Research Article

Amelioration of Isoniazid induced Hepeto-toxicity by Alcoholic and Aqueous Extracts of *Syzygium cumini* in Wistar Rats.

Shweta Sharma*, B. K. Mehta
School of Studies in Chemistry and Biochemistry Department, Vikram University, Ujjain, Madhya Pradesh, India.
***Corresponding author's E-mail:** sharma.harshali10@gmail.com

Accepted on: 27-06-2016; **Finalized on:** 31-08-2016.

ABSTRACT

In this study, studied the comparative hepatoprotective effect of aqueous and ethanolic extracts of *Syzygium cumini* (AESC and EESC respectively) in Isoniazid (INH) induced hepeto-toxicity in wistar rats and the results were compared with the standard hepatoprotective drug silymarin. Hepatotoxicity was induced by regular doses of Isoniazid (100 mg/kg b.w., i.p.) to healthy rats. Standard drug (100 mg/kg, p.o.) and test extracts (500 mg/kg. p.o. for both) were simultaneously for ten days. The effects were observed using different biochemical parameters such as alkaline phosphatase (ALP), serum glutamic oxaloacitic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) and TBil (total biliruin). INH induced animals showed significant increase in all the test parameters ($p<0.001$) than control group. While the animals kept on either herbal extract treatment showed significant reduced values of ALP, SGOT, SGPT and TBil ($p<0.001$ for all) than induced animals. No significant difference was seen in both AESC and EESC. Present findings suggest that the SC extracts can ameliorate INH induced hepatic injury. However, both extracts were observed to be equally effective, might indicate some common active compounds worked in both extracts.

Keywords: hepatoprotective, Silymarin, *Syzygium cumini*, total bilirubin, Isoniazid.

INTRODUCTION

Hepatotoxicity entails liver damage driven by any chemical, drug, microbes etc. Liver plays a fundamental role in drug metabolism, transforming and clearing chemicals thus is more susceptible to the drug indued toxicity[1]. Recently, more than 900 drugs have been reported to cause liver injury which manifest in form of abnormal activities of liver enzymes[1,2]. Epidemiological analysis revealed that drug-induced liver injury is a major cause of 50% of all acute liver failures[3].

Isoniazid (INH) is one of the most commonly used drugs against mycobacterium tuberculosis infection and has been found to be associated with severe hepatotoxicity[4]. Increased serum values of alkaline phosphatase (ALP), serum glutamic oxaloacitic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) and total biliruin (TBil) are often used as biochemical markers of hepatic injury[5].

However, a number of allopathic drugs are available to treat these abnormalities but, their chronic exposure also found to exert detrimental side effects on liver as well as on other organs[6]. Moreover, herbs are rich sources of natural antioxidants, protective secondary metabolites and till now thousands of plants based therapies have been known to exhibit hepatoprotective effects. Therefore, plant based therapy are suppose to be more reliable[7-9]. As, herbal drugs are economic, affordable and safer, are widely used as efficient therapy[8]. For example, the *Syzygium cumini* (SC), is a rich source of several active components such as polyphenolic compounds, quercetin, flavianoids, tannins, etc and also have been reported for its beneficial pharmacological effects[10]. Moreover, scientific reports on its hepatoprotective, immunomoduatory, hypolipidemic, antidiabetic, antibacterial and the anti-inflammatory potentials have also been documented[10-12].

Protective effects of same plants also vary with the extraction system used. Here, the less studied comparative hepatoprotective effective nature of aqueous and ethanolic extracts of SC were studied in INH induced hepatotoxicity. Enzymatic activity such as ALP, SGOT and SGPT and serum total bilirubin (TBil) were taken as studied parameters. Silymarin was used as standard hepato-protective drug to reveal the relative potencies of the test extracts.

MATERIALS AND METHODS

Chemicals

All chemicals were of the highest commercially available purity. The bark of *Syzygium cumini* were collected from D-1 University campus, Dewas road, Ujjain, M.P., India. The identification of plant was done in Dept of Botany, Safia College of science, Bhopal, (M.P.) and the voucher specimen 438/Bot/saf/13, was deposited in the Safia College of Science Bhopal (M.P.).

Preparation of Extracts

To prepare the extract of SC bark, the cleaned and air dried bark was ground to a coarse powder. The powdered bark was exhaustively extracted is in soxhlet extractor with ethanol.

Removal of solvent under reduced pressure afforded solid mass. Ethanol extract was obtained in good amount. For

the preparation of aqueous extract, the bark powder was oaked in distilled water in a flask for 24 hours. After that, the solution was filtered by Whatman filter paper (no. 1) and the filtrate was collected. The residue was again soaked with 20 volume distilled water to get additional extractives. All the filtrates were collected in a round bottom flask.

Gummy substance was obtained by using vacuum rotary evaporator. Then the substance was dried at room temperature. The powdered extract was stored at 40°C for further experiment.

Animals

Healthy in-bred albino wistar rats of either sex (2-2.5 months old) were housed in polypropylene cages under constant temperature (27±2°C) and photo-schedule (14 h light and 10 h dark). They were provided rodent feed (Golden Feeds, New Delhi, India) ad libitum and had free access to boiled drinking water.

The approval of departmental ethical committee for handling and maintenance for experimental animals was also obtained before starting the experiments.

Induction of Hepatotoxicity

Hepatotoxicity was induced by regular doses of Isoniazid (100 mg/kg b.w., i.p.) to healthy rats. Animals with hepatic injury were post treated with silymarin and different herbal extracts.

Experimental Design

In the experiment, a total of 30 rats was used, which were divided into 5 groups having 6 animals in each group as follows:

Group I

Normal control rats received 1ml/100gm of 0.5% CMC using an intragastric tube for 10 days.

Group II

Negative control group given isoniazid (100mg/kg, i.p.) only once at day 1, designated as induced group.

Group III

Rats received isoniazid 100mg/kg, i.p. and silymarin (100mg/kg, p.o.) simultaneously, for 10 days, designated as stand group.

Group IV

Rats received isoniazid 100mg/kg, i.p. and aqueous extract of *Syzygium cumini*, (500mg/kg p.o.) simultaneously, once daily for 10 days, designated as AESC group.

Group V

Rats received isoniazid 100mg/kg, i.p. and ethanolic extract of *Syzygium cumini*, (500mg/kg p.o.) simultaneously, once daily for 10 days, designated as EESC group.

At the end of the experiment on 10th day, animals were kept on overnight fasting and blood was collected by orbital puncture method. This blood was then allowed to clot for 30 minutes at room temperature. The serum was separated by centrifugation at 3000 rpm at 30°C for 15 minutes.

Biochemical Estimation

The serum samples were analyzed spectro-photometrically for ALP, SGOT, SGPT and total bilirubin (TBil) levels using standard kits (Span diagnostics Ltd). Activity of SGOT and SGPT were measured at 505 nm and is expressed as IU/L of serum. In ALP assay the blue color developed which was read at 510 nm against blank and the activity is expressed as IU/L of serum. To estimate the TBil readings were taken at 540 nm, the level of total bilirubin was expressed as mg/dL of serum[5].

Statistical Analysis

Data are expressed as mean ± SE. Statistical analysis was done by using one-way ANOVA followed by unpaired student's t-test and p-values of 5% and less were considered as significant.

RESULTS

INH treatment was observed to be significantly associated with increased values of serum ALP, SGOT, SGPT and TBil (P<0.001, for all) than control animals. However, co-administration of silymarin drug was observed to be significantly effective against INH induced liver injury (P<0.05, P<0.001, P<0.0001 and P<0.001 respectively). The treatment with AESC or EESC showed significant reduction in all the studies parameters (P<0.001, for both) than induced group but these values are significantly less effective (P<0.05) than that of standard drug. Comparative analysis showed that the treatment with ethanolic extract of SC was found to equally effective as AESC for all of the parameters.

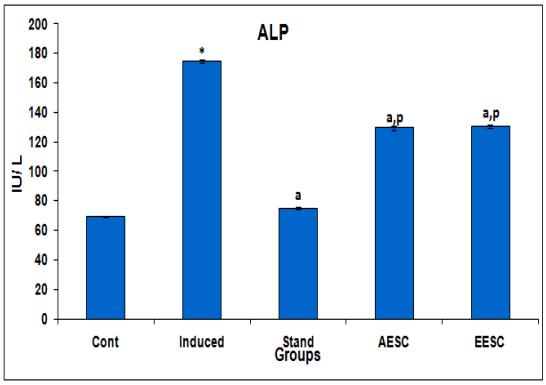

Figure 1: Effects of different extracts of Syzgium cumini (SC) on alkaline phosphatise (ALP) in experimental animals. Cont (Normal control), Induced (Isoniazid treated), STAND (Isoniazid +silymarin), AESC (Isoniazid+ aqueous extract of SC), EESC (Isoniazid+ ethanolic extract of SC). Each bar represents the mean±SE (n=6), *P<0.001, as compared to normal control, [a]P<0.05 and [b]P<0.01 as compared to Isoniazid treated group, [x]P<0.05, [y]P<0.01

and [z]P<0.001, as compared to standard drug while [#]P<0.05 than AESC group.

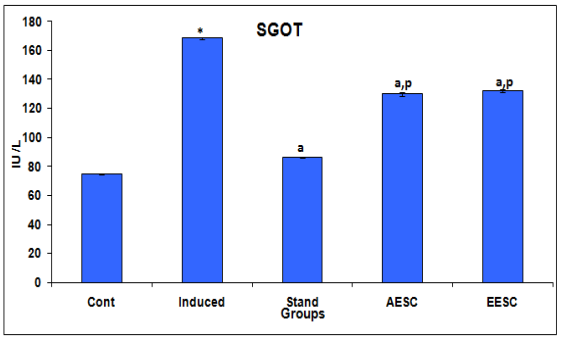

Figure 2: Effects of different extracts of Syzgium cumini (SC) on serum glutamic oxaloacitic transaminase (SGOT) in experimental animals. Cont (Normal control), Induced (Isoniazid treated), STAND (Isoniazid +silymarin), AESC (Isoniazid+ aqueous extract of SC), EESC (Isoniazid+ ethanolic extract of SC). Each bar represents the mean±SE (n=6), *P<0.001, as compared to normal control, [a]P<0.05 and [b]P<0.01 as compared to Isoniazid treated group, [x]P<0.05, [y]P<0.01 and [z]P<0.001, as compared to standard drug while [#]P<0.05 than AESC group.

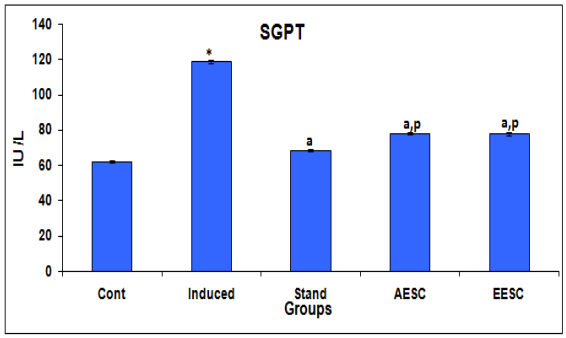

Figure 3: Effects of different extracts of Syzgium cumini (SC) on serum glutamic pyruvic transaminase (SGPT) in experimental animals. Cont (Normal control), Induced (Isoniazid treated), STAND (Isoniazid +silymarin), AESC (Isoniazid+ aqueous extract of SC), EESC (Isoniazid+ ethanolic extract of SC). Each bar represents the mean±SE (n=6), *P<0.001, as compared to normal control, [a]P<0.05 and [b]P<0.01 as compared to Isoniazid treated group, [x]P<0.05, [y]P<0.01 and [z]P<0.001, as compared to standard drug while [#]P<0.05 than AESC group.

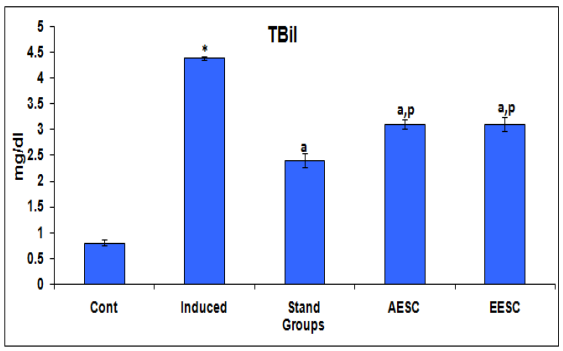

Figure 4: Effects of different extracts of Syzgium cumini (SC) on serum total bilirubin (TBIL) in experimental animals. Cont (Normal control), Induced (Isoniazid treated), STAND (Isoniazid +silymarin), AESC (Isoniazid+ aqueous extract of SC), EESC (Isoniazid+ ethanolic extract of SC). Each bar represents the mean±SE (n=6), *P<0.001, as compared to normal control, [a]P<0.05 and [b]P<0.01 as compared to Isoniazid treated group, [x]P<0.05, [y]P<0.01 and [z]P<0.001, as compared to standard drug while [#]P<0.05 than AESC group.

DISCUSSION AND CONCLUSION

INH treatment was found to cause considerable liver injury, which resembles with earlier investigations[4]. In this research work, we found that the AESC has same hepatoprotective effects as obtained with EESC.

In addition, data also revealed that the protective effects of both the herbs were seemed to be significantly less than that of standard drug used.

The INH used here, is actually an antibiotic drug but also known to cause oxidative injury especially in liver cells[5].

Isoniazid therapy leads to hepatic injury, central nervous system (CNS) toxicity, metabolic acidosis and other abnormal conditions. Its chronic administration observed to be associated with asymptomatic elevated ALP, SGOT and SGPT to hepatic failure[5,13]. The central cause of INH induced toxicity is its conversion into monoacetyl hydrazine by cytochrome P450. This monoacetyl hydrazine is toxic and leads oxidative damages mainly to liver cells[13].

Oxidative damage results in excessive production of free radicals. And free radicals destroy plasma membrane lipids and proteins. Thus this chain reaction enable the leakage of cytosolic proteins into the blood stream[2,4].

In case of liver cell damage SGOT, SGPT, ALP and TBil values are drastically increased which was used analysed as helpful quantitative markers. Here also, the increased levels of SGOT, SGPT, ALP and TBil indicated the toxic effects of INH on hepatic tissues[5,13].

ALP is a hydrolase enzyme in the cells lining the biliary ducts of the liver, responsible for removing phosphate groups from nucleotides, proteins, alkaloids and many other molecules.

It's increased level indicated large bile duct obstruction, intrahepatic cholestasis, or infiltrative diseases of the liver[14].

In this experiment, similar recovery of increased ALP levels in herbal extracts treated groups indicated the equivalent protective efficacy of both the extracts.

Bilirubin is the yellow breakdown product of normal heme-catabolism, Increased TBil serve as an indicator of jaundice or hemolytic anaemia or obstruction of the bile ducts[6,15].

Measurement of total bilirubin includes both unconjugated and conjugated bilirubin and is a reliable marker of liver disease[15]. The extracts mediated inhibition

of INH induced augmented bilirubin level suggests the possibility of the extracts being able to stabilize biliary dysfunction.

In addition, SGOT and SGPT both are pyridoxal phosphate (PLP)-dependent transaminase enzymes and play central role in amino acid metabolism. Both of these are found in the different body's organs such as liver, heart, skeletal muscle, kidneys, brain, and red blood cells. Serum SGOT and SGPT level, and their ratio (SGOT/SGPT ratio) are frequently measured clinically as biomarkers for liver health. Their increased level has been linked with abnormal liver functions, though these are not very specific to liver disease. Moreover, biliary tract disease produces relatively greater increases in ALP than increases in SGOT and SGPT[16]. Here, treatment with the different extracts restored the increased activities of these enzymes in serum. Recovery towards the normalization possibly suggests that these extracts caused parenchymal cell regeneration in liver, thus protecting membrane fragility and thereby decreasing enzyme leakage[17].

So, from the present findings it can be stated that the INH served as potent toxin for liver cells and its regular administration causes severe change in different biomarkers.

These abnormally increased values of test parameter were found to be normalised in animals either given silymarin or test extracts. In addition to this, animals kept on studied ethanolic or aqueous extracts exhibited similar protection might indicate that both extracts bear common active ingredients. To disclose the precise mechanism of these extracts mediated hepato-protective action further studied are needed.

REFERENCES

1. Vadivu R, Vidhya S, Jayshree N. Standardization and evaluation of hepatoprotective activity of polyherbal capsule. International Journal of Pharmaceutical Science Review and Research, 21, 2013, 93-99.
2. Halim AB, el-Ahmady O, Hassab-Allah S, Abdel-Galil F, Hafez Y, Darwish A. Biochemical effect of antioxidants on lipids and liver function in experimentally-induced liver damage. Annals of Clinical Biochemistry, 34, 1997, 656-663.
3. Noda A, Hsu KY, Noda H, Yamamoto Y and Kurozumi T. Is Isoniazid-hepatotoxicity induced by the metabolite, hydralazine? Journal of UOEH, 5, 1983, 183-190.
4. Wu JW, Leev SD, Yeh PF. Isoniazid-Rifampicin induced hepatitis in hepatitis B carriers, Gastroentrology, 98, 1990, 502-504.
5. Hensley K, Robinson KA, Gabbita SP, Salsman S, Floyd RA. Reactive oxygen species, cell signaling and cell injury. Free Radical Biology and Medicine, 28, 2000, 145-162.
6. Pandit A, Sachdeva T, Bafna P. Drug-Induced Hepatotoxicity: A Review. Journal of Applied Pharmaceutical Science, 2, 2012, 233-243.
7. Pari L, Latha M. Antidiabetic effect of Scoparia dulcis: Effect on lipid peroxidation in streptozotocin diabetes. General Physiology and Biophysics, 24, 2005, 13-26.
8. Ronsein GE, Guidi DB, Benassi JC, Filho WD, Pedrosa RC. Cytoprotective effects of carvedilol against oxygen free radical generation in rat liver. Redox Report, 10, 2005, 131-137.
9. Agrawal J, Kar A. Synergistic Action of phytochemicals augments their antioxidative Efficacy: An In Vitro Comparative Study. Asian Journal of Pharmaceutical and Clinical Research, 6, 2013, 121-126.
10. Banerjee A, Dasgupta N, De B. *In vitro* study of antioxidant activity of Syzygium cumini fruit. Food Chemestry, 90, 2005, 727-733.
11. Benherlal PS, Arumughan C. Chemical composition and in vitro antioxidant studies on Syzygium cumini fruit. Journal of the Science of Food and Agriculture, 87, 2007, 2560-2569.
12. Muniappan A, Pandurangan SB. Syzygium cumini (L.) Skeels: A review of its phytochemical constituents and traditional uses. Asian Pacific Journal of Tropical Biomedicine, 2, 2012, 240-246.
13. Jiang Y, Ren-xiu P, Jing Y, Rui K, Juan L. CYP2E1 mediated isoniazid-induced hepatotoxicity in rats, Acta Pharmacologica Sinica, 25, 2004, 699-704.
14. Settya SR, Quereshia AA, Swamyb V, Patila T, Prakasha T, Prabhua K, Goudaa AV. Hepatoprotective activity of Calotropis procera flowers against paracetamol-induced hepatic injury in rats. Fitoterapia, 78, 2007, 451–454.
15. O'Malley SS, Gueorguiev R, Wu R, Jatlow PI. Acute alcohol consumption elevates serum bilirubin: An endogenous antioxidant. Drug and Alcohol Dependence, 149, 2015, 87–92.
16. Achliya GS, Wadodkar SG, Dorle AK. Evaluation of hepatoprotective effect of Amalkadi Ghrita against carbon tetrachloride-induced hepatic damage in rats. Journal of Ethnopharmacology, 90, 2004, 229–232.
17. Gunturu KS, Nagarajan P, McPhedran P, Goodman TR, Hodsdon ME, Strout MP. Ayurvedic herbal medicine and lead poisoning. Journal of Hematological Oncology, 4, 2011, 51.

Source of Support: Nil, **Conflict of Interest:** None.