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PROTECTIVE ROLE OF *NIGELLA SATIVA* OIL IN ISONIAZID INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT

Objectives: Hepatotoxicity is one of the major side effects of the first line anti-tubercular drug Isoniazid. *Nigella sativa* oil is a well researched medicinal plant with reported hepatoprotective activity. The aim of the present study was to evaluate the protective role of *Nigella sativa* oil in hepatotoxicity induced by using Isoniazid. **Methods:** Isoniazid (50mg/kg) was used to induce hepatotoxicity in rats. Silymarin (50mg/kg) was used as a standard drug for this study. Silymarin and two doses of N.sativa oil (0.5ml/kg and 1ml/kg) were given for 31 days; isoniazid was started on the 4th day of the study. All drugs were given orally. After 31 days blood samples were collected from the animals for biochemical analysis and liver tissues were subjected to histopathological examination. **Results:** *N.sativa* oil significantly reduced the liver enzymes and total bilirubin when compared to the negative control group. There was also significant improvement in the histopathological scores in *N. sativa* oil treated group when compared to the negative control group. **Conclusion:** Present study throws light on the usefulness of Nigella sativa oil in hepatotoxicity induced by isoniazid in a dose dependant manner.

KEY WORDS: hepatoprotective, Silymarin, N.sativa.

1. INTRODUCTION

The liver is a vital organ for sustenance of life; it performs many important biochemical and metabolic functions efficiently regulating the internal homeostasis of the body. The different functions of the liver include plasma protein synthesis, production of essential biocemicals (bile) for digestion and also detoxification of substances, which if gets accumulated would be injurious to the living organism. Though the liver effectively maintains the internal chemical environment of an organism, it is also susceptible to host of ailments like hepatitis of different etiologies, cirrhosis of liver, alcohol-related disorders and carcinomas. The functioning of liver as an efficient detoxification unit renders it susceptible to injury by the various toxins which it degrades, neutralizes or eliminates.[1] Drug induced liver injury has been identified to be a leading cause of hepatic dysfunction. The mechanisms of the hepatic injury in most of the cases of drug induced liver damage remains unknown. Few suggested mechanisms are direct injury to the hepatocytes by the drug or its metabolite by generation of free radicals and reactive oxygen species.[2] In some cases the offending drug might trigger an autoimmune reaction targeting different elements of the hepatic tissue leading to disruption in the physiologic functions of the liver.[3]

Antitubercular drugs are major cause of drug induced liver injury. The first line antitubercular drugs having the highest hepatotoxic potential are isoniazid and pyrazinamide.[4] The hepatotoxicity of isoniazid is usually underreported but it has still been reported to be a leading cause of drug induced liver damage.[5] Tuberculosis is a prevalent cause of morbidity and mortality in developing countries like India and the drug isoniazid becomes indispensible for management of this disease as it finds its use in both treatment and prophylaxis of tuberculosis. Despite recent advances in the field of hepatology and medicinal research, there is no effective treatment for majority of the liver ailments. Most of the agents in use are derived from plants with reported hepatoprotective potential.[6]

The seeds of the plant Nigella sativa have been used since ancient time for the treatment of different diseases in various systems of folk medicine.[7] The seeds of this plant have been reported to possess different pharmacological activities like analgesic, anti-inflammatory, anti-ulcer and nephroprotective.[8-10] The potential of N.sativa seeds in alleviating hepatic damage has also been explored.[11-13] But studies demonstrating the hepatoprotective nature of N.sativa against antitubercular drug mediated hepatic damage are few. In

view of the above, the present study was planned to explore the protective effect of N.sativa seed oil in hepatic damage induced by isoniazid.

2. MATERIALS AND METHODS

2.1 Experimental Animals

Adult wistar albino rats of either sex (150-200g) were obtained from Central Animal House, Jawaharlal Nehru Medical College; Aligarh Muslim University, Aligarh, U.P. They were housed under standard conditions (temperature 27 ± 2^0 C, Humidity 30-70% & 12 hour light/dark cycles). They were fed with standard pellet diet and water ad libitum. The rats were acclimatized to the laboratory condition for 1 week prior to the experiments. The study protocol was approved by the Institutional Animal Ethics Committee (IAEC) on 20.12.2014. All the animal experiments were carried out as per the rules and regulations of IAEC & CPCSEA.

2.2 Drugs and chemicals

- Nigella sativa oil (Kalonji oil) was obtained from (Mohammedia products, Aamir nagar, Shah Sahab Mohalla, Karimnagar – 505001, Telengana State., India).

- Isoniazid Dispersible Tablets (Macleod's Pharmaceuticals Ltd. Mumbai, Maharashtra India).

- Normal Saline (Swaroop Pharmaceuticals Pvt. Ltd. Aligarh).

- Silymarin suspension (Micro Labs Ltd. Bengaluru, Karnataka, India) was used as the standard drug for the purpose of this study.

Diagnostic kits used for the purpose of this study were Bilirubin kit (Accurex Biomedicals pvt. Ltd.,Mumbai, India), Aspartate transaminase (AST), Alanine transaminase (ALT) kits - Span Diagnostics Ltd. (Surat, India.) and Alkaline Phosphatase (ALP) kit – Beacon Diagnostics, Gujrat India.

2.3 Induction of hepatotoxicity

Hepatotoxicity was induced in the animals by using isoniazid 50mg/kg orally for 28 days.[14]

2.4Experimental design

The animals were divided into 5 groups containing 6 animals each. The dispersible tablets of isoniazid were dissolved in normal saline (50mg/ml) before administration. Silymarin, the standard drug was also given orally in a dose of 50mg/kg.[15] Nigella sativa oil 0.5ml/kg and 1ml/kg and silymarin suspension were administered for 31 days. Isoniazid in the dose of 50mg/kg orally was administered from 3rd day to 31st day of treatment in groups II, III, IV and V. The standard and test drugs were given 30 minutes before the administration of isoniazid. On the 32nd day, the rats were anaesthetized by pentobarbitone, blood samples were collected from the animals by cardiac puncture for estimation of serum AST, ALT, total billirubin and ALP. The animals were then sacrificed, liver dissected out and subjected to histopathological examination.

2.5 Biochemical analysis

The blood samples were collected by cardiac puncture (open approach) and centrifuged at 5000 rpm for 10 minutes, plasma was separated and subjected to biochemical analysis.Total bilirubin was estimated using the method described by Jendrassik L. et al 1938[16] using reagent supplied by Accurex Biomedicals Pvt Ltd. India. AST and ALT levels were determined by the Reitman and Frankel[17] method using kits acquired from Span Diagnostics Ltd. (Surat, India). Serum ALP levels were estimated by King's method[18] using the ALP determination kit procured from Beacon Diagnostics, Gujrat India.

2.6 Histological Examination

The liver samples were preserved in 10% formalin for 48 hours, fixed in paraffin. The tissue samples were processed according to standard histological techniques and stained with hematoxylin and eosin.[19] The assessment of damage of liver tissue was done by method described by described by Davidson C.S. 1979.[20] The various parameters used were degeneration, necrosis, fibrosis and regeneration.

The percent of hepatoprotection offered by the standard and test drug was calculated using the formula

$$H = \left[1 - \left(\frac{T - V}{C - V}\right)\right] \times 100$$

Where H = Percentage of hepatoprotection, T = Mean value of group treated with test drugs, C = Mean value of group treated with Isoniazid, V = Mean value for normal control group animals.

2.7Statistical analysis

The data of study are expressed as Mean ± Standard Error of Mean (SEM). The groups were compared by one way analysis of variance (ANOVA) followed by post hoc Tukey's test to analyze the statistical significance. P value of less than 0.05 was considered significant for this study.

3. RESULTS

3.1 Effects of N.sativa oil on the liver function parameters in isoniazid treated rats.

For the purpose of this study total billirubin, AST, ALT and ALP were taken as liver function markers In the negative control group (Group II) total bilirubin ($p<0.001$), AST($p<0.001$) and ALT($p<0.001$) showed significant rise and ALP level was also raised significantly($P<0.01$) in comparison to the normal control group (Group I). The liver transaminases and total bilirubin in the animals of the silymarin co-administered group (Group III) showed significant ($p<0.001$) reduction in comparison to the negative control group (Group II), the ALP values in this group

(Group III) were also significantly ($p<0.01$) reduced when compared to the negative control group (Group II). In the rats of the N. sativa oil 0.5ml/kg group (Group IV), total bilirubin levels showed significant ($p<0.001$) reduction in comparison to the negative control group (Group II). Serum AST and ALT levels also exhibited significant ($p<0.01$) reduction, there was also reduction in the serum ALP levels but was not statistically significant .The animals treated with N.sativa oil 1ml/kg (Group V) exhibited significant ($p<0.001$) reduction in the levels of total billirubin, AST and ALT in comparison to the negative control group (Group II). The reduction in ALP levels in comparison to the negative control group (Group II) was not statistically significant. (Table 1).

Table 1.Prophylactic effect of *N.sativa* oil co-administration in INH treated rats.

Group n= 6	T.Bilirubin (mg/dl)	AST (units/l)	ALT (units/l)	ALP (units/l)
Group I (N.saline 1ml/kg)	0.34(±0.048)	32.00(±4.97)	21.22(±5.27)	30.60(±4.56)
Group II (N.saline1ml/kg+INH50mg/kg)	1.54(±0.20)***	106.02(±7.84)***	145.48(±17.49)***	101.76(±12.50)**
Group III (Silymarin 50mg/kg+INH50mg/kg)	0.46(±0.80)***	41.00(±7.60)***	37.13(±6.49)***	50.26(±6.09)*
Group IV (N.sativa oil 0.5ml/kg+INH50mg/kg)	0.57(±0.05)***	61.98(±12.83)**	78.66(±14.65)**	75.48(±15.83)
Group V (N.sativa oil 1ml/kg+INH50mg/kg)	0.50(±0.10)***	45.92(±5.75)***	42.96(±10.35)***	64.54(±13.86)

All data are expressed as Mean± SE. Negative control group was compared with Normal control group and all other groups were compared with Negative control group, * p< 0.05, **p<0.01 and ***p<0.001 were considered significant

3.2 Percentage of Hepatoprotection offered by N. sativa oil low and high dose in isoniazid induced hepatotoxicity.

The hepatoprotection offered by the test drugs was estimated by formula described earlier. Percentage of hepatoprotection was highest for the positive control group, 90%, 88%, 87% and 72 % for bilirubin AST, ALT and ALP respectively. Among the N. sativa oil test groups hepatoprotection for the low dose group was 81%, 59%, 54% and 37% for bilirubin AST, ALT and ALP respectively and 87%, 81%, 82% and 53% for the high dose group. (Table 2).

Table 2: Percentage of hepatoprotection offered by N. sativa in isoniazid treated rats.

S.N.	Groups n=6	Percentage of Hepatoprotection (%)			
		T. Bilirubin	AST	ALT	ALP
1	**Silymarin**	90	88	87	72
2	**N. sativa oil low dose**	81	59	54	37
3	**N. sativa oil high dose**	87	81	82	53

3.2 Effects of N. sativa oil on the histopathological examination in isoniazid treated rats.

The microscopic architecture of the liver in the normal control group (Group I) showed no degeneration, necrosis or fibrosis. In the isoniazid only group (Group II) the liver histopathological score exhibited significant degeneration ($p<0.001$), necrosis ($p<0.001$) and fibrosis ($p<0.001$) with no regeneration. The degeneration ($p<0.001$), necrosis ($p<0.001$) and fibrosis ($p<0.01$) scores were significantly reduced in the rats treated with silymarin (Group III) and the regeneration ($p<0.001$) scores showed commendable improvement. In N.sativa oil low dose group (Group IV) the degeneration score ($p<0.05$) showed significant reduction, there was a decrease in the necrosis and fibrosis scores but were not statistically significant, though there was significant regeneration ($p<0.05$) in comparison to the negative control group (Group II). In N.sativa oil high dose group (Group V) the degeneration ($p<0.001$) and necrosis scores ($p<0.05$) showed significant reduction, there was also decrease in the fibrosis score but it was statistically insignificant whereas significant regeneration ($p<0.001$) was noted in this group. (Group II). (Table 3).

Table 3. Effect of Nigella sativa oil on histopathological scores in Isoniazid induced hepatotoxicity

Group n= 6	Degeneration	Necrosis	Fibrosis	Regeneration
Group I: (N. saline 1ml/kg)	0	0	0	0
Group II: (N.saline1ml/kg+INH50mg/kg)	2.75±0.25***	2.50±0.29***	2.75±0.25***	0
Group III: (Silymarin 50mg/kg+INH50mg/kg)	0.75±0.25***	0.50±0.29***	1.25±0.025**	1.75±0.25***
Group IV: (N.sativaoil0.5ml/kg+INH50mg/kg)	1.67±0.33*	1.67±0.33	2.33±0.33	1.00±0.00*
Group V: (N.sativa oil 1ml/kg+INH50mg/kg)	1.00±0.00***	1.25±0.25*	1.75±0.25	1.50±0.28***

All data are expressed as Mean± SE. Negative control group was compared with Normal control group and all other groups were compared with Negative control group, * p< 0.05, **p<0.01 and ***p<0.001 were considered significant.

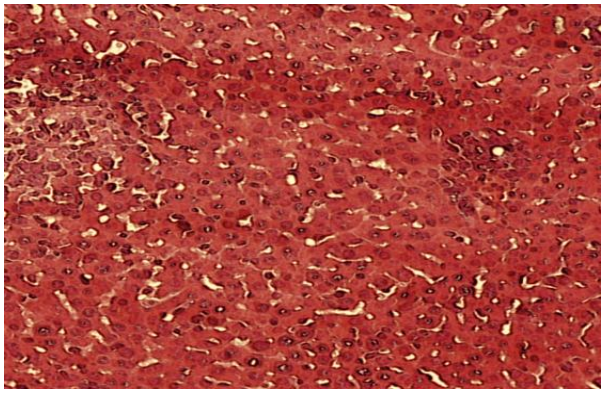

Fig 1.Photomicrograph of rat liver from Group I (Normal Control) showing normal liver microstructure with intact hepatic cords and sinusoids. Hepatocytes show normal contour. (10X. H & E stain).

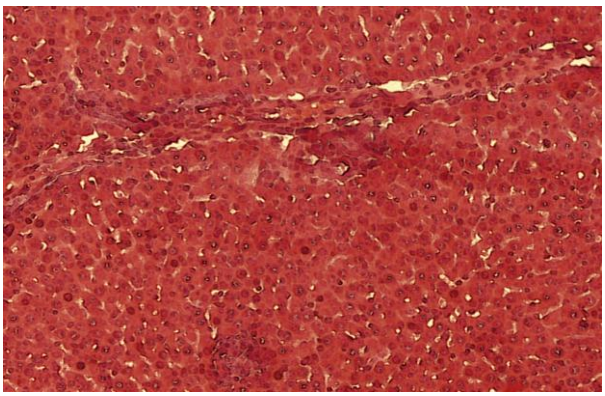

Fig.2 Photomicrograph of rat liver from Group II (Negative Control) shows degeneration of hepatic microstructure. There is also bridging fibrosis and necrosis of the hepatocytes. (10X H & E Stain)

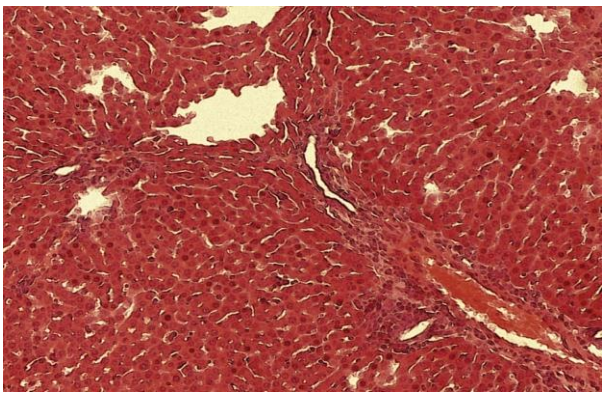

Fig.3 Photomicrograph of rat liver from GroupIII (Silymarin group) showing near normal hepatic microstructure with minimal fibrosis. There are also regenerating nodules along the fibrobitic focii. (10X. H & E stain).

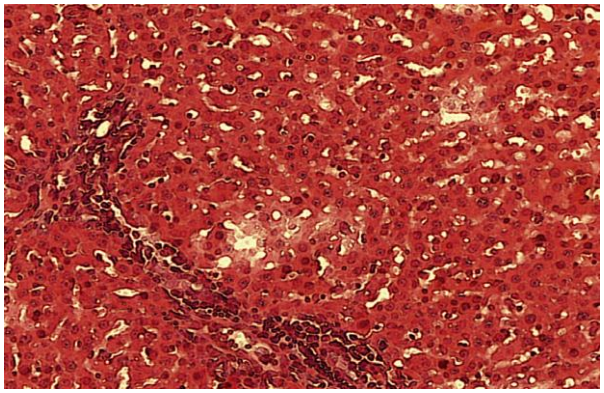

Fig.4 Photomicrograph of rat liver from Group IV (N.sativa oil low dose) showing, occasional hepatocytic degeneration and fibrosis, along with a few regenerating nodules.(10X.H & E stain).

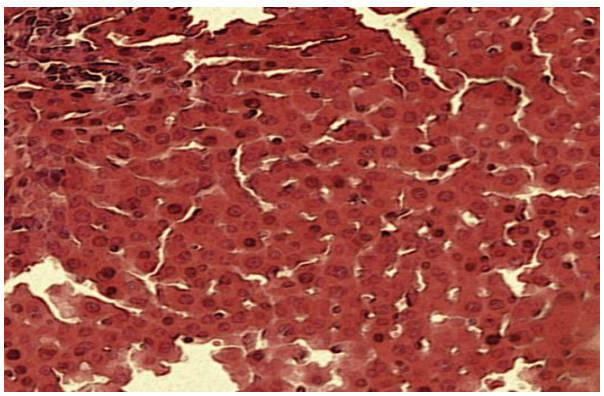

Fig.5 Photomicrograph of rat liver from Group V (N.sativa oil high dose) showing reasonably maintained hepatic microstructure. Occasional hepatocytes show degenerative changes but there are abundant regenerating foci. 10X. H & E stain.

4. DISCUSSION

Isoniazid is a widely used drug for prophylaxis as well as treatment of tuberculosis but significant hepatotoxicity has often been reported with the use of this drug. Hepatotoxicity after isoniazid treatment is encountered in approximately 2% cases, which if not recognized timely can have fatal outcome.[21] Isoniazid gets converted to acetylisoniazid by the enzyme NAT2 which is eliminated by the kidney; acetylisoniazid is further transformed into acetylhydrazine and then to potential hepatotoxic metabolite acetyl diazine by the CYP enzymes which generates reactive acetyl onium ion,acetyl radical and ketene,which causes irreversible damage to the liver tissue.[22]

N.sativa is a well researched medicinal plant and the seeds of this plant have been used in many traditional systems of medicine since antiquity. Many studies have demonstrated the hepatoprotective activity of *N. sativa* plant.[23] There are also reports on hepatoprotective potential of thymoquinone, (the active principle of Nigella sativa seed oil) in antitubercular drug induced hepatotoxicity in rats.[24] So considering the facts the present study was designed to explore the protective role

of Nigella sativa oil in hepatotoxicity induced by isoniazid.

N. sativa oil in both the doses decreased the derangement of liver function parameters but this effect was more pronounced in the high dose i.e. 1ml/kg dose group. The rats treated with Nigella sativa oil 0.5ml/kg there was significant reduction in the serum billirubin, AST and ALT levels, ALP levels though reduced but was not statistically significant in comparison to the negative control group. The percentage of hepatoprotection offered by the 0.5ml/kg dose was 81%, 59%, 54% and 37% for bilirubin AST, ALT and ALP respectively. In the rats treated with Nigella sativa oil 1ml/kg there was also significant reduction in the total bilirubin, AST and ALT levels in comparison to the negative control group. The ALP levels for 1ml/kg group were also reduced but were not statistically significant. The hepatoprotection in percentage for the Nigella sativa oil 1ml/kg group was 87%, 81%, 82% and 53% for bilirubin AST, ALT and ALP respectively which was comparable to the hepatoprotection offered by the standard drug silymarin.
The findings of the biochemical analysis were further supported by histological examination of the liver tissues form the test group. Nigella sativa oil in both the doses protected or maintained the liver tissue morphology evidenced by a reduction in the degeneration, necrosis and fibrosis scores in the histolopathological examination. Regenerative nodules were also seen in the liver samples from both the test groups. So, the findings of the histological analysis also indicate that protection of the hepatocytes against damage induced by isoniazid was higher for the Nigella sativa oil 1ml/kg dose group.

Various mechanisms have been proposed to explain the hepatoprotective activity of Nigella sativa oil and its active principle thymoquinone. It has been suggested that Nigella sativa oil might offer hepatoprotection due to its anti-inflammatory property which might be capable of preventing inflammatory damage to the liver tissue.[25] Also other probable mechanism by which Nigella sativa might be able to prevent the hepatocytic damage may be due to its immunomodulatory and antioxidant activity.[26] Oxidative stress is one the leading mechanisms by which the antitubercular drug isoniazid may inflict damage to the hepatocytic membranes leading to their degeneration and necrosis.[27] The active principle of Nigella sativa seed oil, thymoquine has been reported to possess significant antioxidant and antiperoxidative effects[28] which might counter the oxidative stress to hepatocytes by isoniazid treatment.

The results of our study indicate that treatment with Nigella sativa oil offered significant protection against isoniazid induced hepatotoxicity. The beneficial effect of Nigella sativa oil is well evidenced by significant improvement in the liver function test which was also supported by the results of the histological examination. The findings suggest that Nigella sativa oil supplementation offers protective role in isoniazid induced hepatotoxicity. Further studies are warranted to elucidate the exact mechanism by which Nigella sativa offers hepatoprotection.

REFERENCES

1. Ramadori G, Moriconi F, Malik I,Dudas J. Physiology and pathophysiology of liver inflammation, damage and repair. Journal of Physiology and Pharmacology. 2008; 59(1): 107-117.
2. Khan RA, Khan MR, Sahreen S, Shah NA. Hepatoprotective activity of Sonchus asper against carbon tetrachloride-induced injuries in male rats: a randomized controlled trial. Bio Med Central Complementary and Alternative Medicine. 2012; 12(90): 1-8.
3. Abboud G, Kaplowitz N. Drug-Induced Liver Injury. Drug Safety. 2007; 30(4): 277-294.
4. Durand F, Bernuau J, Pessayre D, Samuel D, Belaiche J, Degott C et al. Deleterious influence of pyrazinamide on the outcome of patients with fulminant or subfulminant liver failure during antituberculous treatment including isoniazid. Hepatology. 1995; 21(4): 929-932.
5. Hayashi PH, Fontana RJ, Chalasani NP, Stolz AA et al. Under-reporting and Poor Adherence to Monitoring Guidelines for Severe Cases of Isoniazid Hepatotoxicity. Clinical Gastroenterology and Hepatology. 2015; 13(9): 1676–1682.
6. Abhilash G, Maheswari YU, Gopal JA, Chanda D. Review on Some Medicinal Plants with Hepato-protective Activities. Research and reviews: Journal OF Pharmacognsoy and Phytochemistry. 2014; 2(2): 10-21.
7. Paarakh PM. Nigella sativa L. A comprehensive review. Indian Journal of Natural Products and Resources 2010; 1(4): 409-429.
8. Rawoof KG, Suresh V, Dey T, Ata S, Singh JSD. Evaluation of antidiabetic, analgesic and antimicrobial activities of Nigella sativa. Contemporary Investigations and Observations in Pharmacy. 2013; 2(1): 13-16.
9. Hasan M N, Khan R A, Nasiruddin M, Khan A A. Protective effect of Nigella sativa against aspirin induced gastric damage in rats. Int J Pharm Pharm Sci., 2014; 6(5): 275-278.
10. Hadjzadeh MAR, Keshavarzi Z, Yazdi SAT, Shirazi MG, Rajaei Z, Rad AK. Effect of alcoholic extract of Nigella sativaon Cisplatin-induced toxicity in rats. Iranian Journal of Kidney Diseases 2012; 6(2): 99-104.
11. Essawy AE, Abdel-Moneim AM, Khayyat L , Elzergy AA. Nigella sativa seeds protect against hepatotoxicity and dyslipidemia induced by carbon tetrachloride in mice. Journal of Applied Pharmaceutical Science. 2012; 2(10): 021-025.
12. Al-Suhaimi EA. Hepatoprotective and immunological functions of Nigella sativa seed oil against hypervitaminosis A in adult male rats.

International Journal for Vitamin and Nutritional Research 2012; 82(4): 288-297.
13. Hassan A.S, Ahmed J .H, Al-Haroon S. S A study of the effect of Nigella sativa (Black seeds) in isoniazid (INH)-induced hepatotoxicity in rabbits Indian Journal pf Pharmacology. 2012; 44(6): 678–682.
14. Attri S, Rana SV, Vaiphei K, Sodhi CP, Katyal R, Goel RC et al. Isoniazid- and rifampicin-induced oxidative hepatic injury — protection by N-acetylcysteine. Human & Experimental Toxicology. 2000; 19(9): 517-522.
15. Singh G, Goyal R, Sharma P. Pharmacological potential of Silymarin in combination with Hepatoprotective plants against experimental Hepatotoxicity in rats. Asian journal of Pharmaceutical and Clinical Research. 2012; 5(s1): 128-133.
16. Jendrassik, L, Grof, P.Method on photometric systems for in vitro determination of direct and total bilirubin. Bioche Zeitschrift 1938; 297: 82-89.
17. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamicoxalaceticandglutamicpyruvictransaminases .AmericanJournal of ClinicalPathology. 1957; 28: 56-63.
18. King EJ, Kind PRM. In-vitro determination of serum alkaline phosphatase. Journal of Clinical Pathology. 1972; 7: 321-322.
19. Culling CFA, Alliston RT, Barr WT. Cellular Pathology Technique [Internet]. 4th ed. London:Butterworth&Co.Ltd.;1985[cited29May2016].Availablefrom:http://samples.sainsburysebooks.co.uk/9781483192192_sample_813090.pdf.
20. Davidson CS. Guidelines for detection of hepatotoxicty due to drugs and chemical. USA. USA: NIH publication, U.S. Department of Health and Education and Welfare NIH; 1979.
21. Sarich T, Zhou T, Adams SP, Bain AI, Wall RA, Wright JM. A model of isoniazid-induced hepatotoxicity in rabbits. Journal of Pharmacological and Toxicological Methods. 1995; 34(2): 109-116.
22. Ramappa V, Aithal GP. Hepatotoxicity Related to Anti-tuberculosis Drugs: Mechanisms and Management. Journal of Clinical and Experimental Hepatology. 2013; 3(1): 37-49.
23. Yesmin F, Rahman Z, Dewan JF, Helali AM, Rahman NIA, Ahmed AG et al. Hepatoprotective role of aqueous and n-hexane extracts of Nigella sativa L. in experimental liver damage in rats. Asian Journal of Pharmaceutical and Clinical research 2013; 6(3): 205-209.
24. Trivedi M, Chansoria AK, Dixit AK. Protective effect of Thymoquinone against Antitubercular Drug Induced Hepatic Toxicity in Rats. International Journal of Universal Pharmacy and Bio Sciences 2013; 2(1): 1-8.
25. Houghton PJ, Zaraka R, Delas Heras B, Hoult JR. Fixed oil of Nigella sativa and derived thymoquinone inhibit eicosanoid generation in leucocytes and lipid peroxidation. Planta Medica. 1995; 47(3): 119-126.
26. Mansour MA, Ginawi OT, EL-Hiday T, EL-Khatib AS et al. Effects of volatile oil constituents of Nigella sativa on carbon tetrachloride induced hepatotoxicity in mice: evidence for antioxidant effects of thymoquinone. Research Communication in Molecular Pathology and Pharmacology. 2001; 110(3-4): 239-251.
27. Sodhi CP, Rana SV, Mehta SK, Vaiphei K et al. Study of oxidative stress inisoniazid-rifampicin induced hepatic injury in young rats. Drug and Chemical Toxicology. 1997; 20(3): 255-269.
28. Kanter M, Coskun O, Uysal H. The antioxidative and antihistaminic effect of Nigella sativaand its major constituent, Thymoquinone on ethanol-induced gastric mucosal damage.Archives of Toxicology. 2006; 80(4): 217-24.