Orginal Article

Hepatoprotective Effect of Nigella Sativa Linn (Kalajira) On Paracetamol-induced Liver Damage

D R Pal[1], S Nahar[2], K R Rimi[3], S A Talukder[4],
M M Hossain[5], P C Paul[6], E O Eva[7]

Abstract

Paracetamol is the widely used non-steroidal anti-inflammatory drugs for the treatment of mild to moderate pain. It causes hepatotoxicity in therapeutic dose for prolonged time. It can induce centrilobular hepatic necrosis in large over doses. Nigella sativa (kalajira) is a medicinal plant has a protective role against hepatotoxicity. Therefore, the present study was designed to observe the protective role of Nigella sativa on paracetamol induced liver damage biochemically in Long Evans rats. The experiment was carried out in the Department of Anatomy, Dhaka Medical college, in the period of July 2003 to June 2004. Sixty matured Long Evans rats of either sex, age of 10-12 weeks and weighing between 150-200 gm were used in this study. They were divided into four equal groups. Group A was vehicle (distilled water) control group, Group B was Nigella oil treated group, Group C was paracetamol treated group and Group D was Paracetamol & Nigella sativa oil treated group. Paracetamol in a single dose of 3gm/kg body weight orally causes hepatotoxicity which was assessed bio-chemically. Nigella sativa oil at a dose of 800mg/kg body weight was administered orally for 4 weeks. It was found that significant elevation of serum alanine aminotransferase, serum aspartate aminotransferase, serum alkaline phosphatase and serum bilirubin level in paracetamol treated group. It was observed that 4 weeks oral treatment of Nigella sativa oil in Group D, decrease the level of serum alanine aminotransferase, serum aspartate aminotransferase, serum alkaline phosphatase and serum bilirubin. The result revealed that Nigella sativa oil able to give protection against paracetamol induced liver damage. However, more sophisticated biochemical studies like glutathione content and malondialdehyde level should be studied further.

Key words: Paracetamol, Nigella sativa

Introduction

Many environmental factors, chemicals, drugs and other contaminated food affect the liver physiology up to a certain extent, which may lead to other secondary physiological changes.[1] Inappropriate use of drugs, excessive consumption of alcohol and certain toxin lead to a various kind of liver disorders. In spite of tremendous research in modern medicine, there are hardly any drugs that stimulate liver functions, offer protection to the liver from damage or help regeneration of hepatic cells.[2] Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used of all therapeutic agents. They are frequently prescribed for rheumatic musculoskeletal complaints and are often taken without prescription for minor aches and pain. Paracetamol is one of the most important and also popular drugs among NSAIDs. It can causes hepatotoxicity in a low dose and therapeutic dose for prolong time.[3] It can induce centrilobular hepatic necrosis in large overdoses.[4] Use of herbal drugs in the treatment of liver diseases has a long tradition, especially in eastern medicine5. It is a widely held belief that herbal preparations are natural and are therefore intrinsically harmless.[6] Nigella sativa (N. sativa) is an herbaceous plant commonly known as black seed, belongs to botanical family of Ranunculaceae.[7] It is commonly used as a natural food additive. Seeds of N. sativa are frequently used in folk medicine in the Middle East and some Asian countries for the promotion of good health & treatment of many diseases. In recent years, it has been suggested that oil of N. sativa has protective role against carbon tetrachloride and D-galactosamine-induced hepatic damage in rats.[8] In rabbits, prior administration of N. sativa has prevented experimental liver cirrhosis and fibrosis induced by carbon tetrachloride.[9] It has been reported that N. sativa oil protects liver against Schistosoma mansoni induced liver damage.[10] Thymoquinone is the main constituent of N. sativa essential oil.[11] The possible hepatoprotective action of thymoquinone is related to preservation of intracellular glutathione[12]. It has been reported that thymoquinone protects organs against oxidative damage induced by free radical generating agents and conditions like CCl4-hepatotoxicity.[13] cisplantin nephropathy[14], Diabetes mellitus.[15] Therefore, in the present study, hepatoprotective effects of N. sativa were examined bio-chemically.

Materials and Methods

The experiment was carried out on 60 adult healthy Long Evans rats of both sexes. They were 10-12 weeks and weighing between 150-200gm and was kept in metallic cages in the animal house of the department of Pharmacology, Dhaka Medical College. They were allowed to live at room temperature with standard rat feed and water. Nigella sativa linn: The oil of Nigella sativa was used. Extraction of N. sativa oil from N. sativa seeds: N. sativa seeds were cleaned, washed in water and dried. The seeds were crushed in a blender. Dried and powdered seeds (1000) were extracted with petroleum ether (40-60ºC)

1. Dr. Dipali Rani Pal, Associate Professor and Head, Department of Anatomy, Jahurul Islam Medical College.
2. Dr. Shamusn-Nahar, Associate Professor(c.c.), Department of Anatomy, Rangpur Community Medical College.
3. Dr. Kanta Roy Associate Professor(c.c.), Department of Anatomy, Dinajpur Medical College.
4. Dr. Shah Alam Talukder, Professor (Retd.), Department of Anatomy, Shaheed Sawardhy Medical College.
5. Dr. Md. Motahar Hossain, Professor and Head, Department of Anatomy, Ibrahim Medical College.
6. Dr. Paritosh Chandra Paul, Professor and Head, Department of Pharmacology, Jahurul Islam Medical College.
7. Dr Eliza Omar Eva, Associate Professor(c.c.), Department of Pharmacology, Shaheed Sawardhy Medical College.

Corresponding Author

Dr. Dipali Rani Pal, Associate Professor and Head, Department of Anatomy, Jahurul Islam Medical College. e-mail:

(4×3L) at room temperature for 72 hours in the BCSIR Laboratory. The solvent was removed from the extract under reduced pressure (40ºC). Evaporation of petroleum ether left brownish residue (23.1%).

Experimental procedure

The animals were divided into four groups consisting of 15 rats each. Grouping of animal, their dietary and drug allotment is presented in table-I.

On 30th day after being fasted overnight except water, 2cc blood was withdrawn from the heart by a 5cc disposable syringe. The blood was collected in plain test tube for estimation of serum bilirubin, serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST) and serum alkaline phosphatase (ALP). The collected blood samples were kept vertically in a test tube rack for separation of serum. Serum was prepared from the collected blood samples by centrifuging the blood at 300 rpm for 8 minutes.

Statistical evaluation

Students unpaired't' test was performed to evaluate the degree of significance.

Results

Serum bilirubin, ALT, AST and ALP level in different groups of rat is presented in Table-II. As evident from study, the mean serum bilirubin, ALT, AST and ALP level was normal in Group-A (0.80±0.18, 18.26±5.24, 25.26±5.09 and 8.90±1.85 accordingly) and in Group-B (0.88±0.12, 17.86±4.92, 24.80±5.83 and 8.74±1.72 accordingly). In Group-C, serum bilirubin, ALT, AST and ALP

level (3.28±0.61, 71.33±5.71, 68.40±7.67, 31.40±4.07 accordingly) was significantly elevated after paracetamol administration. These elevated serum level was significantly antagonized by prior administration of N. sativa oil (800mg/kg body weight) in Group-D animals. Serum bilirubin, ALT, AST and ALP level (0.84±0.13, 37.20±5.69, 37.93±6.61, 15.61±3.15 accordingly) was decrease in Group-D as compared to Group-C.

Table I: Grouping of the animals, doses of drug/vehicle and Nigella sativa oil, duration of experiment and sacrificing schedule.

Group	Drug	Dose	Route of administration	Duration of treatment	Day of sacrifice
A (n=15)	Distilled water	3 cc	oral	Single dose	30 th day of experiment
B (n=15)	*Nigella sativa* oil	800mg/kg bw		Daily dose for 28 days	
	Distilled water	3 cc		On 29 th day	
C (n=15)	Paracetamol Distilled water	3gm/kg bw		Single dose on 29 th day	
D (n=15)	*Nigella sativa* oil	800mg/kg bw		Daily dose for 28 days	
	Paracetamol	3gm/kg bw		Single dose on 29 th day	
	Distilled water	3 cc			

Table-II: Serum bilirubin, ALT, AST, ALP level in different groups of rats

Group	No of rats	Serum bilirubin mg/dl Mean±SD	Serum ALT U/L Mean±SD	Serum AST U/L Mean±SD	Serum ALP U/L Mean±SD
A	15	0.80±0.18	18.26±5.24	25.26±5.09	8.90±1.85
B	15	0.88±0.12	17.86±4.92	24.80±5.83	8.74±1.72
C	15	3.28±0.61	71.33±5.71	68.40±7.67	31.40±4.07
D	15	0.84±0.13	37.20±5.69	37.93±6.61	15.61±3.15

Discussion

The present study, hepatotoxicity was induced by single oral administration of paracetamol (3gm/kg body weight). The dose was selected according to Sharma et al.16 N. sativa oil was used at a dose of 800mg/kg body weight/day for 28 days. The dose, route of administration and duration was selected according to El-Dakhakhny et al.[8] The result of bio-chemical studies suggested that paracetamol 3gm/kg body weight, in a single dose produced hepatotoxicity which was assessed by significant (P<0.001) increase in serum bilirubin, ALT, AST and ALP in Group-C animals. Same enzyme level elevation was found by Davidson and Eastham in human.[17] Boyer and Rouf.[18] Johnson and Tolman[3], Gerber et al.[19] In the present study, N. sativa oil at the given dose has no significant bio-chemical changes in Group-B as compared to Group-A. This result agrees with that reported by Tenekoon et al.[20] In this study, effects of N. sativa oil on paracetamol-induced hepatotoxicity were evaluated bio-chemically. 28 days treatment with N. sativa oil in Group-D, the mean serum bilirubin, ALT, AST and ALP level was significantly (P<0.001) lower as compared to Group-C. Data of the present study were closely associated with the result done by El-Dakhakhny et al.[8] Al-Gharably et al[13] and Nagi et al[21] also observed the similar findings measured by ALT level and malondialdehyde (MDA) level. They suggested that thymoquinone (the active constituent of N. sativa volatile oil) is an efficient cytoprotective agent against chemically induced hepatic damage. Meral et al [15] also suggested that N. sativa treatment increase antioxidant defense system.

Conclusion

N. sativa oil did not produce any significant changes in serum enzymes (ALT, AST and ALP) and serum bilirubin level in normal rats. Paracetamol in a toxic dose produces hepatic damage to the liver which can be prevented by N. sativa oil. However, more studies are recommended for establishing as potent, safe and useful antihepatotoxic herbal plant as well as its mechanism of action regarding hepatoprotective properties.

Acknowledgment

I express my deepest regards to my respected teacher, Professor Dr. Md. Motahar Hossain and Professor(Rtd.) Dr. Md. Shah Alam Talukder and also grateful to Mrs. Monowara begum, chief scientific officer, BCSIR and Gour Chandra Saha, senior scientific officer, BCSIR, Dhaka laboratory.

References

1. Sharma S, Tripathi P, Singh VP, Tripathi YB (1995) Hepatoprotective and toxicological evaluation of hepatomed, An aurvedic drug. Indian J Exp Biol; 33: 34-37.

2. Boyd W. (1970) Structure and functions in disease. Text book of Pathology. 8th ed. Lea & Febiger, Philadelphia. 359.

3. Johnson GK, Tolman KG (1977) Chronic liver disease and acetaminophen. Ann Intern Med; 87. 302.

4. Legros J (1976) Animal studies-a theoretical basis for treatment. J Int Med Res; 4, supple (4) 46-54.

5. Schuppan D, Ji-Dong Jia, Brinkhaus B, Hahn EG(1999) Herbal products for liver disease; A therapeutic challenge for new millennium. Hepatology.

6. Bateman J, Chapman RD, Simpson D (1998) possible toxicity of herbal remedies. Scott Med J; 43: 7-15.

7. Saad SI (1975) Classification of the flowering plants. The General Egyptian Book Company, Alexandria; 412-413.

8. El-Dakhakhny M, Barakat M, Abd El-Halim M, Aly SM (2000) Effects of Nigella sativa oil on gastric secretion and ethanol induced ulcer in rats. J Ethnopharmacol; 72: 299-304.

9. Turkdogan MK, Agaoglu Z, Yener Z, Sekeroglu R, Akkan HA, Avci ME (2000) The role of antioxidant Vita mins (C and E), selenium and Nigella sativa in the prevention of liver fibrosis cirrhosis in rabbits: new hopes Dtscch Tierarztliche Wochenschr; 108: 71-73.

10. Mahmoud MR, El-Abhar HS, Saleh S (2002) The effects of Nigella sativa oil against the liver damage induced by schistosoma mansoni infection in mice. J Ethnopharmacol ; 79: 1-11.

11. Lautenbacher LM (1997) Schwarzkummelol. Dtsch Apoth Ztg; 137: 68-69.

12. Daba MH and Abdel Rahman MS (1998) Hepatoprotective activity of thymoquinone in isolated rat hepatocytes. Toxicol lett; 95: 23-29.

13. Al-Gharably NM, Badary O, Nagi M, et al. (1997) Protective effect of thymoquinone against carbon tetrachloride-induced hepatotoxicity in mice. Res Comm Pharmacol Toxicol; 2: 41-50.

14. Badary OA, Nagi MN, Al-Shabanah OA, Al-Shawaf HA, Al-Sohaibani MO, Al-Bekairi AM (1997 Thymoquinon ameliorates the nephrotoxicity induced by cisplantin in rodents and potentiates its antitumor activity. Can J Physiol Pharmacol; 75: 1356-1361.

15. Meral I, Yener Z, Kahraman T, Mert N (2001). Effect of Nigella sativa on glucose concentration, lipid peroxidation, anti-oxidant defence system and liver damage in experimentally induced diabetic rabbits. J Vet Med Physiol Pathol Clin Med; 48: 593-599.

16. Sharma S, Bhatia A, Das PK (1983) Role of microsomal drug detoxifying enzyme systems in paracetamol induced liver injury in rats. Indian J Med Res; 78: 134-141.

17. Davidson DGS and Eastham WN (1966) Acute liver necrosis following overdose of Paracetamol. BMJ; 2:497-499..

18. Boyer TD and Rouff SL (1971) Acetaminophen induced hepatic necrosis and renal failure. JAMA; 218: 440-441.

19. Gerber MA, Kaufmann H, Klion F et al. (1980) Acetaminophen associated hepatic injury. Human Pathol; 11: 37-42.

20. Tenekoon KH, Jeevathayaparan S, Kurukulasooriya AP, Karunanayake EH (1991) Possible hepatotoxicity of Nigella sativa seeds and Dregea volubilis leaves. J Ethnopharmacol; 31: 283-289.

21. Nagi MN, Alam K, Badary OA (1999). Thymoquinone protects against carbon tetrachloride-induced hepatotoxicity in mice via an antioxidant mechanism. Biochem Mol Biol Int; 47: 143-159.