ORIGINAL ARTICLE

Effect of Nigella Sativa on Biochemical Changes in Doxorubicin- induced Nephrotoxicity in Albino Rats

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ABSTRACT:

Objective: To evaluate the protective effects of Nigella Sativa on biochemical changes in the kidney of Albino rat treated with Doxorubicin.

Methodology: This experimental study was undertaken in April 2015 for a duration of 05 weeks at BMSI, JPMC. Forty Albino rats were divided into four groups, ten animals in each group. Group-A was taken as control. Group-B was treated with Doxorubicin (DOX). Group-C was given doxorubicin and Nigella Sativa (NS), and Group-D was treated with Nigella Sativa only. At the end of the study, the serum urea and creatinine levels were measured.

Results: The mean values (mg/dl) of serum urea and creatinine levels in group-A and B were 23.40±3.07, 0.61±0.059, 85.50±7.93, and 1.06±0.071 respectively. There was highly significant increase in the mean values of serum urea and creatinine levels in group-B when compared with group-A. Further, the mean values (mg/dl) of serum urea and creatinine levels in group-C were 56.10±6.87 and 0.96±0.087 respectively, which showed highly significant increase when compared with group-A, and significant decrease when these values were compared with group-B. The mean values (mg/dl) of serum urea and creatinine levels in group-D were 25.01±3.39 and 0.67±0.057 respectively. There was insignificant increase when these values were compared with group-A, and highly significant decrease when compared with the groups-B and C.

Conclusion: This study concludes that Doxorubicin induced biochemical changes can be minimized by the administration of aqueous powder of Nigella Sativa. The free radical scavenging effects of Nigella Sativa might be attributed to the presence of flavonoid, alkaloids and steroids which are powerful antioxidant and anti-inflammatory agents.

Keywords: Doxorubicin, Nigella sativa, Serum Urea, Serum Creatinine

INTRODUCTION:

Doxorubicin is an Anthracycline Antibiotic, and represents a class of anticancer drug used as antineoplastic agent for a variety of human neoplasms[1,2]. Doxorubicin is isolated from cultures of Streptomyces peucetius and it is used in the management of various hematological malignancies and neoplastic diseases, such as renal, cardiac, breast cancer, soft tissue, leukemia[3]. The clinical use of Doxorubicin is restricted due to its toxic effect on various organs including kidney and heart[4], it targets both the normal and tumour cells[5].

Doxorubicin is a highly potent chemotherapeutic agent that causes cell damage by a variety of biochemical changes in the body[6]. The exact mechanism of doxorubicin-induced nephrotoxicity remains unclear[7]. The main anticancer activity of Doxorubicin is thought to occur due to DNA damage through inhibition of topoisomerase II[8]. According to various researches, Doxorubicin-induced cellular damage occurs due to plasma membrane injury caused by free anthracycline radicals[9]. Doxorubicin causes an imbalance between free radicals and antioxidants. The disruption in oxidant-antioxidant systems has been shown with lipid peroxidation and protein oxidation resulting in tissue injury[10]. Doxorubicin induced renal damage is manifested as raised serum urea and creatinine levels and histological damage to proximal tubular cells[11]. Doxorubicin induced nephrotoxicity in rats is evidenced by an increased glomerular capillary permeability and tubular atrophy due to oxidative stress[12].

Therapeutic plants and herbs have an important role in the treatment and prevention of renal diseases[13]. Nigella Sativa is a kind of seed, also known as black seed, or kalonji, is a small annual spicy herb belonging to the family Ranunculaceae, native to South west Asia, North Africa, and cultivated in many countries in the world like India, Pakistan, Syria, Turkey and Saudi Arabia[14]. The nutritional composition of Nigella Sativa is carbohydrates, proteins, minerals, vitamins and fats, and also include eight or nine essential amino acids[15]. Significantly, it is narrated with saying of Prophet Muhammad (PBUH) that the black seed can heal every disease except death[16]. Aqueous Nigella sativa has been used as a natural cure for more than 2000 years to enhance health and treat diseases, such as gastrointestinal disorders, intrinsic haemorrhage and amenorrhoea, asthma, cough, bronchitis, diabetes, inflammation, headache, eczema, fever, dizziness and hypertension[17]. Nigella Sativa has various therapeutic effects such as

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antioxidant, nephroprotective, anti-inflammatory, anticancer, antiallergic and antibacterial[18]. Thymoquinone (Nigella sativa seed) improved serum urea and creatinine levels, renal glutathione depletion and lipid peroxide accumulation in Doxorubicin-induced nephropathy[19]. As Nigella Sativa seeds are widely available, at affordable price and being a safe product, its scavenging effects can be utilized in Doxorubicin-induced nephrotoxicity.

METHODOLOGY:

This experimental study was conducted in the department of Anatomy, in collaboration with the Department of Pathology Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre (JPMC), Karachi,in April 2015 for a duration of 35 days. The research was initiated after approval from ethical committee of JPMC. Forty Albino rats (about 180 to 250 gram), 90-120 days were obtained from the animal house of JPMC. Nigella Sativa seeds were dried, freed of dust and crushed in the grinder (WestPoint, WF-9221 France). Prepared extract powder was collected and stored in refrigerator till use. Doxorubicin (Pfizer Pharma Pvt. Ltd. Pak) was used in the form of an injectable commercial product (Adriblastina vials). Each vial contained Doxorubicin hydrochloride as a freeze-dried powder 50mg/25ml. The contents of each vial were freshly dissolved in sterile saline solution just before use. It was administered at the doses of 3mg/kg/week ip for five weeks[20]. Rats were divided into four groups. Each group consisted of 10 animals. Group-A served as control. Group-B received Doxorubicin injection in a dose of 3mg/kg/body weight/week[20] intraperitoneal (IP) for five weeks. Group-C was given Doxorubicin injection at the same dose as in group-B with aqueous suspension of powdered Nigella Sativa 1000mg/kg body weight orally daily[21] for five weeks[22]. Group-D received aqueous suspension of powdered Nigella Sativa 1000mg/kg body weight orally daily for the experimental duration.

Blood samples for serum urea and creatinine levels (1 ml) from each animal were drawn under chloroform anaesthesia by cardiac puncture with disposable syringe. After clotting, the serum was centrifuged at 3000r/pm for 10 minutes and stored at -20°C till the estimation of serum urea and creatinine. Data was analyzed with SPSS version 20. P value less than 0.05 was considered as significant.

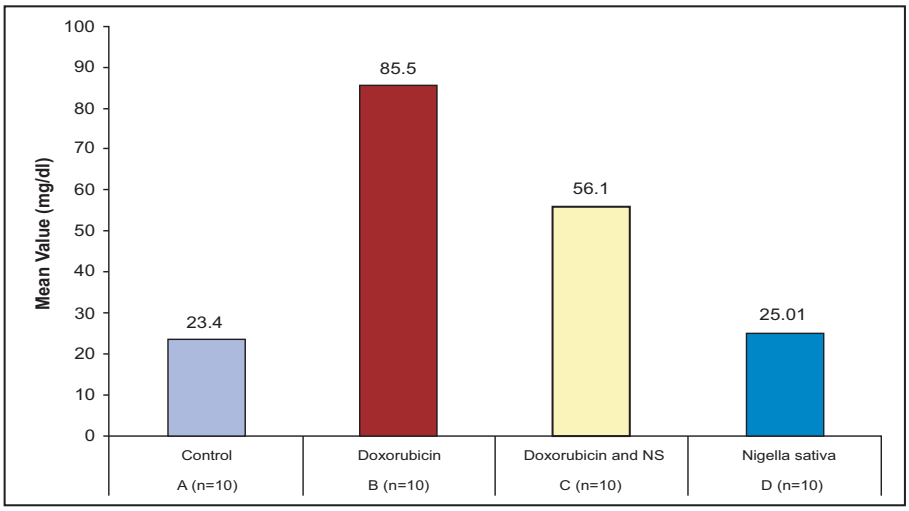
RESULTS:

The mean values of serum urea level in different groups of experimental animals is given in Figure-1, where as mean values of serum Creatinine levels is shown in Figure-2. There was highly significant increase in the mean value of serum urea and creatinine levels in group-B when compared with similar values of group-A.

The data showed a highly significant increase in the mean values of serum urea and creatinine levels in group-C when compared with group-A, whereas highly significant decrease in urea level and a significant decrease in serum creatinine values when these levels were compared with corresponding values in group-B animals (Table-1 &2).

The results demonstrated an insignificant increase in the mean value of serum urea level in group-D when compared with group-A, and highly significant decrease when compared with group-B and C. There was also an insignificant increase in the serum creatinine level in group-D when compared with group-A, and highly significant decrease in these levels when compared with group-B and C (Table-1 & 2).

Figure-1: Mean serum urea level (mg/dl) in different groups of albino rat



Data is presented as Mean

n= number of albino rat

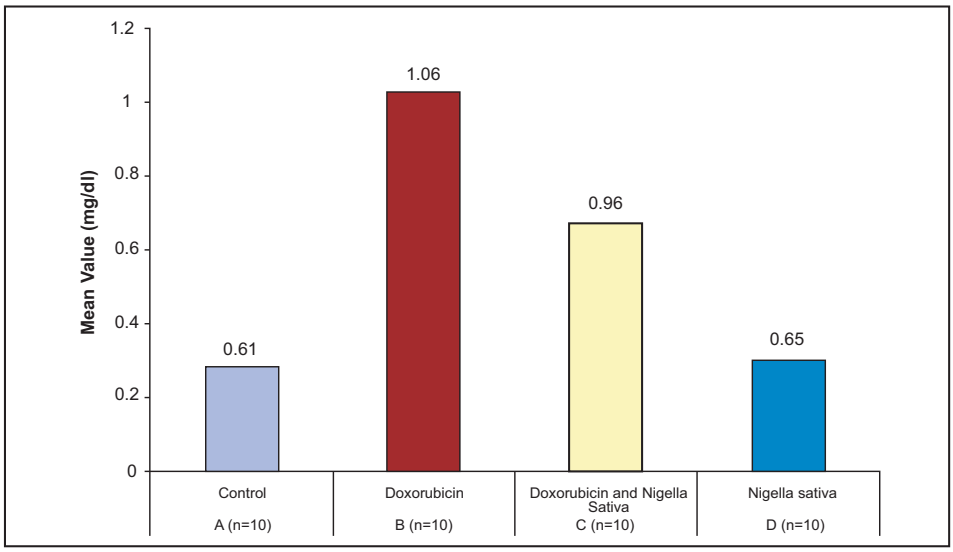
Table-1: Statistical analysis of mean serum urea level between different groups

Statistical Comparison	P-value
B vs A	<0.001**
C vs A	<0.001**
D vs A	0.280
B vs C	<0.001**
B vs D	<0.001**
C vs D	<0.001**

P<0.05 (*) significant difference

P<0.01 (**) highly significant difference

Figure-2: Mean serum creatinine level (mg/dl) in different groups of albino rats



Data is presented as Mean

n= number of albino rat

Table-2: Statistical analysis of mean serum creatinine level between different groups

Statistical Comparison	P-value
B vs A	<0.001**
C vs A	<0.001**
D vs A	0.140
B vs C	0.011*
B vs D	<0.001**
C vs D	<0.001**

P<0.05 (*) significant difference

P<0.01 (**) highly significant difference

DISCUSSION:

The nephrotoxicity produced by Doxorubicin in group-B was demonstrated by highly significant increase in serum urea and creatinine levels when compared to control group-A. Our results were in agreement with Shinde et al[23] who reported that a single dose of Doxorubicin (15mg/kg/body weight) induced acute nephrotoxicity in rats manifested by increase in blood urea and creatinine after 72hours; and with Ayla et al[12], who showed that Doxorubicin caused a marked increase in serum urea and creatinine, and sodium and potassium levels in albino rats treated with single dose of Doxorubicin. Our results were also in agreement with Al- Saedi et al[24], who reported an increase in serum urea and creatinine levels, indicating a decrease in glomerular filtration rate in albino rats treated with single dose of Doxorubicin (25 mg/kg body weight ip) on the day 3; and with Roomi et al[25], who showed significantly increased levels of renal markers, including serum creatinine, blood urea nitrogen and uric acid in mice treated with single dose of Doxorubicin (20 mg/kg body weight ip). Our results were also supported by Refaie et al[7], who found nephrotoxic effects of Doxorubicin characterized by decreased glomerular filtration rate leading to increased serum urea and creatinine levels at the single dose of 15mg/kg body weight on 11th day of treatment in albino rats.

Aqueous suspension of powdered Nigella sativa administered with Doxorubicin in group-C resulted in significant decrease of serum urea and creatinine levels when compared to group-B; evidently, showing protective role of Nigella sativa. Our results were in agreement with Al-Azzawi and Baraaj[26], who reported that oral administration of aqueous suspension of Nigella Sativa at the dose of 2g/kg body weight prevented toxic effects of Rifampicin, as indicated by significant reduction in serum creatinine, urea and uric acid levels in albino rats; and with Majeed and Tahir[27], who used Nigella Sativa (500mg/kg body weight orally daily for 7 days) combined with Amphotericin-B producing a significant decrease in serum urea and creatinine levels. Group-D treated with aqueous suspension of powdered Nigella Sativa 1000mg/kg body weight daily orally, did not show any significant difference in serum urea and creatinine levels when compared to group A. Our observation was comparable with the results of Dollah et al[22], who observed that the supplementation of Nigella Sativa to the diet of rats (1.0g/kg body weight) for five weeks did not alter the renal function i.e. serum urea and creatinine levels. Our results were also in agreement with Onoshe and Madusolumuo[28], who found no significant difference in serum urea and creatinine levels between group-i (control) and group-iii (Nigella sativa) 250mg/kg body weight for 14 days in albino rats.

CONCLUSION:

The present study demonstrated that co-administration of Nigella sativa with Doxorubicin caused significant decrease in the serum urea and creatinine levels, indicating that considerable protection is afforded. Further investigations on the mechanism of improvement of aqueous suspension of powdered Nigella sativa is required and may have a considerable impact on future clinical treatment of patients with acute renal failure.

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