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COMPARISON OF AMELIORATIVE EFFECTS OF ESSENTIAL OIL AND ETHANOLIC EXTRACT OF *NIGELLA SATIVA* SEED IN ACETAMINOPHEN INDUCED LIVER DAMAGE.

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ABSTRACT

Adverse drug effects are a major limitation of modern medical practices. Herbs play an important role in the management of various liver disorders. This study was designed to investigate the hepatoprotective activity of ethanolic extract and essential oil of *Nigella sativa* (kalonji) in paracetamol induced acute liver toxicity in rats. For this purpose fasted male Wistar rats, 225-250 g in weight, were orally treated with *Nigella sativa* extract (NSE) in graded doses, silymarin or vehicle for 7 days followed by paracetamol (PCM) 3 g/kg on 8^{th} day. 24 hrs after PCM administration rats were sacrificed and histopathology of liver sample was done. Blood was withdrawn before and after the treatment for estimation of circulatory liver markers using semiautoanalyzer. PCM caused a significant increase in serum alkaline phosphatase, glutamic pyruvic transaminase and glutamic oxaloacetic transaminase levels, which were significantly ameliorated in groups receiving NSE or silymarin in a dose dependant manner. Liver histopathology showed marked reduction in sinusoidal dilatation, midzonal necrosis, portal triditis and occasional apoptosis in NSE treated group as compared to group receiving only PCM. The present results indicate that NSE possesses hepatoprotective action against PCM induced acute hepatoxicity. Further research is needed to advocate its use in prevention of drug induced hepatotoxicity.

KEYWORDS : NIGELLA SATIVA, HEPATOTOXICITY, PARACETAMOL

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INTRODUCTION

Among the promising medicinal plants, Nigella sativa, also known as Black seeds and Black cumin, has been called the "Blessed Seed" for its miraculous curing ability. The results of extensive pharmacological studies justify the broad, traditional therapeutic value of Black Seeds. These studies found Black Seed to have analgesic[1] antilipemic[2, 3] post coital contraceptive[4], diuretic and antihypertensive[5], bronchodilator and calcium antagonist[6], histamine release inhibitor[7], hepatoprotective[8], anthelmintic[9], antifungal[10], antimicrobial (against a wide range of organisms) [11], anticancer[12], and anti-inflammatory activities[13]. Drug-induced hepatic injury is the most frequent reason cited for the withdrawal from the market of an approved drug. Although considered safe at therapeutic doses, in overdose, acetaminophen produces a centrilobular hepatic necrosis that can be fatal[14]. Recently U.S. Food and Drug Administration (FDA) asked drug manufacturers to limit the strength of acetaminophen in prescription drug products. In addition, a Boxed Warning is highlighting the potential for severe liver injury and a warning is highlighting the potential for allergic reactions (e.g., swelling of the face, mouth, and throat, difficulty breathing, itching, or rash) are being added to the label of all prescription drug products that contain acetaminophen. This gives rise to an urgent need for searching an agent for better pharmacotherapy for acetaminophen induced hepatotoxicity. Since oxidative stress play an important role in acetaminophen-induced hepatotoxicity [15, 16, 17] and Nigella sativa possess strong anti-oxidative properties, therefore, it was hypothesized that Nigella sativa oil and extract could protect against acetaminophen-induced hepatotoxicity by improving GSH level. To investigate this hypothesis, this study was undertaken to investigate whether or not pretreatment of oral Nigella sativa oil and extract ameliorates acetaminophen-induced acute hepatotoxicity as well as authors establish a comparative evaluation between oil and extract for the same.

METHODS

Plant extraction and oil

In the present study we used seeds of *Nigella sativa* which is used by local population. 250 gm of *Nigella sativa* seeds were purchased from the local market of Lucknow and authenticated by a botanist at National botanical research institute, Lucknow(NBRI). Seeds was dried in shade for 7 days and then ground. The grounded seeds were then extracted with 99% ethanol. The mixture was left in closed jar for 15 days with periodic stirring. After 15 days the mixture was filtered using wattmann's filter paper no.1, and then the mixture was left in an open jar for 4 days to allow evaporation of ethanol. The final product was a dark brown coloured that bears *Nigella* aroma. The extract was stored in 50ml closed glass tubes away from direct light sources at 4^{o}c till further use. *Nigella sativa* oil was purchased from Mohammedia products, a GMP certified company, Hyderabad, Andhra Pradesh.

Animals

Male Wistar rats, 225-250 g in weight, were purchased from central drug and research institute (CDRI), Lucknow(U.P.), India, and housed in a temperature controlled room (21±1°C) with a 12 hr light-12 hr dark cycle; and allowed free access to a standard rat chow and filtered tap water for at least 5 days of acclimatization period. The solid food, but not the water, was removed 12 hours prior to an experiment. The study received the approval of the Institutional Animal ethics Committee of Era's Lucknow medical college & hospital, and the animals were cared for in accordance with the internationally accepted principles for laboratory animal use and care.

Treatment and samples

The rats were randomly divided into 8 groups, and were treated as follows, The control group received normal saline for 9 days. The paracetamol group animal received normal saline for 6 days, then paracetamol 3mg/kg on day 7 and 8 consecutively at the dose of 3 g/kg. *Nigella sativa* extract group's animal received *a Nigella sativa extract* (NSE) for 6

days at the dose of 1ml/kg or 2ml/kg and then paracetamol at 7 and 8 days consecutively at the dose of 3 g/kg. Nigella sativa oil group's animal received *Nigella sativa* oil (NSO) for 6 days at the dose of 1ml/kg or 2ml/kg and then paracetamol at 7 and 8 days consecutively at the dose of 3 g/kg. Nigella sativa oil or extract alone group animals received NSO or NSE for 6 days in the dose of 2ml/kg and 2ml/kg respectively. Administration of all doses was done with the help of oral feeding tube. At the end of the study (at the end of 9 days), the rats were sacrificed and dissected. The occurrence of hepatocellular damage induced by paracetamol (3 g/kg) and the influence of a pretreatment with NSE was investigated by measuring the hepatic ALP, SGOT, SGPT and reduced glutathione into the circulation. Blood and liver tissue samples were taken for biochemical investigations.

Biochemical Study

Measurements of serum Alkaline phosphatase (ALP), Serum glutamic oxaloacetic transaminase (SGOT), and Serum glutamic pyruvic transaminase (SGPT) and total bilirubin were determined using a semi auto analyser.

Liver homogenate

A liver homogenate for the assay of GSH was prepared by mixing some part of liver sample with 4 times its volume of 25% metaphosphoric acid plus 14 times its volume of PBS, pH 8.0, followed by homogenization on ice with the help of a manual electric blender. The suspension was centrifuged at 5,000 rpm and 4oC for 6 min, and the resulting supernatant was stored on ice. For the remaining assays, a liver homogenate was prepared by mixing a part of liver sample with 20 times its volume of 0.01% phenylmethylsulfonyl fluoride in buffer pH 7.0 followed by homogenization with a hand held electric blender while on an ice bath. The suspension was centrifuged at 12,000 rpm and 4°C for 25 min.

Assay of GSH

The concentration of GSH in a liver sample was measured by a fluorometric method that uses OPT as a fluorescent reagent [34]. An aliquot of liver homogenate in 25% metaphosphoric acid (or plasma) was mixed with 9 times its volume of 10 mM phosphate buffer pH 8.0, and an aliquot of this mixture was treated with an equal volume OPT solution (1 mg/ml). After standing at ambient temperature for 15 min, the fluorescence of the solution was measured on a fluorometer set at an emission wavelength of 420 nm and an excitation wavelength of 350 nm.

Statistical analysis

The different groups were compared using ANOVA followed by post hoc dunnett's test. All test were performed using SPSS (17.0 version). P value <0.05 was considered significant.

RESULTS

The effects of *N. sativa* on PCM (3 g/kg p.o.) induced hepatotoxicity in rats were evaluated By recording changes in serum ALP, SGOT, SGPT and GSH levels. Administration of PCM caused a significant increase in ALP, SGOT, SGPT and total Bil. And a significant decrease in GSH as compared to control. Pre-treatment with NSE as well NSO significantly prevented the increase in liver enzymes and total bilirubin and the decrease in GSH level as compared to PCM group in a dose dependent manner. NSE was found to be more effective than NSO in all the doses tested. All the parameters were found to be not significantly different from control group in NSE pre treated groups. ALP, total bilirubin and GSH levels were found to be similar to control group in NSO treated rats. However SGOT levels in NSO1 group and SGPT levels in NSO1 and NSO2 groups were significantly elevated in comparison to control. On the other hand, ALP, SGOT, SGPT and GSH of animals treated with N. sativa alone remained within normal levels as that of the control group. No significant difference in all the tested parameters was seen in groups receiving NSO or NSE only.

Table.1

Groups	ALP		SGOT		SGPT		Total bil.		GSH	
	mean±SD	95%CI	mean±SD	95%CI	mean±SD	95%CI	mean±SD	95% CI	mean±SD	95% CI
Control	[B]70.92±1.58	69.60-72.24	[A]39.85±1.79	38.35-41.35	[B]38.22±2.21	36.36-40.07	[A].12±1.76	.11-.14	[B]50.23±1.59	48.90-51.57
PCM	[2]248.25±62.07	196.36-300.1	[1]184.60±64.72	130.49-238.71	[2]177.86±19.59	161.48-194.23	[1].57±.22	.40-.76	[2]40.28±1.07	39.38-41.18
NSE(1 ml/kg)+PCM	[B]76.54±6.67	70.96-82.12	[A]42.73±4.41	39.04-46.41	[B]41.00±2.84	38.63-43.38	[A].15±2.07	.13-.16	[B]57.54±2.42	55.51-59.57
NSE(2 ml/kg)+PCM	[B]72.63±4	69.30-75.98	[A]40.51±3.3	37.75-43.27	[B]38.54±2.3	36.61-40.46	[A].14±1.55	.13-.15	[B]57.53±6.09	52.44-62.62
NSO(1 ml/kg) +PCM	[B]82.12±9.96	73.80-90.45	[2,A]47.08±2.46	45.03-49.14	[1,B]43.29±1.96	41.65-44.93	[A].14±1.3	.13-.15	[B]54.01±2.70	51.76-56.26
NSO(2 ml/kg) +PCM	[B]76.07±5.54	71.43-80.70	[A]42.26±5.64	37.54-46.98	[2,B]80.67±2.56	78.53-82.81	[A].14±1.51	.13-.15	[B]56.04±3.30	53.29-58.40
NSE(2ml/kg)	74.67±2.52	72.56-76.78	[A]37.36±1.53	36.08-38.64	[B]35.58±1.31	34.48-36.67	[A].12±1.46	.11-.13	[B]50.64±1.79	49.15-52.14
NSO(2ml /kg)	[B]71.75±2.33	69.80-73.71	[A]39.48±1.37	38.34-40.63	[B]34.67±3.25	31.95-37.38	[A].12±1.06	.11-.13	[B]49.14±3.35	46.34-51.95

[1]*P<0.05 in comparison to control*
[2]*P<0.005 in comparison to control*
[a]*P<0.05 in comparison to PCM*
[b]*P<0.005 in comparison to PCM*

DISCUSSION

Our data of present study strongly suggest that oral administration of NSO and NSE might protect the liver against toxicity induced by PCM (3 mg/kg, p.o.) in male Wistar rat. The hepatotoxicity induced by PCM manifested biochemically by significant elevation of serum enzyme activities such as ALP, SGOT and SGPT, and decrease in GSH level. Marked histopathological destruction of hepatic structure further evidenced the liver cell damage. Pretreatment of animal with NSO, NSE (1ml/kg, 2ml/kg p.o.) significantly ameliorates the elevated level of these enzymes which was even comparable with control group. When used in overdoses it is well known that acetaminophen (APAP) is a common cause of morbidity and mortality in humans. Therapeutic doses of the analgesic drug acetaminophen (APAP) are readily detoxified by hepatic phase II drug metabolizing systems mediating glucuronidation and sulfation[20], with a small portion undergoing a cytochrome P-450-mediated bioactivation to the highly reactive electrophilic arylating intermediate N-acetyl-p-benzoquinoneimine (NAPQI)[21]. In rats and humans, NAPQI is detoxified principally by conjugation with reduced glutathione (GSH) under spontaneous or glutathione Stransferase (GST)-mediated conditions to the 3-glutathione- S-yl-APAP conjugate[20]. In the event of the intake of an overdose of APAP, the increased production of NAPQI rapidly overwhelms GST, eventually exhausts GSH, UDP-glucuronic acid and inorganic sulfate[22], inhibits GSH synthesis[22,23] and decreases cytosolic GST activity[24]. More importantly, this APAP metabolite is a major cause of hepatocellular damage, centrilobular hepatic necrosis and even fatalities upon entering in adduct formation with liver macromolecules, especially proteins[25]. It appears to be fairly well accepted that in acetaminophen-induced hepatotoxicity GSH plays a key role in the detoxification of the reactive toxic metabolites of acetaminophen, and that liver necrosis begins when GSH stores are markedly depleted[26]. As expected acetaminophen

treatment caused the remarkable depletion of cellular GSH level in present study. This may be related to its direct conjugation with acetaminophen metabolite, NAPQI and/or to acetaminophen-induced lipid peroxidation. Our results agree with the other reports pertaining to acetaminophen-induced GSH depletion. Present data clearly states that Nigella sativa improves GSH level which is in conformity to the well established results of previous studies[27, 28].

APAP may also cause hepatotoxicity by mechanisms leading to the formation of reactive oxygen species (ROS), such as superoxide anion (O2 -•), hydrogen peroxide (H2O2) and hydroxyl radical (HO•), reactive nitrogen species (RNS), such as nitric oxide and peroxynitrite (ONOO-), and peroxidation reaction products[25,29,30]. Burits M et al.[31] was tested the essential oil of black cumin seeds, Nigella sativa L., for a possible antioxidant activity. They have showed that thymoquinone and the components carvacrol, t-anethole and 4-terpineol demonstrated respectable radical scavenging property. These four constituents and the essential oil possessed variable antioxidant activity when tested in the diphenylpicrylhydracyl assay for non-specific hydrogen atom or electron donating activity. A large number of studies already presented to provide enough evidences about hepatoprotective activity of Nigella Sativa plant and its active constituents Thymoquinone against CCL_4 induced liver damage. In the present study authors were induced the liver cell injury by Acetaminophen, one of the most selling over the counter drug in India, which was associated with a significant increase in hepatic ALP, SGOT and SGPT and intense tissue destruction with this dose. These findings are consistent with previous reports[32]. In conclusion, NS is effective in protecting rats against acetaminophen induced hepatotoxicity possibly via increased resistance to oxidative stress and by reverse cellular damage. This study provides a notion to use this plant's active constituent as a prophylactic measure in liver diseases, but to stem these results further research is required in another species and also clinical trials before it can be used in human. The main limitations of the study is that the only two doses of oil and extract have been used, but for establish dose-response relationship three or more doses are required and also in the present study NSO and NSE was supplemented for 6 days before acetaminophen. Therefore, another study is required to find out if plant will be curative when given after acetaminophen-induced hepatotoxicity.

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