Biochemical evaluation of *Nigella sativa* L. seeds on fluconazole toxicity in Wistar rats

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ABSTRACT

*Nigella sativa* L. seed commonly known as black seed, extensively used as a folk medicine in Middle East and Asian subcontinent. Traditionally it is used as an adjunct therapy along with modern medicine to counter the adverse effects. Pure thymoquinone or isolated compounds are not free from adverse effects. The present study was undertaken to evaluate *N. sativa* seeds powder impact on the fluconazole (FLZ) induced toxicities in the liver and kidneys. Male Wistar rats were acclimatized, divided into four groups each group with six animals. Experimental animals were subjected to an FLZ dose of 50 mg per kg of body mass. The toxic effects of FLZ treatment were manifested and animals were given 1000 mg of *N. sativa* seeds powder per kg of body weight to observe any retrieval. Dissected animals were evaluated for biochemical and histopathology alterations. Based on findings, it can be concluded that *N. sativa* seeds powder restored various altered biochemical parameters induced by fluconazole and can be used along with fluconazole in the treatment of fungal infections.

1. Introduction

Hepatic injury caused by fluconazole may vary from asymptomatic to slight liver dysfunction which may act as a potentially fatal fulminant hepatic failure [1]. Fluconazole generally causes cholestasis, acute hepatocellular or mixed cholestasis and hepatocellular reactions [2]. The exact mechanisms of action of hepatotoxicity of fluconazole are unknown [3]. Fluconazole shows toxic effect on hepatocytes. It damages hepatocytes, produces inflammation with alteration in the level of liver enzymes. It is mainly eliminated from kidney and also shows effect on kidney pharmacokinetics. Further, mechanism of renal toxicity of fluconazole is not reported till date [4]. According to previous studies, *N. sativa* reduce various drug and chemical-induced toxicity. The protective effects of this plant against toxic drugs in different organs including lung, heart, brain, kidney and liver have been established from previous researches [5]. A lot of studies available on the investigation of the efficacy of *N. sativa* in toxic manifestations due to the use of some common medicines. It restores the multiple organ toxicity caused by cyclophosphamide, cisplatin-induced nephrotoxicity, doxorubicin induced cardio and nephrotoxicity, paracetamol-induced hepatotoxicity, to name a few. These alterations were restored by *N. sativa* [6–9]. Mechanisms of reduction of toxicity by *N. sativa* involve anti-inflammatory, antioxidant, restore of antioxidant defence systems, free radical scavenging, improvement in the disturbed levels of biochemical markers, regulatory effects on genes expression and inhibition of apoptosis, and various signalling pathways.

Fluconazole is an FDA approved, highly water-soluble triazole family antifungal drug most commonly used throughout the world. It inhibits the cellular formation of ergosterol which plays a critical role in the formation of fungal plasma membrane [10]. The drug is widely used to treat respiratory and urinary tract fungal infections [11]. Only a small proportion of fluconazole is processed by the liver and about 60–80% is eliminated from the body by the renal system [12]. Nevertheless, there are several reports of fluconazole induced hepatic and kidney toxicity [13, 14]. According to recent information, the pre-existing liver toxicity is further complicated by the antifungal use of fluconazole [15]. Severe to mild liver injury has been reported among the patients subjected to azole antifungal drugs including fluconazole [16], the toxicity is often represented by an elevated plasma level of liver enzymes (AST and ALT) [14]. *Nigella sativa* is a small nutrient-rich herb that has been extensively studied worldwide for its medicinal applications. Traditionally, the seeds of *N. sativa* have been discussed in ancient books with different names Habbatul Barakah (Arabic) Tizkur azmud (Amharic), it was also used by...
people in Europe, Far East Asia and Africa [17]. The seeds were described as an herb from 1 heaven by [18]. In Islamic literature (hadith), N. sativa mentioned as a seed which has a remedy for every illness [19]. The curative property of seeds is also described in holy bible labelled as Melanthion by Hippocrates and in Indian system of medicine [20, 21]. In some countries, N. sativa seeds are sold to treat conditions that include liver diseases. Furthermore N. sativa seeds are also referred as diuretic and antiurinary retention in books. In Persian literature, black seed has been traditionally used for the treatment of hepatic disease and is referred as hepatic tonic [22]. Many reports are available where the active constituent thymoquinone is used to regulate the normal kidney [23] and liver function [24]. The chemical composition of its seeds is much studied for various activities in animals, the active components and essential oils found in the N. sativa seeds have a wide range of medicinal applications [25–27]. These pure compounds are not free from adverse effects [28] but most of the research is concentrated on the pure or isolated compounds and this may be a reason that as on date no clinical translation of this active compound reached any phase of the clinical trial in the pharmaceutical industry. The antioxidant effects of seeds have been reported [29], the activity of glutathione peroxidase, Glutathione-S-transferase and superoxide dismutase were increased post-treatment with N. sativa seeds [30]. Anti-diabetic [31, 32], anti-hypertensive [33], anti-inflammatory [34] and anti-cancer activities of N. sativa seeds have been reported by several researchers [35]. This study is an attempt to reduce or alter the toxic effects of FLZ from complete seed powder and not the pure compound that can promote the healing effect of N. sativa seeds on the fluconazole induced hepatic and renal toxicity in Wistar rats.

2. Materials and methods

2.1. Animals and materials

Twenty four albino Wistar rats ranging in weight from 100 to 120 g were obtained from King Fahad Medical Research Centre (KFMRC) at King Abdulaziz University Jeddah 21589, Saudi Arabia. The animals were acclimatized at our lab conditions for a week and segregated in groups according to the experimental design. The study plan was approved as per the University Ethical Committee for the animal. Flunazolone was purchased from Ontop Pharmaceuticals Pvt. Ltd. (Trade name: AF-150 dispersible tablets) and N. sativa seeds were purchased from the local market and were authenticated by Dr. Imran Kazmi, Pharmacognosy specialist. Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia, with reference no. FOS/KAU/2019/001. A copy of specimens is submitted to the herbarium of the department. The seeds were purchased from local market of Jeddah.

They were sieve to remove any unwanted impurities, washed and shade dried for 1 day. The seeds were grounded with Panasonic Super Mixer Grinder Model (MX-AC210SWTZ). The coarse was obtained was sieved and stored in a glass container for further use. Powder Dose was calculated for the animal as per the existing dose 1 g/kg/day, orally [36].

2.2. Experimental design

Totally 24 male Wistar rats were divided into 4 groups, each group has 6 animals. The group I was untreated, group II was treated with 1000 mg of N. sativa seeds per kg body weight by mixing power in water and force-feeding rats [37], group III was treated with 50 mg fluconazole per kg of body weight (oral dose) once a week, (modified from our previous protocol) [38] and group IV was treated with 50 mg of FLZ as above with and 1000 mg of N. sativa seeds powder per kg body weight daily. All the group animals were subjected to the above conditions for 12 weeks under normal laboratory conditions (22 ± 3°C, 55 ± 5% humidity, 12 h dark/light cycle) and with a normal diet. The experimental protocol was approved by animal ethical committee, King Abdulaziz University (Approval no. BIOC-90A-130-1440).

2.3. Animal growth and blood biochemistry

The total body and liver weight of animals from all groups were recorded during dissection. The rats were subjected to a 12-h fasting period and dissected under mild anaesthesia. Blood samples were collected by cardiac puncture method and saved in EDTA coated tubes. Before collection, the site cleansed with alcohol (70%), kept under control, and then blood is withdrawn by using a needle of 21–22 gauge from the lateral vein of the tail. Quick after collection, the flow of blood was stopped with the application of pressure with sterile gauze for stopping blood flow [39]. Collected blood was centrifuged serum separated and processed for further biochemical study. The blood plasma was isolated and biochemical analysis was made. The studies on the plasma enzyme activity measurements, glucose level, lipid profiles and other parameters were conducted by an automated analyser (Dimension-clinical chemistry System USA) using standard kits [40].

2.4. Histopathology

During the animal dissecting process, liver and kidney tissues were immediately fixed in formalin (10%) and were subsequently dehydrated by passing through a graded series of alcohol and paraffin infiltration. The semi-automated microtome was used to prepare 5 µm sections then the samples were dried in an oven at 37°C overnight. Hematoxylin and eosin were used for staining and images were captured by light microscopy.
The photographs of both tissues were compared for all groups.

2.5. Statistical analysis

The mean values of data were presented in the tabulated form with standard error values. The significance of results in between more than two groups was measured via one-way analysis of variance and then Student’s t-test was used with Graph Pad Prism 5.0 software. The differences of ($p < 0.05$) were considered to be statistically significant. (*) Group (Fluconazole Control) compared with Normal Control; (*) Groups (Fluconazole + Nigella and Nigella Control) as compared to Disease Control (Fluconazole Control); *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ns = non-significant.

3. Results

3.1. Blood electrolytes

In Fluconazole Control group, the plasma level of K+ ions was significantly ($p < 0.01$) decreased to 3.1 ± 0.03 mmol/L as compared to Normal Control (4.6 ± 0.02) mmol/L. This altered level was significantly ($p < 0.05$) restored up to 3.8 ± 0.06 mmol/L by the treatment with FLZ. Very small changes found in the plasma levels of Na+, Ca²⁺, Mg²⁺, PO₄⁻, and Cl⁻ after the treatment with N. sativa and FLZ treated groups as compared to the Fluconazole control group (Table 1).

3.2. Liver profile

3.2.1. Alanine aminotransferase (ALT)

The level of ALT was significantly ($p < 0.001$) increased up to 127.5 ± 0.56 U/L in Fluconazole Control group as compared to Normal Control 44.1 ± 0.82 U/L. Almost 3 times increase in the plasma level of ALT was recorded indicating the liver toxicity by FLZ treatment. This altered level was significantly ($p < 0.001$) restored up to 64.3 ± 0.4 U/L with administration of N. sativa in Fluconazole + Nigella group. There was very little change in Nigella Control Group (Table 1).

3.2.2. Aspartate aminotransferase (AST)

A little significant ($p < 0.01$) change in the mean value of plasma AST level of FLZ administered group was recorded (88 ± 2.3 U/L) as compared to the normal control group (51 ± 3.3 U/L) and significantly ($p < 0.05$) to those treated with FLZ and N. sativa seeds (73 ± 4.1 U/L). These changes in the AST levels were statistically insignificant as indicated in Table 1.

3.2.3. Alkaline phosphatase (ALP)

The ALP level was remarkably increased (188 ± 8.3 U/L) as compared to the normal control group (72 ± 1.2 U/L). ALP level in FLZ + N. sativa group was significantly ($p < 0.01$) controlled and decreased (104 ± 1.9 U/L) in

Table 1. Effect of fluconazole and N. sativa seeds on the blood plasma values of electrolytes (mmol/L), liver and kidney specific enzymes (U/L), blood glucose (mg/dL), blood urea nitrogen (BUN) (mg/dL), LDL/HDL/TC/TG (mg/dL), and TB/DB (mg/dL) is described. The mean values from 6 animals of each group are given in the table below.

| Sample         | Blood Electrolytes | Enzyme markers | Creatine, BUN, Bilirubin and Lipid profile |
|----------------|--------------------|----------------|------------------------------------------|
|                | Na⁺ | K⁺ | Ca²⁺ | Mg²⁺ | Cl⁻ | ALT | AST | Amy | ALP | Creatinine | BUN | DB | TB | LDL | HDL | TC | TG |
| Normal Control | 146 | 41 | 3.9 | 0.6 | 102 | 72 | 64 | 3.0 | 1.0 | 51 | 44 | 3.8 | 58 | 1.0 | 160 | 1.1 |
| Fluconazole Control | 141 | 46 | 3.6 | 0.7 | 101 | 127.5 | 64.3 | 3.8 | 1.0 | 51 | 44 | 4.8 | 58 | 0.8 | 160 | 1.1 |
| Fluconazole | 144 | 46 | 3.7 | 0.6 | 101 | 147.5 | 64.3 | 3.8 | 1.0 | 51 | 44 | 4.8 | 58 | 0.8 | 160 | 1.1 |
| Nigella | 141 | 46 | 3.6 | 0.7 | 101 | 127.5 | 64.3 | 3.8 | 1.0 | 51 | 44 | 4.8 | 58 | 0.8 | 160 | 1.1 |
| Nigella Control | 144 | 46 | 3.7 | 0.6 | 101 | 147.5 | 64.3 | 3.8 | 1.0 | 51 | 44 | 4.8 | 58 | 0.8 | 160 | 1.1 |

*Values are expressed as mean ± N (N = 6). (#) Groups as compared to normal control; (*) Groups as compared to Disease Control; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ns = non-significant.
this group of treatment (Table 1). The results indicate reasonable recovery of ALP level by the treatment of *N. sativa* seeds.

### 3.2.4. Amylase

An elevated plasma level of amylase in the Fluconazole treated group was significantly (*p* < 0.001) increased found 181.6 ± 1.57 U/L, as compared to the normal control group 101 ± 2.01 U/L. There was a significant (*p* < 0.01) healing impact of *N. sativa* on the pancreatic disorder indicating a plasma level of amylase 135.3 ± 1.39 U/L.

### 3.3. Renal profile

#### 3.3.1. Total and direct bilirubin (TB and DB)

A significant (*p* < 0.01) increase in the levels of TB was found in the FLZ group (1.7 ± 0.21 mg/dL) as compared to normal control (0.8 ± 0.3 mg/dL). There was considerable significant (*p* < 0.01) recovery in the plasma level of TB in the treated group (FLZ + *N. sativa*) (1.15 ± 0.25 mg/dL). The level of direct bilirubin was also altered by FLZ treatment, however, it was not significant (Table 1).

#### 3.3.2. Blood urea nitrogen (BUN) and creatinine

The BUN level was elevated significantly (*p* < 0.001) with a plasma value of 41.5 ± 0.32 mg/dL as compared to its value in the normal rats (13.1 ± 0.12 mg/dL). There was considerable significant (*p* < 0.01) recovery in the BUN level (21.5 ± 0.41 mg/dL) in the *N. sativa* treatment group. The values of creatinine were changed among animal groups but the variation was non-significant (Table 1).

### 3.4. Lipids profile analysis

There was no considerable effect of FLZ treatment on the plasma levels of TC, TG and HDL. However, a non-significant decrease in the HDL level was observed in this group (Table 1).

### 3.5. Histopathology

All the animals were euthanized after the withdrawal of blood by diethyl ether and ketamine. The liver was isolated from each animal and was fixed in formalin 10%. The tissues were dehydrated by passing through graded series of alcohol and paraffin infiltration. Semi-automated microtome was used to obtain the samples/sections of 5 µm and were dried at 37°C overnight. Hematoxylin and eosin were used for staining. The images were captured by light microscope [38, 41]. The kidneys from each group were removed, fixed in formalin (10%) for 24 h, embedded in paraffin. 4–5 µm thick sections made by microtome, stained with hematoxylin–eosin dye and sections were evaluated for the pathological changes in the kidney (Figures 1 and 2). All the slides were examined under a light microscope. The morphological changes were observed under a light microscope. The morphological changes were recorded for tubular casts, glomerular congestion, epithelial desquamation and blood vessel congestion (Table 2).

### 4. Discussion

Pure or isolated compounds are not free from toxicity or adverse effects [42]. These compounds may show their therapeutic effect on one disease and on one organ but may have an adverse effect on other organs [43]. Anti-cancer drugs whether natural or synthetic are the best example that substantiates our statement. This work is an attempt to exactly the ethanopharmacological use of *N. sativa* powder. Azole antifungal drugs including fluconazole are used to treat invasive fungal infections. These drugs have well established for chronic toxic side effects to the patients along with their therapeutic role [44, 45]. As an inhibitor of CYP 3A4, FLZ applications result in the elevations in plasma levels of biomarkers [46]. Hence, during long-term application of fluconazole like medicine, the monitoring of plasma enzymes is a common practice.

The present study was aimed to evaluate the liver and kidney toxicity in Wistar rats caused by the fluconazole application and to determine the role of *N. sativa* powder as whole in countering these toxic effects by the natural drug. *N. sativa* oil has been reported to recover liver injuries [47]. Pure compounds from any plant including *N. sativa* have shown adverse or side effects. Thymoquinone is the most active compound obtained from *N. sativa* and is well studied for many metabolic and non-metabolic diseases. Recent studies have confirmed the adverse effects of thymoquinone [43]. This work is, therefore, aimed to decipher the role of *N. sativa* seed as a whole without any treatment with chemicals to counter the fluconazole toxicity. In the present study, the plasma electrolytes level of normal, fluconazole treated and FLZ + *N. sativa* groups were compared.

The plasma electrolyte levels including Ca$^{2+}$, Mg$^{2+}$, Cl$^-$, PO$_4^{3-}$ were not changed to a considerable level. However, the plasma level of K$^+$ was decreased in the rats treated with FLZ, the *N. sativa* seed application along with FLZ has shown a significant recovery in the levels of potassium ions (Table 1). The plasma concentration of potassium is maintained by the selective filtration and reabsorption process in the kidneys [48], hence a decrease in the potassium concentration indicates malfunctioning of kidneys after FLZ treatment. Post-FLZ treatment level of liver-specific plasma enzyme biomarkers was evaluated and compared with other research groups.
Figure 1. Effect of *N. sativa* in FLZ administered hepatotoxicity in different groups of rats: (A) FLZ + *N. sativa*; (B) FLZ control; (C) *N. sativa* control; (D) Normal control. Note: The pictures were selected randomly. The FLZ treated animal have well defined binucleated cells (yellow arrow) balloonated hepatocytes (black arrow), steatosis (brown star), cellular injury (blue arrow). *N. sativa* treated group shows well defined structure with best recovery of injury (blue arrow). Further, there is almost no binucleated cells compared to FLZ control group. *N. sativa* controlled slide is almost same as that of normal controlled. The slides were haematoxylin and eosin stained and the original magnification was 20X.

Figure 2. Effect of *N. sativa* in FLZ administered nephrotoxicity in different groups of rats: (A) FLZ + *N. sativa*; (B) FLZ control; (C) *N. sativa* control; (D) Normal control. The normal histopathological studies of the kidneys are generally based on cellular structure including tubular casts, glomerular congestion, epithelial desquamation and blood vessel congestion. Normal controlled rats showed no evidence for tubular casts, glomerular congestion, epithelial desquamation and blood vessel congestion. Whereas in FLZ treated rat a clear demarcation in tubular casts, glomerular congestion (yellow arrow), epithelial desquamation (red arrow) and blood vessel congestion (blue arrow) were observed compared to normal control rats. The slides were haematoxylin and eosin stained and the original magnification was 20X.
The histopathological studies of the FLZ treated kidney of albino rat expressed epithelial desquamation tubular casts, glomerular and blood vessel congestion, when compared to normal control rats. Administration of 1000 mg of Nigella powder showed almost complete normalization of the tissues which clearly indicates a potent nephroprotective activity of Nigella and results are depicted in Figures 1, 2 and 3. (−) normal; (+) mild effect; (+++ ) severe effect.

| S. No. | Effect                          | Control | FLZ       | FLZ + Nigella (1000 mg) | Nigella control |
|--------|--------------------------------|---------|-----------|------------------------|----------------|
| 1      | Epithelial desquamation         | −       | +++       | ++                     | +              |
| 2      | Blood vessel congestion         | −       | +++       | +                      | −              |
| 3      | Intestinal edema                | −       | +++       | +                      | −              |
| 4      | Glomerular congestion           | −       | +++       | +                      | −              |
| 5      | Tubular casts                   | −       | +++       | ++                     | −              |
| 6      | Peritubular congestion          | −       | +++       | +                      | −              |
| 7      | Inflammatory cells              | −       | +++       | ++                     | −              |

The level of ALT was determined in FLZ treated (127 ± 3.4 U/L), normal control (44 ± 1.5 U/L) and FLZ + N. sativa groups (64 ± 3.1 U/L). These findings indicate a significant increase in the plasma ALT level. Many times elevation in the plasma ALT activity has been reported in the case of azole antifungal drugs [49]. The blood plasma activity of AST in the FLZ was increased but to a non-significant level. These findings are different from the recent literature [49, 50]. Where a many-fold increase in the plasma activity of AST has been reported. Elevation in the plasma ALP activity has been observed in the present study which is in accordance with the previous reports onazole antifungal drug applications [51]. However, our study presents the first report on the reversal of hepatotoxicity by the N. sativa seeds.

We found a significant increase in the levels of TB in the FLZ group as compared to normal control. There was considerable recovery in the plasma level of TB in the treated group (FLZ + N. sativa). Bilirubin is a protein produced by the processing of haemoglobin in the liver, hence indicating the working efficiency of the liver [52]. It is used as a marker for liver health and function in the clinical laboratories [53]. In the present study, there was no considerable variation in the lipid profile components such as TC, TG and LDL. However, the value of HDL was slightly decreased in the FLZ treated rats. An increase in the TG levels and a decrease in the plasma levels of HDL has been reported during medication [54].

The BUN and creatinine levels are used as important plasma markers to evaluate the kidney function. The elevated levels of these biomolecules are associated with the kidney disease [55]. In the present study, the level plasma value of plasma DT was 41.5 ± 0.32 mg/dL as compared to its value in the normal rats (Table 1). There was considerable recovery in the BUN level in the N. sativa treatment group. However, the values were still above the normal range. The values of creatinine were changed among animal groups but the variation was non-significant. The creatinine level was not altered significantly (Table 1). The blood urea nitrogen (BUN) and creatinine (Cr) are nitrogen-containing substances in the human body. These are small molecules, easily filtered and reabsorbed in the kidneys. Hence, these are used as the biomarkers of kidney function [56]. Our findings indicate the functional disability of kidneys in the animals treated with FLZ.

Histopathology provides valuable information on the nature and the severity of injury, possible pathogenesis and some expected outcome from it [57]. The results of our histopathological findings have clearly depicted the reduction in liver injury by N. sativa. Cellular injury and binucleated hepatocytes along with the deposition of fats or steatosis with hepatocyte ballooning, variable degrees of inflammation and fibrosis are few well stable markers for the tissue injury [58]. The treatment group is well free from the binucleated hepatocytes, cellular injury, reduction in steatosis and inflammation of liver cells. The FLZ treated animal has well-defined binucleated cells (yellow arrow), balloonated hepatocytes (black arrow), steatosis (brown arrow), cellular injury (blue arrow) (Figure 1). N. sativa treated group shows well-defined structure with best recovery of injury (blue arrow). Further, there is almost no binucleated cells compared to FLZ control group. N. sativa controlled slide is almost same as that of normal controlled. The relation between the N. sativa and Histological and biochemical injury is correlated in pregnant albino rats [59] but as on date there were no reports that supported the role of N. sativa in reduction of FLZ-induced drug toxicity [60] a very common phenomenon associated with FLZ in fungal disease treatment.

5. Conclusion

The results of the study clearly indicate the reduction in adverse effects of fluconazole by N. sativa both at the cellular and biochemical levels. On the basis of findings of results, it can be concluded that N. sativa seeds are useful in the reduction of drug and chemical-induced toxicity. Further clinical study required to establish the claim in human being.

Author contribution

Firoz Anwar and Fahad A. Al-Abbasi conceived the idea, designed the experiment, prepared the manuscript; Sharifa Al-Ghamdi, Abdukadir Kurban and Muhammad Shahid Nadeem treated the animals, participated in data analysis and manuscript writing.

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