. sativa* administration to rats as a result of pre- and post-treatments depicted by the increased serum bilirubin (1.27 fold and 1.4 fold respectively) levels (fig.5c). However, the *N. Sativa* pre-treatment showed slightly better activity in normalizing the bilirubin levels.

***Status of some trace elements***

We have noticed the same response pattern of decrease in trace metal levels after administration of quinine to rats to induce thrombocytopenia, as elicited by the dengue patients. However, the *N. sativa* pre-treatment to rats significantly improved the status of trace metals in comparison to the *N. sativa* post treatment (fig. 6).

## DISCUSSION

***Studies on serum samples from dengue infected patients***

***Total platelet count***

Dengue Hemorrhagic Fever (DHF) results in severe decline in total platelet count followed by the plasma leakage. Hemograms of patients infected with dengue virus showed leucopenia with atypical lymphocytes and thrombocytopenia (<100,000/$mm^3$) (Souza, 2007). The platelet counts and packed cell volume have been used to monitor the imminent decline (Tai *et al.*, 1999).

As a result of rapid decrease in platelet count, the platelets transfusions have shown useful therapy against the dengue virus (Lam *et al.*, 2007). The abrupt and significant decrease in platelet count, the outcome of dengue fever, as noted has earlier been reported by several studies (Kadir *et al.*, 2013; Cecilia, 2014).

***Evaluation of oxidative parameters***

Oxidative stress during maladies has been the hot issue for researchers. The reasons may be the recognition of biomarkers for oxidative damage and the existing stress-disease relationship. Oxidative stress is the result of the homeostatic disturbance caused by overproduction of free radicals, leading to cellular injury and tissue damage. This damage may involve cellular DNA, protein, lipid per oxidation of membranes, calcium influx, mitochondrial swelling and lysis (Younes, 1999). The normal status of reactive oxygen species (ROS) are maintained at low levels by various endogenous enzymatic and non-enzymatic molecules by *in vivo* biological systems (Rahal *et al.*, 2014). An antioxidant deficiency can also result in oxidative stress which may lead to generation of reactive oxygen and/or nitrogen species (Brown *et al.*, 1997). Therefore, characterization and control of the altered biochemical homeostasis would be helpful in managing the disease condition.

***Catalase activity***

Endogenous and exogenous antioxidants comprised of some high molecular weight (Catalase, SOD, albumin) and some low molecular weight substances (uric acid, ascorbic acid, lipoic acid, glutathione and tocopherol/ vitamin E). Rise in reactive oxygen species induce a significant damage to virus infected cells. Now enzymatic antioxidants such as glutathione (GSH), superoxide dismutase (SOD), thioredoxin (Trx), and catalase (CAT), which constitute the cellular system, neutralize the ROS and maintain the reductive intracellular environment. Among different markers of oxidative stress, catalase is the natural antioxidants and currently considered as an important markers (Singh *et al.*, 2011). In eukaryotic organisms, the ubiquitous primary antioxidant enzymes catalyze a complex cascade of reactions to convert ROS to molecules such as $H_2O$ and $O_2$. $H_2O_2$ is a particular intracellular signaling molecule because of its neutral and membrane permeable nature (Forman *et al.*, 2010). Specifically, $H_2O_2$ can oxidize thiol (-SH) of cysteine residues (Finkel, 1998). These modifications amend the target protein(s) activity (e.g. MAPK phosphatases) thereby changing its function in the signaling pathway (Tonks, 2005). Catalase activity for its crucial role in alleviating the oxidative stress in cell milieu has been analysed in the study. We have found a marked decrease of catalase activity form serum of dengue patients. Ohta and Nishida, (2003) describe the protective effect of co-administered native SOD and catalase against gastric mucosal lesions in rats with water immersion restraint (WIR) stress. According to them, the protective effect could be due to the SOD and catalase activity to scavenge

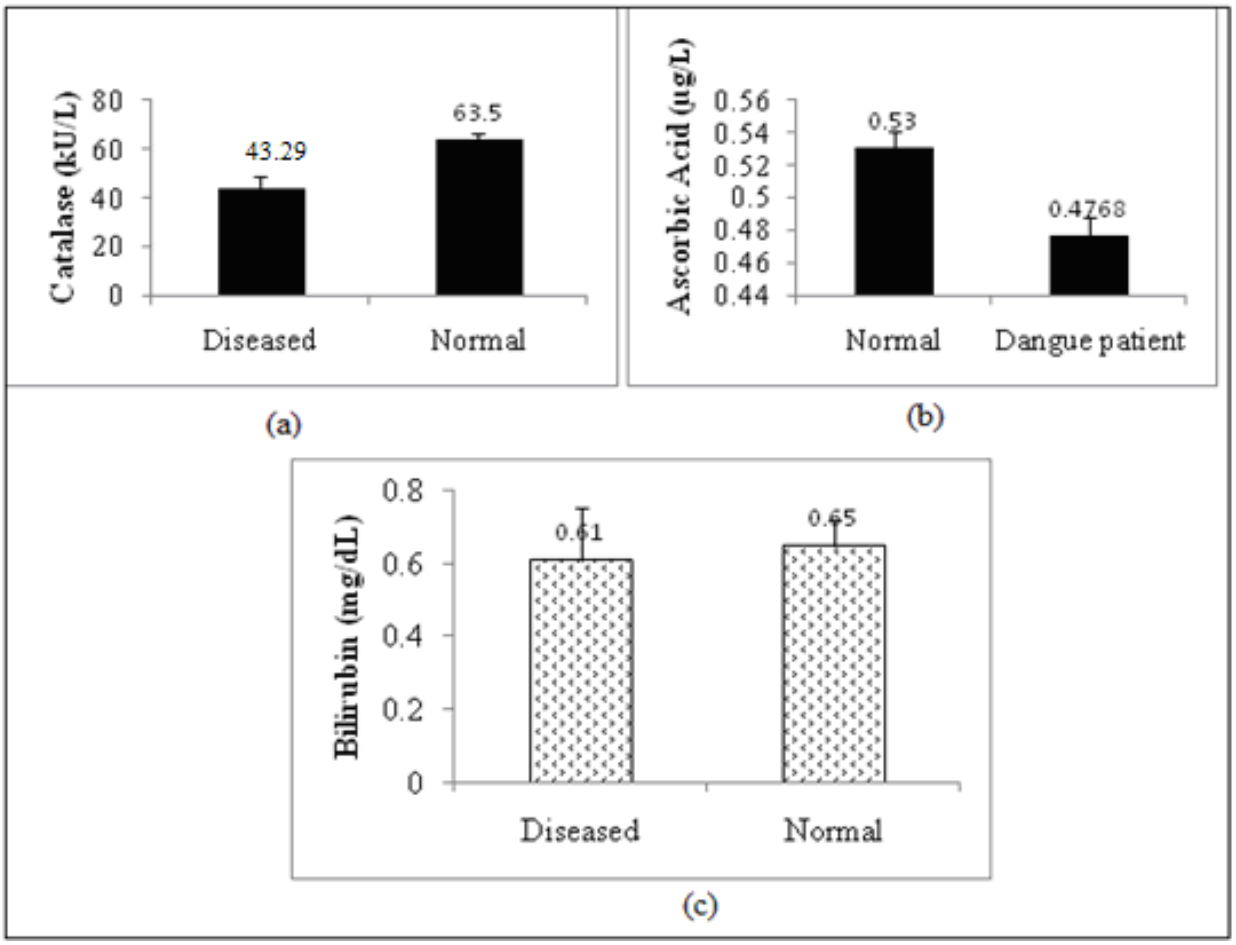


**Fig. 2a,b,c**: Serum antioxidant activities: A significant decrease in catalase activity and ascorbic acid contents was observed in serum from dengue infected patient (a and b). However, very slight decrease in bilirubin levels was noted in serum from dengue patient as compared to the normal healthy individuals (c). Bars represent the standard deviation, means were compared at $p<0.05$.

XO-derived active oxygen species that have increased in the blood.

Enzymatic antioxidants have been found in low concentrations in response to oxidative stress during a disease. A significant decrease in the activities of superoxide dismutase, catalase, glutathione reductase and glutathione per oxidase has been observed by Chitra and Jayakumar (2012). Rao *et al.* (2012) found a significantly decreased value for GPx, GST, SOD and catalase activity in Simvastatin -intoxicated rats. The protective effects of *N. sativa* oil (NSO) against dimethoate-induced lipid per oxidation and oxidative stress in rats have been reported by Al-Tayib *et al*, (2009). The protection suggested to be mediated by increased activities of SOD and catalase, and decreased TBARS levels.

***Ascorbic acid***

The interplay of enzymatic and non-enzymatic antioxidant systems in cell maintains an intracellular redox balance to prevent cellular damage caused by ROS (Durackova, 2010). These small molecular-weight non enzymatic antioxidants (e.g. vitamin E and C) also function as direct scavengers of ROS. Vitamin E and C supplementation has directed its role as stimulant to immune system function (Hennekens, 1994). Ascorbic acid (vitamin C) exhibits both its antioxidant and pro-oxidant effects; depending upon the dose (Seo and Lee, 2002). The low electron potential and resonance stability of ascorbate and ascorbyl radical have given the ascorbic acid an antioxidant property (Buettner and Jurkiewicz, 1996). The hydrophilic antioxidant, ascorbic acid reacts with free radicals to produce semidehydro- or dehydroascorbic acids (DHA) that is regenerated by antioxidant enzymes present in the organism (semidehydroascorbic acid reductase and dehydroascorbic acid reductase) back to ascorbate. Recently, toxicity of ascorbic acid has also been reported due after auto oxidation (Rahal *et al.,* 2014). The increased oxidative stress during dengue fever has also been reported for its pathogenesis (Klassen *et al.*, 2004).

In the present study a small decrease in ascorbic acid contents was noted from serum of dengue patients as compared to the normal individuals.

Severe bleeding is the outcome of thrombocytopenia (Chairulfatah *et al.,* 2003). It has been reported that a reduced level of plasma ascorbate and tocopherol may contribute to oxidative destruction of thrombocytes. del Valle *et al.,* (2012) showed that the oxidative alterations and antioxidant capacity has decreased significantly ($p<0.05$) in patients compared to healthy individuals related in age and gender. The antioxidant supplementation with Vimang® in 40 and 81 HIV Cuban patients respectively showed significant beneficial changes ($p<0.05$) in 56% and 43.9% of cases respectively. Chandra *et al.* (2013) found that supplementation of vitamin E and C may contribute to an increase in platelet count and early recovery in dengue fever, with the mean platelet count of $1.8\pm0.3\times10^5$ on day 1 that was increased to $2.3\pm0.5\times10^5$ on day 8, after supplementation.

***Total bilirubin***

In search of the differences existed in total bilirubin values in dengue infected and normal individuals; we have found a slight decrease in total bilirubin levels in serum of patients. Wahid *et al.* (2000) in a retrospective study described that the C-reactive protein (CRP) and total bilirubin (T-bil) were useful indicators to distinguish between DF and malaria. The significant differences may require a long exposure to disease. Madiedo *et al.* (1995) found thrombocytopenia associated with serious bleeding. Hyperbilirubinemia occur frequently during malarial infection reflecting the severity of intravascular hemolysis. Similarly, serum from dengue patients has revealed the abnormal levels of total bilirubin, alkaline phosphatase (ALP), and gamma-glutamyl transpeptidase (GGT) (Wong and Shen, 2008). High bilirubin, ALT and ALP levels in DHF patients as poor prognostic markers for dengue infection were described by different researchers (Singh *et al.,* 2008).

***Status of some trace elements***

Trace metals in biological system are involved in a number of metabolic activities; especially serving as cofactors for enzymes, neuroconduction, transport and excretory processes. Little information is available about the serum trace metal concentrations in many diseases, mainly limited by methodology. Atomic absorption spectrophotometery has remedied the situation to a major extent (Ghoreshi *et al.,* 2001). Variation in the concentrations of essential trace elements has been reported in several pathological conditions (Ciftci *et al.,* 2003; Hamed *et al.,* 2004). During infectious disease, deficiency of micronutrient adds oxidative stress, as free oxygen radicals may protect against virus attack by tissue damage action triggering the inflammation (Romero *et al.,* 1995). The influence of trace elements have been studied in a large number of viral infections; zinc in HCV (De Francesco *et al.,* 1998), selenium in HIV (Diamond *et al.,* 2001), cobalt (Funseth *et al.,* 2000) and nickel (Ilback *et al.,* 1994) in CoxBV. The variation in serum concentrations of some essential trace elements; Iron, cobalt, and nickel, has not yet been documented.

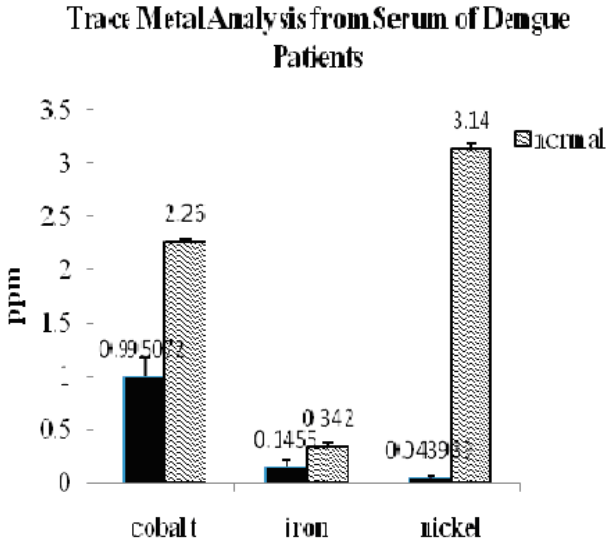


**Fig. 3**: Trace metal status of dengue patients: Highly significant decrease in nickel concentrations has been observed in patients, remarkable decrease was noted for cobalt concentrations, while less significant impact of dengue severity was found on iron concentrations. Bars represent the standard deviation and means were compared at $p<0.05$.

In the following study we described the impact of dengue infection on normal status of three of the important essential trace metals, iron, nickel, and cobalt.

***Cobalt***: Status of trace elemants in the plasma may be a useful indicator for development of infectious diseases. Cobalt (III) Schiff base complexes have been shown to inhibit the replication of ocular herpes virus (Takeuchi *et al.,* 1999). We have found a significant decrease in cobalt concentration in sera form dengue patients. A decrease in cobalt concentration would be the cause of anaemia due to $B_{12}$ deficiency. It also reffers that there may be erythrocyte deficiency accompnaied with the white blood cells during the course of dengue infection. Further research would be usuful for more accurate infrence.

***Iron***: Variations in serum trace element concentration during viral infection were studied by many researchers. In the present study, the serum iron and cobalt concentrations were investigated to correlate with disease severity and also as a predictive marker for severity of the disease. We also found slight decrease in iron trace metal concentrations in serum from dengue infected patients, as for cobalt levels. This decrease in serum cobalt and iron concentrations may indicate the possible outcomes of

anemia, may caused due to $B_{12}$ deficiency and iron deficieny both. According to Ballinger, (2007) depletion in RBCs count and Hb content cause iron deficiency anemia, characterized by a microcytic hypochromic blood image. Therefore, it is worth mentioning that the patients with dengue infection may manifest iron deficiency, and the possible supplementation should be included in the prescription.

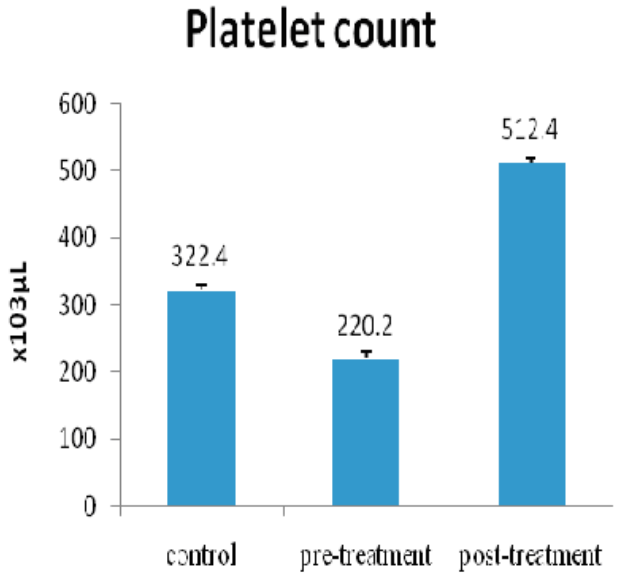


**Fig. 4**: Total platelet count of thrombocytopenic rats after *N. sativa* treatment: *N. sativa* post treatment has found very effective in elevating the TPC in rats after induction of thrombocytopenia by quinine. Bars represent the standard deviation, means were compared at $p<0.05$.

***Nickel***: The micronutrient nickel is found in blood and tissues, associated with DNA and RNA, function in modulation of immune system and development of brain, and required for normal growth and reproduction in mammals. We found a significant decrease in nickel concentrations from serum of dengue patients. Ilback *et al.,* (1994) studied the immunotoxic effect of a low dose (10-weaks) administration of $NiCl_2$ prior to CBV infection. The infection has shown to induce the increased concentration of nickel ($^{63}Ni$) in pancreas and heart, increased spleen B- and T-cell activities, and the increased spleen NK blood cell activity. Nickel treatment resulted in a decrease in number of cytotoxic T-cells and helper T-cells in the lesions of heart. It was suggested that nickel may contribute to the progression of target organ pathology in CBV infection-induced diseases, e.g. diabetes and myocarditis.

***Studies on animal model***

*Total platelet count*

During the early stage of dengue infection, thrombocytopenia occurs as a result of bone marrow hypocellularity resulting from direct infection of haematopoietic progenitor cells and stromal cells (Nakao *et al.,* 1989). As the fever settles, bone marrow hypercellularity is seen, and increased destruction from immune-mediated clearance of platelets becomes the primary mechanism for thrombocytopenia (Mitrakul *et al.,* 1977).

It has been observed that quinidine and quinine appear to cause Drug-Induced Immunologic Thrombocytopenia (DITP) more often than other medications, with the exception of heparin (Brinker and Beitz, 2002). Earlier studies showed its effects in lowering thrombocytes in blood (Perdomo *et al.,* 2011). Plant-based medicines have now been widely accepted against a variety of vector ailments as to be safer, non-toxic and less harmful than synthetic drugs. Ahmad *et al.,* (2011) investigated the activity of aqueous extract of papaya leaves against DF for its potential to increase the platelet (PLT) count, white blood cells (WBC) and neutrophils (NEUT) in blood samples of a 45-year-old patient. *Nigella sativa* has shown antiviral properties *in vitro* against infectious laryngotrachietis virus and cytomegalovirus virus in mice (Ballinger, 2007).

In the present study, the oral dose of *N. sativa* to rats was found very effective in raising the level of thrombocytes in blood. It was also observed that *N. sativa* post-treatment was effective in elevating the platelet (PLT) count of the treated rats. Recently, *Carica papaya* leaves extracts was found helpful in treating dengue by improving PLT counts, with a striking increase in thrombocyte count (4.9 folds) within 5 consecutive days of administration (Siddique *et al.,* 2014). However, *N. sativa* seed juice has not been reported yet. This will be the first ever report with positive role of *N. sativa* seed water extract for treating dengue. *N. sativa* was preferred over papaya juice as more nutritionally valuable food condiment processing various pharmacological activities (Al-Naggar *et al.,* 2003; Zaher *et al.,* 2008). Barakat *et al.,* (2013) also described the *N. sativa* oil as tolerable and safe to decrease viral load and to reduce oxidative stress.

***Effect of Nigella sativa treatment on oxidative parameters***

Imbalance in cell's redox status has been related to diverse clinical entities. Redox balance may be characterized by oxidative biological damage and the antioxidant activity in human samples, e.g. serum, as the progression markers for diverse clinical conditions.

***Catalase*** activity was determined as an indicator of enzymatic antioxidants. The levels of enzymatic antioxidants were found decreased under stress conditions (fig. 5a). Same trend was noticed when the experiment was conducted *in vivo*. In the present study we found the more effective role of *N. sativa* post treatment in increasing the catalase activity. Recently, Wang *et al.,*

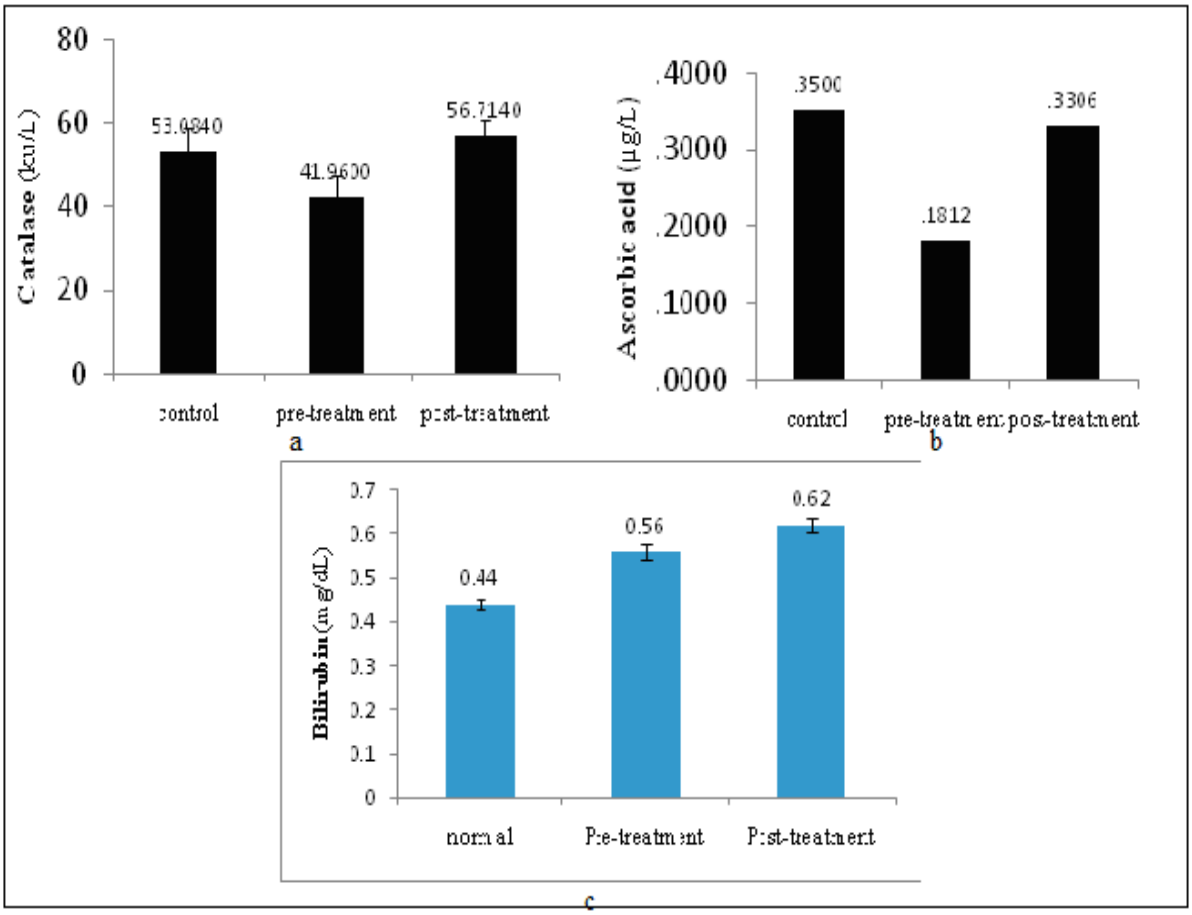


**Fig. 5a,b,c**: Antioxidant status of thrombocytopenic rats after *N. sativa* treatment: *N. sativa* post treatment has shown significant increase in antioxidant levels; catalase activity (a), ascorbic acid (b), and bilirubin levels (c) when compared to *N. sativa* pre treatment. Bars represent the standard deviation. Means were compared, and $p<0.05$.

(2014) used linezolid to induce a decrease of WBC, RBC and platelet numbers in rat blood. There observed a dose-dependent increase in enzymatic activities of superoxide dismutase and catalase in rat serum as well. Change of redox status mediated by depletion of glutathione (GSH) in human hepatocarcinoma cell line HepG2 is a common result of viral infection. The results of Wang *et al.*, (2013) suggested the glutathione (GSH) antioxidant as a promising therapeutic agent to prevent oxidative liver damage during DV infection in mice by inhibiting pro-inflammatory cytokine production.

***Ascorbic acid*** (vitamin C) act *in vivo* in connection with vitamin E and other prooxidant/ antioxidant e.g. betacarotene and lycopene (Young and Lowe, 2001). The antioxidant and pro-oxidant role of ascorbic acid in low (30 and 100 mg/kg body weight) and high doses (1000 mg/kg body weight), respectively, has been described under oxidative stress (Seo and Lee, 2002).

We have found that again in normalizing the levels of ascorbic acid in serum of rats, the *N. sativa* post-treatment was more effective as compared to the *N. sativa* pre-treatment to rats.

***Bilirubin*** levels are related mainly to hepatic cell function. The increase in liver marker enzymes and bilirubin level may indicate liver tissue damage by altered cell membrane. However, Adaramoye *et al.* (2008) found no profound differences ($P>0.05$) in the levels of serum ALP, conjugated and unconjugated bilirubin of the test groups when compared to control. In this study the *N. sativa* pre- treatment was found effective in bringing serum bilirubin level of rats close to normal values. Lawrence *et al.*, (2014) showed that the quinine (26 mg/kg b.w) administration to rats resulted in liver necrosis and peripotal inflammation with a significant ($P<0.05$) increase in aspartate and alanine amino transferease and total bilirubin levels of treated rats compared with the control animals. The findings thus suggested that *N. sativa* administration maintains the genuine antioxidant (enzymatic and/ non enzymatic) status of a cell by regularizing the metabolism under oxidative stress induced by dengue disease.

***Effect of Nigella sativa treatment on levels of trace elements***

Deficiencies of trace elements and progression of infectious diseases exhibit complex interaction. The

present study was undertaken to evaluate the alterations in serum levels of some trace elements in rats under thrombopenic conditions. Serum concentrations of three biologically important trace metals; iron, nickel and cobalt were investigated for their supportive role.

Quinine induced thrombocytopenia in rats showed same response to trace metal concentrations as elicited by the dengue patients. However, the *N. sativa* pre-treatment to rats significantly improved the status of trace metals in comparison to the *N. sativa* post treatment.

Nutritional deficiency of trace elements is lethal for biological life as they combine with vitamins, required for enzyme function, and for almost every physiological function of a cell. For example, mice deficient in selenium are more susceptible to infection with coxsackie virus, as well as with influenza virus (Beck *et al.,* 2003). Cobalt chelates also act as antiviral agent. Deficiency of cobalt suppresses the immunity by restraining the neutrophils and macrophages. Funseth *et al.,* (2000) found a decrease in plasma zinc levels of adult male A/J mice during the pre-inflammatory stage of CBV myocarditis, studied for different trace metal levels. Infection of female C3H/HeJ or CD+ mice with a sublethal dose of murine cytomegalovirus, and exposure to nickel chloride, resulted in enhanced mortality and reduction in virus augmented NK cell activity at a dose of 10 mg Nicl2/kg as depicted by (Daniels *et al.,* 1987). New insights about the participation of trace elements in controlling cell activities and their role in enhancing the immune-competence and decrease in incidence and severity of some infections, has thus suggested their potential benefits to reduce the risk of infectious diseases.

## CONCLUSION

The findings of present study suggest that serum antioxidant and complementary trace metal levels in severely ill dengue virus infected patients may serve as a predictive marker for disease severity. Deficiencies of antioxidants; catalase, ascorbic acid, bilirubin and trace metals; iron, cobalt and nickel may enhance susceptibility to infection. Herbal treatment, for example *N. sativa* seed aqueous extract as proposed, may ameliorate the symptom severity by regularizing the normal antioxidant and trace element status. Platelet count (PLT) is of the central importance in determining the progression of dengue disease and *N. sativa* post treatment has showed the significant effectiveness in raising the PLT count in rats with quinine-induced thrombocytopenia. Therefore, it is suggested that *N. sativa* seeds as food/ or drug supplement to medication (as rich in antioxidants and trace metals) may help in reducing the risks associated with this life threatening disease.

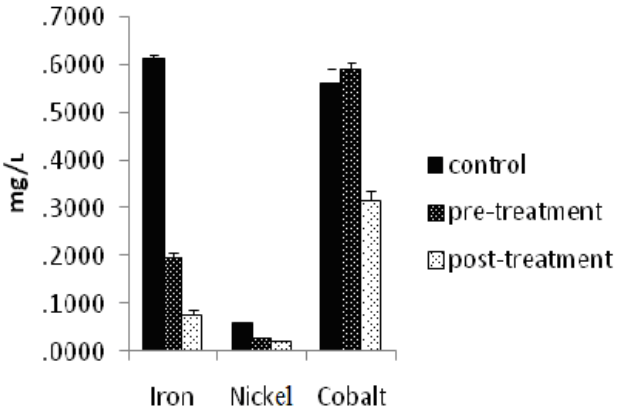


**Fig. 6**: Trace metal status of thrombocytopenic rats after *N. sativa* treatment: *N. sativa* pre treatment has shown profound activity in normalizing the serum concentrations of iron, nickel and cobalt when compared to *N. sativa* post treatment. Bars represent the standard deviation. Means were compared at $p<0.05$.

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