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HEPATOPROTECTIVE ACTIVITY OF ETHANOLIC EXTRACT OF *TERMINALIA CHEBULA* FRUITS ON ETHANOL INDUCED HEPATOTOXICITY IN WISTAR RATS

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ABSTRACT

The purpose of this study was to assess the effect of ethanolic extract of *Terminalia chebula* fruits experimentally in ethanol induced hepatotoxicity in rats. Rats were divided into six different groups each having six. Group 1 served as a control, Group 2 received 40% ethanol(2ml/100g), Group 3, 4 and 5 served as extract treatment groups and received 50, 100, & 200 mg/kg, orally, ethanolic extract of *Terminalia chebula* fruits and Group 6 served as standard group and received Silymarin 25 mg/kg orally. After 21 days of all the treatments, rats were sacrificed, blood and liver were taken for biochemical and histological studies, respectively. The ethanol treated group rats (G2) showed variable increase in serum AST, ALT, ALP, and total bilirubin levels and decrease in total proteins levels. Administration of ethanolic extracts of *Terminalia chebula* fruits significantly prevented ethanol-induced elevation in the levels of serum diagnostic liver marker enzymes aspartate amino transferase (AST), alanine amino transferase (ALT) and alkaline phosphatase (ALP), total bilirubin levels in experimental groups of rats. Moreover, total protein levels were significantly increased in treatment groups. The effect of ethanolic extracts of *Terminalia chebula* was compared with marketed drug, Silymarin. The changes in biochemical parameters were supported by histological profile. It is concluded that the ethanolic extract of *Terminalia chebula* fruits protects against ethanol-induced hepatotoxicity liver injury in rats.

KEYWORDS: *Terminalia chebula*, ethanol, Silymarin, hepatotoxicity.

INTRODUCTION

Hepatotoxicity means chemical-driven liver damage. Drug-induced liver damage is a cause of acute and chronic liver disease. *Terminalia chebula* Retz. (Fam. Combretaceae), is called the 'King of Medicine' because of its extraordinary power of healing. The plant possesses high medicinal value and traditionally used for the treatment of various ailments for human beings. The plant is used in the treatment of bleeding piles, hiccough, asthma, sore throat, vomiting, diarrhea, dysentery, ulcers, gout, heart and bladder diseases by the traditional healers.[1] Despite the numerous scientifically proven pharmacological activites of *Terminalia chebula*[2,3,4,5] there was no scientific data on its potential as hepatoprotective agent. Ethano medical information has been reported hepatoprotective activity of *Terminalia chebula*. Hence, the present study was undertaken to assess ethanolic extract of fruits of *Terminalia chebula* for its hepatoprotective activity using animal model.

MATERIALS AND METHODS

COLLECTION

The fresh fruits of *Terminali chebula* Retz were collected from the medicinal plants form Mulugu, Warangal district surroundings, Telangana state, India. The fruits was air dried at room temperature. The powdered fruit was used for the preparation of ethanolic extract.

IDENTIFICATION & AUTHENTICATION

The plant material was taxonomically identified and authenticated by Dr. P. Satyanarayana raju M.sc, M. Phil, Ph.D. Plant Taxonomist Department of Botany and Microbiology, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur, India.

EXTRACTION

The fruits of *Terminalia chebula* were carefully washed under running tap water followed by distilled water. These were air dried and then ground to coarse powder. This dried coarse powder(500g) was used for extraction. The Extraction of powder was done with 95% ethanol by using the soxhlet apparatus at a temperature of 60^{o}C for 48 hours. The extracts were concentrated under reduced pressure and stored in vaccum dessicators for complete removal of solvent. The obtained extract was weighed and percentage yield was calculated.[6]

Qualitative Phytochemical Analysis

Phytochemical analysis of ethanolic extract of fruits of *Terminalia chebula* was carried out by using standard procedures to identify the presence of various phytoconstituents.[7]

IN-VIVO STUDIES

EXPERIMENTAL ANIMALS

The wistar rats (150-250 g) of either sex were housed in polypropylene cages and maintained under standard conditions (12 h light and dark cycles, at 25±3° C and 35-60% humidity). Standard pellet diet and tap water were provided *ad libitum*. All the animals were acclimatized to the laboratory environment five days prior to experiment. All the pharmacological experimental protocols were approved by the Institutional Animal Ethics Committee of Sipra Labs Limited Hyderabad. The study was conducted after obtaining ethical committee clearance from the Institutional Animal Ethics committee No:SLL/PCT/IAEC/43-19.

HEPATOPROTECTIVE ACTIVITY

1. Ethanol induced hepatotoxicity model

Thirty six Wistar Albino male rats of weight 150gms-250gms were selected for this study. Rats were divided into 6 groups. Each group contained of six animals,. The 1st group was served as control and treated with distilled water for 21 days. The 2ndgroup was treated with 40% ethanol (2ml/100g body weight) for 21 days. The 3rd, 4th, and 5th groups were treated with ethanolic extract of *Terminalia chebula* fruit in doses 50mg/kg, 100mg/kg, 200mg/kg along with 40% ethanol (2ml/100g b.wt). Group 6th was treated with standard drug (Silymarin 25mg/kg) along with 40% ethanol (2ml/100g b.wt).

Group 1: Control group (distilled water) for 21 days.
Group 2: Inducer(Ethanol 2ml/100g body weight, p.o) for 21 days.
Group 3: *T.chebula* fruit extract 50 mg/kg body weight p.o +Ethanol 2ml/100g body weight, p.o for 21 days.
Group 4: *T.chebula* fruit extract 100 mg/kg body weight p.o +Ethanol 2ml/100g body weight, p.o for 21 days.
Group 5: *T.chebula* fruit extract 200 mg/kg body weight p.o+Ethanol 2ml/100g body weight, p.o for 21 days.
Group 6: Silymarin 25 mg/kg body weight p.o +Ethanol 2ml/100g body weight, p.o for 21 days.

BIOCHEMICAL ASSAYS

At the end of the drug treatment period, all the animals were anaesthetized by light ether anaesthesia and blood samples were collected from the animals from retro orbital plexus

puncture. The collected blood samples were allowed to coagulate for 30 min in vacutainer tubes and Serum was separated by centrifugation at 3000 rpm for 20 minutes. Serumsamples were kept at – 20˚C and the different biochemical analysis of all the serum samples were estimated for the levels of ALT, AST, ALP, Total bilirubin and Total proteins.[8]

HISTOPATHOLOGY EXAMINATION

At the end of the experiment (day 22), all rats were anesthetized by ether and the Liver were excised out and fixed in formalin (10%). Five micron thick section were prepared by using microtome and these section were stained with hematoxyline and eosin. For histological alterations these slides were observed under light microscope.

Statistical analysis

Results were analyzed by one-way ANOVA followed by Dunnett's multiple comparison test and the values P< 0.05 were considered significant (Table 1).

Table 1: Effect of *Terminalia chebula* fruit extract on serum parameters (ALT, ALP, AST, Total proteins, Total bilirubin) in ethanol induced hepatotoxicity in rats.

Groups	Treatment	ALT(IU/L)	ALP(IU/L)	AST(IU/L)	Total proteins (mg/dL)	Totalbilirubin (mg/dL)
I	Normal control	31.5 ±5.37	120.1±5.76	40±4.49	6.74±0.27	1.1±0.32
II	Inducer(ethanol)	56.5±5.77	174±5.77	68.8±0.052	2.67±0.14	2.12±0.49
III	TCE(50mg/kg)+ethanol	41.2±5.77*	156±5.77**	60.80±0.03**	3.29±0.10*	1.92±0.37*
IV	TCE(100mg/kg) +ethanol	29.1±5.768**	141.3±5.768*	47.06±0.239**	4.12±0.13**	1.74±0.35*
V	TCE(200mg/kg)+ethanol	18.9±5.77**	130.3±5.74**	32.7±0.096**	5.68±0.19**	1.41±0.29**
VI	Silymarin(25mg/kg) +ethanol	21.8±5.80**	138.8±5.74**	30.29±0.033**	5.92±0.25**	1.37±0.38**

All values are mean ± SEM. (n=6).One-way ANOVA followed by Dunnet's test.*P< 0.05,** P<0.01 when compared to vehicle treated (control) animals.

RESULTS AND DISCUSSION

The dried and powered fruit material of *Terminalia chebula* was weighed, packed in soxhlet apparatus and extracted with 95% ethanol for 48 hours. The solvent thus obtained was evaporated under vaccum to get a semi-solid form of the extract. Percentage yield was 12.1% with respect to dried powder.

Preliminary phytochemical analysis revealed the presence of Tannins, Triterpenoids, Anthraquinones, Flavonoids, Glycosides, Phenolic compounds, Carbohydrates and Sterols in ethanolic extract of fruits of *Terminalia chebula.*

Alcohol is used as one of the hepatotoxic agent in the experimental study of liver related disorders. The hepatotoxic effects of alcohol are largely due to its active metabolite trichloromethyl radical. Serum ALT, AST and ALP are important sensitive markers used in the diagnosis of hepatic damage because these are cytoplasmic in location and are released into the circulation after cellular damage. The increased activities of AST, ALT, ALP and Total bilirubin level in serum manifested the ethanol induced hepatocellular damage. Treatment with *Terminalia chebula* significantly decreased the activities of ALT, AST, ALP and Total bilirubin in serum indicating that test agents offer protection by preserving the structural integrity.

In this study, we observed the hepatoprotective effect of *Terminalia chebula* in ethanol induced hepatotoxicity in rats. A significant elevation was observed in the levels of serum AST, ALT, ALP, Total bilirubin and significant decrease level Total protein in ethanolic group which received ethanol as compared to control group rats who received distilled water.

Administration of various doses (50mg/kg, 100mg/kg & 200mg/kg) of ethanolic extract of fruits of *Terminalia chebula* with ethanol in various treatment groups, maintained the levels of AST, ALT, ALP, and serum total Protein and Total bilirubin towards normal when compared to ethanol induced rats group. This was most likely due to the anti-oxidant effect of *Terminalia chebula* phytconstituents. On morphological examination in low dose *Terminalia chebula* showed partial inflammation in hepatic cells.

While in high dose (200mg/kg) of ethanolic extract of *Terminalia chebula* showed a highly recovery compared to normal.

The investigations of ethanolic extract of fruits of *Terminalia chebula* (50mg/kg, 100mg/kg, 200mg/kg; p.o) in Ethanol induced method in rats demonstrated significant *in-vivo* hepatoprotective activity. The effects of *Terminalia chebula* extract was comparable to that of standard Silymarin.

CONCLUSION

From our studies it can be concluded that ethanolic extract of *Terminalia chebula* fruit exhibited hepatoprotective activity. However, further research is required for isolation of the active principles responsible for hepatoprotective activity.

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