Original Article

The Hepatoprotective Effect of Ethanolic Extract of Syzygium Aromaticum (Clove) on Alcohol Induced Hepatotoxicity in Rats

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ABSTRACT

Objective: The aim of this study was to assess the effect of ethanolic extract of Syzygium aromaticum(clove) buds in alcohol induced hepatotoxicity in rats.

Study Design: Randomized controlled trial

Place and duration of study: Department of Pharmacology, Fatima Memorial College of Medicine and dentistry, Lahore from 1st July 2011 to 31st December 2011.

Materials and Method: The rats were divided into five groups, i.e. control group (A), ethanol positive group (B), experimental groups (C and D) and standard group (E). Group C experimental rats received ethanol (3 g/kg body weight daily intraperitoneal injection) and ethanolic extract of Syzygium aromaticum at 250mg/kg of body weight daily orally for a period of forty five days. Group D experimental rats received ethanol and ethanolic extract of Syzygium aromaticum at 500 mg/kg of body weight. Group E received ethanol and silymarin (100mg/kg orally).Blood samples were taken at 45th day and liver in each was taken out for histopathological examination.

Results: The ethanol group rats showed variable increase in serum ALT (Alanine Transaminase), AST(Aspartate transaminase)), ALP(Alkaline Phosphatase) and total bilirubin levels. In group C and group E rats the levels of these parameters become slightly decreased while in group D rats the levels decreased more towards normal. The morphological examination of experimental groups C and E rats showed slight recovery whereas the rats in experimental group D showed a significant recovery. Syzygium aromaticum constituents, especially flavonoids and polyphenols have strong anti-oxidant activity which provides hepato-protection against alcohol induced hepatotoxicity.

Conclusion: High dose of Syzygium aromaticun ethanolic extract (500 mg/kg body weight) showed better hepatoprotection against alcohol induced hepatotoxicity than low dose Syzygium aromaticum extract and silymarin in rats.

Key Words: Hepato-protection, ethanol, Syzygium aromaticum, Silymarin, oxidative stress

INTRODUCTION

The liver is the largest solid organ, the largest gland, and the main metabolic organ of the human body.[1] The liver performs not only physiological functions but also protects body against adverse effects related with drugs and chemicals.[2] Alcohol is a hepatotoxicant that induces a diffuse type of liver injury closely resembling human viral hepatitis. Oxidative stress plays a major role in pathogenesis of alcohol induced liver injury. The major factor in cytotoxic action of alcohol has shown to be peroxidation of endogenous lipids.[3] The toxicity of alcohol was later on shown to be related to its metabolism by alcohol dehydrogenases(ADHs) and also to the metabolism by CYP2E1(Cytochrome).[4] Free radicals have been implicated in alcoholic liver disease. Mechanisms that are thought to be involved are impairment of antioxidant defenses, as well as production of reactive oxygen species by the mitochondria and the CYP2E1 enzyme, and by activated phagocytic cells.[5] Oxidative compounds are the stimulator of lipid peroxidation which ultimately leads to cell membrane damage. It is reported that administration of alcohol decreases the antiperoxidative enzymes.[6-7] Due to high cost and adverse effects of drugs, peoples are now diverting to certain natural substances. The use of such substances are grown faster over the past few years which is undoubtedly driven by the belief that they are relatively safe, easily available and affordable.[8]

Syzygium aromaticum are the aromatic dried flower buds of a tree in the family Myrtaceae. Syzygium aromaticum is widely cultivated in Indonesia, Sri-Lanka, Madagascar, Tanzania, Brazil, Pakistan, Sri lanka and India. It is used in limited amounts in food products as a fragrant, flavoring agent and condiment. Syzygium aromaticum, usually called as clove, is used as a topical antiseptic and local anaesthetic in dentistry.[9] It is used in the form of a paste or mixture as dental cement.[10] It is also used as antibacterial, antifungal, antimicrobial, antiinflammatory and insecticidal.[11-12] Clove oil mainly constitutes eugenol, isoeugenol and caryophyllene contributing pharmacological role to Syzygium aromaticum.

Eugenol comprises 72-90% of the essential oil extracted from cloves. Other important constituents of clove include polyphenols, beta-caryophyllene, tannins, flavonoids, kaempferol, rhamnetin, terpenoids like oleanolic acid, stigmasterol and campesterol.[13]

The purpose of the present experimental model was to observe the effect of Syzygium aromaticum in alcohol induced hepatotoxicity in wistar rats

MATERIALS AND METHODS

Animals: Fifty adult male Wistar rats weighing 200-250g were procured for this study. They were kept in the experimental research laboratory of Fatima Memorial college of medicine and Dentistry, Lahore under day and night conditions. Prior to the commencement of the experiments, all animals were kept for one week under the same laboratory conditions, at a temperature of 22 ± 2 ^{0}C, relative humidity of 70 ± 4% and 12 hour light/day cycle. They received nutritionally standard diet and tap water. The care and handling of rats were in accordance with the internationally accepted standard guidelines for use of experimental animals. The recommendations of Animal Ethics Committee for the care and use of animals were strictly followed throughout the study.

Chemicals/Instruments: Commercially available kits (Randox) for biochemical analysis of ALT,AST,ALP, bilirubin, 95% ethanol, 10% formalin, paraffin wax, Haematoxylin and eosin stains, pre-coated TLC(Thin Layer Chromatography) plate silica gel GF254 and toluene. The standard compounds used are ellagic acid, gallic acid and protocatechuic acid. The instruments used were soxhlet and rotary evaporator and centrifuge (Germany), TLC scanner III (Camag, Switzerland) with win CATS software.

Plant materials and preparation of the extract: Clove buds were collected from local market of Lahore and were authenticated from a botanist. Clove buds were coarsely powdered using a grinder. 500 g powdered form of clove was dissolved in 2L of 95% ethanol. The extraction was carried out by mixing the powdered clove in ethanol by Soxhlet apparatus for 72 hr. The extract was filtered and the solvent i.e., ethanol was allowed to evaporate using rotary evaporator at temperature 40-45°C. Thus the highly concentrated ethanolic extract was obtained .The yield of extract was 6.2% w/w in terms of dried starting material. The extracts obtained were stored at -20°C till used for experimental purposes.[14`] The clove buds (voucher no. 0282) and extract (voucher no. 0283) were deposited in Pharmacology laboratory, Fatima Memorial College of Medicine and Dentistry, Lahore. This extract got standardized from PCSIR (Pakistan Council for Scientific and Industrial Research) laboratories, Lahore.

Standardization of plant extract: TLC (Thin Layer Chromatography) was used for standardization. TLC was performed on a pre-coated TLC plate silica gel GF_{254}. Sample was applied on the plate as 8 mm wide bands with an automatic TLC sampler. The development was carried out in trough chamber (20 cm × 10 cm),which was pre-saturated with mobile phase(solvent system, toluene-ethyl acetate-formic acid-methanol (30:30:8:2), for 20 min at room temperature (25 ± 2°Cand 40% relative humidity). Subsequent to the development, TLC plates were dried under stream of hot air and then subjected to densitometric scanning using a TLC scanner III (Camag, Switzerland) with win CATS software (version 1.4.1) in the absorbance- reflectance scan mode. Quantitative evaluation of the plate was performed in absorption-reflection mode at 338 nm. The standard compounds used are ellagic acid, gallic acid and protocatechuic acid.

Experimental Procedure: After acclimatization, all rats were divided into five groups each having 10 animals. The control rats (Group A) were fed on standard diet with tap water and received no drug. Group B positive control rats received 3g/kg body weight of ethanol prepared as 35% v/v solution in 0.9% w/v NaCl as I.P(intraperitoneal) injection daily for forty five days. Group C i.e. experimental group rats received 3g/kg body weight of ethanol prepared as 35% v/v solution in 0.9% w/v NaCl I.P injection daily and ethanolic extract of Syzygium aromaticum buds in a daily oral dose of 250 mg/kg for a period of forty five days. Group D i.e. experimental group rats received 3g/kg body weight of ethanol prepared as 35% v/v solution in 0.9% w/v NaCl as I.P injection daily and ethanolic extract of Syzygium aromaticum buds in a dose of 500 mg/kg body weight daily (orally) for a period of forty five days. Group E(standard) group rats received 3g/kg body weight of ethanol prepared as 35% v/v solution in 0.9% w/v NaCl as I.P injection daily and silymarin(100mg/kg) daily orally for forty five days.

Sample collection: Blood sampling through tail vein was performed at 2 intervals (0 day &at 45th day) following same protocol every time. Twenty four hour after administration of the last dose of extract i.e. on 45th day and after overnight fasting, the animals were weighed and anaesthetized under ether vapours. A sample of 2ml blood was drawn from tail vein from all animals. Blood was transferred to the sterile vacuotainers with gel and allowed to clot at room temperature for one hour. It was then centrifuged for ten minutes at a speed of 3000 rpm. Serum was separated and stored in sterile eppendorf tubes at -20°C for analysis of biochemical parameters.[15]

Biochemical Analysis: ALT, AST, ALP and total bilirubin levels were estimated by commercially available kits (Randox of UK). Serum ALT, AST, ALP was estimated by IFCC method. [16-19] Total bilirubin was estimated according to Calorimetric method. [20]

Liver tissue for morphology: When anaesthetized, the liver of all animals were exposed and a wedge was removed after their gross examinations and they were preserved.

Statistical Analysis: The data was entered and analyzed using SPSS 17.0 (Statistical Package for Social Sciences). All data are shown as mean ± S.E.M (standard error of mean). One way ANOVA (analysis of variance) was applied to observe group mean differences. Post Hoc Tukey test was applied to observe mean differences among the groups. A p-value of <0.05 was considered as statistically significant.

RESULTS

We observed that 3g/kg body weight of ethanol prepared as 35% v/v solution in 0.9% w/v NaCl as I.P injection, caused significantly increased($p<0.05$) levels of serum AST,ALT,ALP and bilirubin in rats of group B as compared to group A (control) rats. On the other hand, simultaneous administration of ethanolic extract of *Syzygium aromaticum* resulted in a significant ($p<0.01$) decrease in the serum AST,ALT,ALP and bilirubin levels in rats of groups C, D and E when compared with that of group B.

Table No.1: Mean± SEM values of different biochemical parameters in all groups (A, B, C , D and E)

Parameter	Group A	Group B	Group C	Group D	Group E
Serum ALT (u/l)	40.19±1.10	69.60± 1.82 *	48.18 ± 1.27 **	40.25± 2.38 **	49.53±1.08**
Serum AST (u/l)	82.20±4.09	155.49±6.01*	116.10± 4.57 **	86.14±2.90**	119.76±4.42**
Serum ALP (u/l)	117.73±8.11	278.77±6.96*	235.42±6.20**	202.19±2.28**	240.83±6.71**
Serum Bilirubin(mg/dl)	0.33±0.03	0.83±0.06*	0.61±0.03**	0.37±0.03**	0.63±0.03**

* $p<0.05$ when compared with group A (control)

** $p<0.05$ when compared with group B (diabetic)

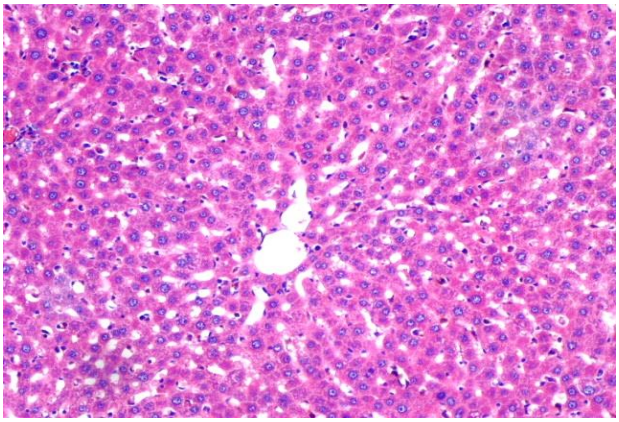

Figure No.1: Photomicrograph of normal liver (control group A) showing normal lobular pattern and normal architecture. The hepatocytes and portal system are normal (H& E ×40)

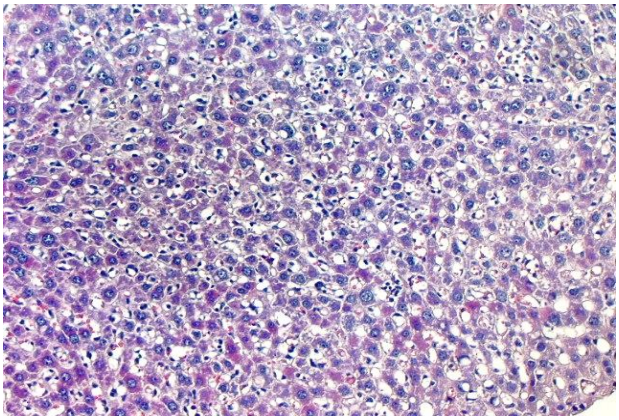

Figure No.2: Photomicrograph of liver of group B rats (treated only with ethanol) showing liver damage characterized by clear cytoplasm, vascular congestion, fatty changes, apoptosis and focal areas of necrosis (H& E ×40).

Histopathological Examination: In histopathological studies of livers of male albino rats, the control group showed normal lobular pattern and normal architecture. The hepatocytes and portal system are normal (Fig.1). The liver of Group B rats (treated only with ethanol) showed liver damage characterized by clear cytoplasm, vascular congestion, fatty changes, apoptosis and focal areas of necrosis (Fig.2)

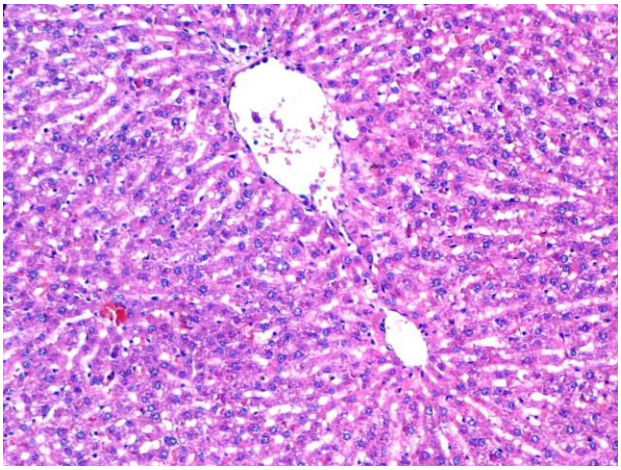

Figure No.3: Photomicrograph of liver of group C rats (treated with ethanol and *Syzygium aromaticum* extract 250mg/kg)) showing mild fatty change along with few apoptotic bodies. (H& E ×40)

These changes were found to be reduced in livers of group C rats treated with ethanol plus Syzygium aromaticum extract in a dose of 250mg/kg (Fig.3) and group E treated with ethanol plus silymarin (Fig.5) The liver of Group D rats treated with ethanol and 500mg/kg of Syzygium aromaticum extract showed vascular congestion and evidence of regeneration

(Fig. 4).This group of rats showed reversal towards normal liver architecture.

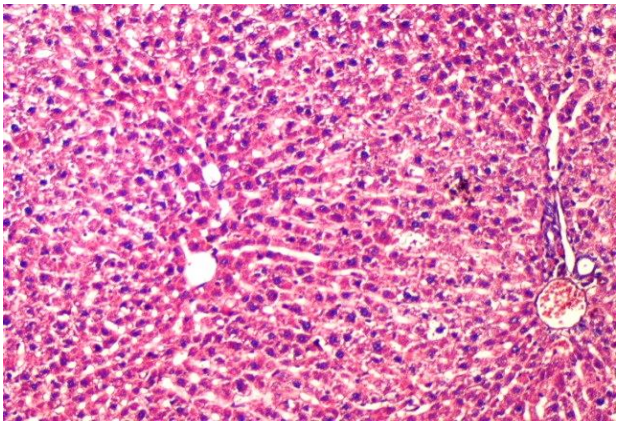

Figure No.4: Photomicrograph of liver of group D rats (treated with ethanol and Syzygium aromaticum extract 500mg/kg)) showing reversal to normal hepatocytes with occasional apoptotic bodies (H& E ×40).

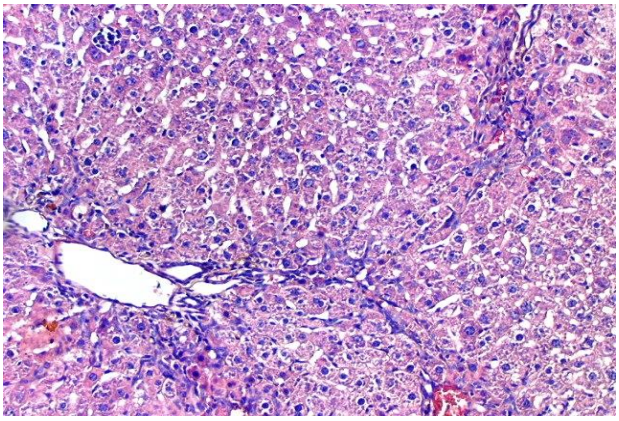

Figure No.5. Photomicrograph of liver of group E rats (treated with ethanol and Silymarin) showing mild fatty change, vascular congestion and apoptotic bodies. (H& E ×40)

DISCUSSION

The ingestion of alcohol for prolong time may lead to alcoholic hepatitis, fatty infiltration, accelerated progression of liver disease and higher frequency of liver cirrhosis. If left untreated, it leads to fibrosis and ultimately to cirrhosis. [21] Alcohol administration in rats basically disrupts permeability of plasma membrane. This lead to leakage of enzymes from the cells which results in elevation of lipid peroxides. It has been reported that liver damage induced by alcohol is due to instability of cell membrane as a result of lipid peroxidation. Lipid peroxidation arising from reaction of free radicals with lipids is considered to be an important feature of cellular injury brought by free radicals attack. Oxidative damage caused by reactive oxygen species is considered to be an important pathophysiological condition promoting cell injury. Antioxidant enzymes also decrease in alcohol induced liver injury. [22-23] Currently available drugs for the treatment of hepatotoxicity have a number of limitations including adverse effects and high rate of secondary failure. A number of plants are being assessed for their therapeutic potential as there is a growing trend towards the use of natural remedies as adjuncts to conventional therapy. Modulations of oxidative stress through treatment with antioxidants can help in hepatotoxicity due to alcohol.

The present study showed a significant elevation in the levels of serum AST,ALT, ALP and bilirubin in rats of group B as compared to group A (control) rats. On the other hand, simultaneous administration of ethanolic extract of Syzygium aromaticum to groups C and D and silymarin to group E resulted in a significant ($p<0.01$) decrease in the serum AST,ALT,ALP and bilirubin levels in rats of groups C , D and E when compared with that of group B (Table 1). When we compare mean values of group C and D with group E, although level decreases but levels reduced more in group C and D as compared to group E showing better effectiveness of Syzygium aromaticum over silymarin. When we compare mean values of group C with group D, although both decrease levels, but group D reduced the levels more as compared to group C. The morphological examination in group C and E animals showed moderate change towards normalcy whereas in group D animals, high dose Syzygium aromaticum ethanolic extract resulted in a significant morphological reversal towards normal. Our results are in accordance with the reports by others who used chemical antioxidants and diet of natural antioxidant plants.[24-25]

The main constituents in syzygium aromaticum are eugenol, polyphenols and flavonoids. The proposed mechanism of syzygium aromaticum in hepatoprotection could be due to the antioxidant mechanism. Atawodi et al. in 2011 showed that polyphenols in syzygium aromaticum have antioxidant activity.[26] Robards and Antolovich in 1997 have critically reviewed the analytical chemistry of bioflavonoid and it was found that flavonoids possess antioxidant activity, they are potent free radical scavengers and metal chelators and they also inhibit lipid oxidation which is a key step in the liver cell injury.[27] Therefore, in our study polyphenols and flavonoids in syzygium aromaticum might have a role in decreasing liver enzymes levels and hepatoprotection in rats. Further studies are needed to observe if higher doses and variable routes of administration have better protective effect on alcohol induced hepatotoxic liver.

CONCLUSION

The results of the present study indicate that the treatment with syzygium aromaticum ethanolic extract provide hepatoprotection against alcohol induced

hepatotoxicity both biochemically and morphologically. The high dose of syzygium aromaticum ethanolic extract, showed better results as compared to its low dose and standard drug silymarin.

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