ORIGINAL ARTICLE

EFFECT OF *NIGELLA SATIVA* EXTRACT ON RENAL FUNCTIONS IN AMPHOTERICIN B INDUCED NEPHROTOXICITY IN MICE

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ABSTRACT

Introduction: Amphotericin B is considered as one of the most effective antifungal drugs presently used to treat systemic fungal infections; however, nephrotoxicity remains the major side effect. The current study is therefore, designed to determine Amphotericin B induced nephrotoxicity and its prevention by administration of Nigella sativa (NS) extract in albino mice.

Materials and Methods: Thirty two albino mice, 6 – 8 weeks of age, 30 ±5 gm body wt, were randomized into four groups of eight mice each. Group A (control) was injected 3.7 ml/kg of 5% dextrose solution intraperitoneally daily for 7 days. Group B was treated with Amphotericin B (18.5 mg/kg dissolved in 3.7ml of 5% dextrose solution) intraperitoneally daily for 7 days. Group C received Amphotericin B (18.5 mg/kg dissolved in 3.7ml of 5% dextrose solution) intraperitoneally along with Extract of Nigella sativa (500 mg/kg) orally daily for 7 days. Group D received Amphotericin B (18.5 mg/kg dissolved in 3.7 ml of 5% dextrose solution) intraperitoneally for initial 7 days; after completing the injection regimen, extract of Nigella sativa 500 mg/kg was given orally daily for next 7 days. At the end of the experiment, cardiac puncture was performed to draw blood from each animal for renal function tests.

Results and Conclusions: Nigella sativa when co-administered with Amphotericin B significantly contained serum urea and serum creatinine levels ($p < 0.001$) implying thereby that Amphotericin B induced nephrotoxicity was significantly reduced. However, when Nigella sativa extract was given after Amphotericin B, the toxic effects of the drug persisted unabated thus indicating that Nigella sativa protects but do not ameliorates the toxic effects of the drug.

Key Words: Amphotericin B, Nephrotoxicity, Nigella sativa, Mice, Serum urea and creatinine.

INTRODUCTION

Amphotericin B (AmB), a polyenic antifungal antibiotic, has been extensively used in the clinical cure of systemic mycoses for more than 45 years.[1] However, its clinical use is often restricted on account of its potential nephrotoxic effects which remains a serious dose – limiting factor.[2] The antifungal mechanism of this drug has been believed to be mediated by association of AmB with ergosterol in the fungal cell membranes, forming ion-permeable pores.[3,4] Unfortunately, as a result of poor selectivity by Amphotericin B between fungal and mammalian cells, it is one of the most toxic drugs used clinically.[5] Amphotericin B induce harmful effects on kidney cells by the formation of Amphotericin B channels (non-aqueous or aqueous pores), which are permeable to protons, salt and calcium ions.[6]

The pathophysiology of Amphotericin B induced nephrotoxicity involves (a) direct interaction with epithelial cell membrane, resulting in creation of pores, leading to tubular dysfunction (b) vasoconstriction, decreasing renal blood flow and glomerular filtration rate producing ischemic injury. It has been reported that ischemia accelerates production of free radicals causing oxidative damage of membranes.[7] Collectively these two mechanisms induce acute renal dysfunction producing azotaemia.[8] Wingard et al., (1999) retrospectively studied the effect of AmB administered for fungal infections in patients who received bone – marrow transplant, 29% of the patients showed serum creatinine levels > 2.5 mg/dl, whereas 53% of patients showed doubling of baseline creatinine.[9] Fatefully, there have been a few effectual strategies implied for prevention or treatment of nephrotoxicity induced by AmB, except for saline loading, aggressive hydration, alternate day administration and Amphotericin B dose reduction.[10]

Herbal medicines obtained from plant extracts are being used increasingly to cure a broad variety of clinical ailments.[11] *Nigella sativa* (NS), a dicotyledon of *Ranunculaceae* family, is a herb that have been traditionally utilized for centuries in the Middle East, Northern Africa and India for the cure of a variety of diseases.[12] *Nigella sativa* seeds possess 36% – 38% fixed oils, proteins, alkaloids and 0.4% – 2.5% essential oil. Clinical and experimental studies have reported that *Nigella Sativa* extract has a variety of remedial effects including antihypertensive, antidiabetic, renoprotective, anti-neoplastic, anti-histaminic, immunomodulative, antimicrobial, hepatoprotective and gastroprotective.[13-20] The experimental and clinical studies perfor-

med on *Nigella sativa*, shows that the majority of its pharmacological effects are owing to its antioxidant activity which is primarily due to its ability to scavenge free oxygen radicals or inhibit lipid peroxidation.[21] It has also been reported that *Nigella sativa* extract and its active constituents showed protective effects against nephrotoxicity produced by either disease or chemicals.[22] Begum et al., (2006) has shown that the n-hexane extract of *Nigella sativa* was able to produce considerable alleviation from the nephrotoxic effect of gentamicin in the adult male rats on account of its free oxygen radical scavenging and antioxidant properties.[15] It has also been observed that concomitant treatment of lead exposed rats with *Nigella sativa* prevented the increase in serum urea and creatinine and showed reduction of damaged areas in kidney tissues.[23]

The current work was, therefore, carried out to assess the possible protective effect of *Nigella sativa* extract on AmB induced nephrotoxicity which has hitherto not met with enough attention.

MATERIALS AND METHODS

Chemicals and Preparation of *Nigella sativa* Extract

Amphotericin B was obtained from Bristol – Myers Squibb Company, USA and was dissolved in 5% dextrose solution.[24]

Nigella sativa extract was prepared at PCSIR Laboratory, Lahore. One kg of Black seeds was purchased from Punjab Seed Cooperation Lahore. The seeds were dried under shade, freed of dust and crushed in a domestic grinder and were soaked in absolute alcohol for 4 days at room temperature with intermittent stirring. The soaked crushed seeds were filtered under UV light using filter paper. The filtrate was put into Rotary evaporator (Heidolph Apparatus) so as to evaporate ethanol. Prepared extract was collected, weighed and stored in refrigerator till use. Yield was calculated 8.33%.

Animals

Thirty two male albino mice of 6-8 weeks of age weighing 30 ± 5 g, were obtained from Veterinary Research Institute, Lahore. They were kept in the Animal House of the University of Health Sciences, Lahore under controlled environment (temperature 23 ± 2˚C, humidity 55 ± 5%) and light and dark cycle of 12 hours each. They were provided with standard pellet rodent diet and tap water ad libitum and were acclimatized for a week prior to the beginning of the experiment. The mice were randomized into four groups of 8 mice each.

Group A: Received 3.7 ml/kg of 5% dextrose solution intraperitoneally daily for 7 days.

Group B: Received Amphotericin B at dosage of 18.5 mg/kg dissolved in 3.7 ml of 5% dextrose solution intraperitoneally daily for 7 days.[24]

Group C: Received Amphotericin B at dosage of 18.5 mg/kg dissolved in 3.7 ml of 5% dextrose solution intraperitoneally along with the extract of *Nigella sativa* 500 mg/kg orally daily for 7 days.[25]

Group D: Received Amphotericin B at dosage of 18.5 mg/kg dissolved in 3.7 ml of 5% dextrose solution intraperitoneally daily for first 7 days and then extract of *Nigella sativa* (500 mg/kg) was given orally daily for next 7 days.

Blood Sample Collection

Following the experiment blood samples from each group were drawn under chloroform anesthesia by cardiac puncture. One ml of blood was drawn in a 3ml disposable syringe and after clotting centrifuged at a speed of 3000 r/pm for 10 minutes. The clear serum was collected and stored at -20°C until used for biochemical estimation.

Biochemical Analysis

Serum urea and serum creatinine levels were measured by using commercially available kits prepared by Human Company (Germany).

Statistical Analysis

SPSS version 16.0 was used for data analysis. One way ANOVA was used to evaluate mean differences among groups and post hoc Tukey's test was used to assess which group mean differs. A p-value of ≤0.05 was considered statistically significant.

RESULTS

Effect of Nigella sativa extract on serum urea levels (Table 1)

The results showed that serum urea in group B which received intraperitoneal injection of Amphotericin B was significantly raised when compared with group A ($p < 0.001$) but these values were significantly reduced in mice of group C, which received *Nigella sativa* extract simultaneously with Amphotericin B in contrast to that in group B ($p < 0.001$). Group D mice which received NS extract after treatment with AmB did not show significant reduction in serum urea when compared to that in group B ($p = 0.85$).

Effect of *Nigella sativa* extract on serum creatinine levels (Table 1)

A significantly increased ($p < 0.001$) level of serum creatinine of group B receiving Amphotericin B as compared to control group A was seen. These levels were significantly reduced ($p<0.001$) in mice of group C treated with *Nigella sativa* extract simultaneously with Amphotericin B. Mean value of serum creatinine of group D which received NS extract after treatment with AmB did not show significant reduction when compared to that in group B ($p = 0.57$).

Table 1: *Comparison of mean value of serum urea and creatinine in (mg/dl) among groups A, B, C and D.*

	Serum Urea	*Serum Creatinine*
Group A: Mean ± SEM	57.96 ± 2.90	0.92 ± 0.015
Group B: Mean ± SEM	149.73 ± 7.70	2.35 ± 0.102
Group C: Mean ± SEM	67.69 ± 4.13	1.02 ± 0.052
Group D: Mean ± SEM	144.08 ± 4.05	2.18 ± 0.066
p-value	0.001*	0.001*

P-value ≤ 0.05 is statistically significant

DICUSSION

Nigella sativa seeds, oil and its main constituents are extensively described to protect several organs against oxidative damage depending on its high antioxidant activity.[26] Both clinical and experimental studies have demonstrated nephrotoxic profiles following AmB desoxycholate treatment.[27] In the current work, therefore, we evaluated the possible protective effects of Nigella sativa extract on nephrotoxicity produced by Amphotericin B in albino mice.

In the current investigation, nephrotoxicity produced by Amphotericin B was indicated by statistically significant elevation in serum urea and creatinine levels in group B in contrast to those in group A; this was reported to be due to Amphotericin B induced oxidetive destruction of cellular membranes causing tubular damage resulting in renal impairment and azotemia.[8] These observations were similar to those of Tonomura *et al*., (2009) who reported similar biochemical changes suggestive of nephrotoxicity after single intravenous daily dose of AmB repeated for 1 week in mice given simultaneously with Nigella sativa.[28]

Nigella sativa extract administered simultaneously with Amphotericin B in mice of group C resulted in significantly reduced levels of serum urea and serum creatinine when compared to that of group B; evidently, showing protective role of Nigella sativa extract. However, in group D post treatment with *Nigella sativa* extract did not show significant alleviation of nephrotoxic effects indicating that Nigella sativa is not effective in reversing the AmB induced nephrotoxicity. Comparable results were also reported earlier by Yaman and Balikci (2010) who reported that treatment with NS prevented the toxic effects of gentamicin, as indicated by significant reduction in levels of plasma urea and creatinine. They explained it on the antioxidant actions of NS which prevented the renal damage produced by reactive oxygen species.[29] Similar findings had been reported earlier by Ali (2004) who observed that treatment of rats with NS oil showed significant alleviation in the biochemical parameters of gentamicin nephrotoxicity, implying a rise in the scavenger defense system and the total anti-oxidant condition in kidney parenchyma.[30]

Amphotericin B induced nephrotoxicity involves direct toxic effect on epithelial cell membrane, resulting in creation of pores, leading to tubular damage and vasoconstriction induced ischemic injury.[8] Free radicals are reported to be the chief contributors to ischemic injury resulting in increase lipid peroxidation that induces oxidative damage at the cellular level of the renal cortex.[7]

Studies performed on NS in various models of oxidetive stress showed that the majority of its pharmacological effects are owing to its antioxidant potential which is chiefly a result of its capability to hunt reactive oxygen radicals and to slow down the process of lipid peroxidation.[21] In our investigation, presumably, the antioxidant action of the *Nigella sativa* prevented the oxidative damage of renal tissue with subsequent reduction in serum levels of urea and creatinine. Studies have shown that NS seed extracts and its active constituents possess beneficial effects in kidney and liver damage caused as a result of exposure to various pharmacological agents.[22] It has also been reported that *Nigella sativa* oil inhibited lipid peroxidation in liposomes and act as a scavenger of free radicals.[31] In view of the action of *Nigella sativa* on lipid peroxidation and antioxidant defense system, it was also reported that administration of *Nigella sativa* oil in carbon tetrachloride induced toxicity in rats resulted in an improvement of the antioxidant defense system.[20]

In ***conclusion*** the present study demonstrated that *Nigella sativa* co-administration with AmB caused significantly reduced serum urea and creatinine, indicating that considerable protection is afforded by *Nigella sativa* extract. However, when mice were given NS extract after the period of treatment with AmB, the toxic effects of the drug persisted thus indicating that NS did not reverse the toxic effects produced earlier by the drug. Further, investigations on the mechanism of action of NS extract are required and may possibly be used as an adjunct to the treatment of patients with renal impairment.

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