Research Article

Effect of aqueous extract of *Citrullus lanatus* Rind on ethanol-induced hepatotoxicity in rats

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Abstract

Objective: The present study was conducted to investigate that aqueous extract of *Citrullus lanatus* rind (CLRE) would ameliorate the hepatic damage caused by Ethanol. **Materials and Methods:** Thirty male wistar rats were divided into 5 groups as follows, Group-1 served as Control group, Group-2 as Disease control (20 ml/kg of 40% Ethanol on 28th day) and Groups-3, 4, 5 served as treatment groups 100 mg/kg, 200 mg/kg, 400 mg/kg of CLRE for 27 days and on 28th day 20 ml/kg of 40% Ethanol respectively. The hepatoprotective activity was evaluated by determining the liver serum parameters i.e., alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), anti-oxidant parameters like superoxide dismutase (SOD) and catalase (CAT), oxidative stress parameters - Malondialdehyde (MDA) and histopathological examinations. **Results:** Ethanol toxicity led to significant increase ($P<0.001$) of serum levels of AST, ALT, ALP and increased levels of MDA and decreased levels of SOD, catalase with multiple histological damage. Interestingly, Pre-treatment with CLRE, dose-dependently and significantly attenuated the changes caused by Ethanol administration. **Conclusion:** This study reveals that CLRE possesses hepatoprotective activity against 40% ethanol in dose dependent manner in rats.

Keywords: *Citrullus lanatus* rind, hepatotoxicity, ethanol, hepatoprotective, malondialdehyde

Introduction

Liver defends the body from xenobiotic substances. Primary functions of liver includes bile synthesis, food metabolism, detoxification, storage of vitamin (A, D and B-12) and glucose regulation (Zong et al., 2014). Hepatotoxicity (HTX) is defined as liver injury or damage induced by exposure to medication or other non-pharmacological substance (Cifuentes and Francisco, 2010). Use of drugs may have some negative effects on the patient's health; of all the vital organs affected, liver is one, because substances or metabolites formed during the biotransformation process; drugs can cause liver injury (Paniagua et al., 2017).

It is well known that alcohol causes HTX (Alcohol Liver Diseases, fatty infiltration, hepatitis & cirrhosis). Lipid peroxidation is the important cause of liver damage. Increased Glutamyl transpeptidase (GluTr) is the result of excessive lipid peroxidation, which also tends to alter the lipid balance in cell membranes (which are composed of lipid bilayers). Cell membrane integrity is lost as a result. GluTr also lowers the levels of natural antioxidants like GSH and SOD in the body (Iqubal et al., 2016). According to WHO 2019, about 3.3 million deaths are contributed due to over consumption of Alcohol, out of which 7.1 % are males and 2.2 % are females. Global alcohol consumption per person may reach 6.6 liters in 2020 and 7.0 liters in 2025 if anticipated rising patterns in alcohol use in the Region of the Americas, South-East Asia, and Western Pacific Regions are not stopped and reversed.

Citrullus lanatus, commonly called as watermelon. It is a horticultural crop belonging to the family Cucurbitaceae. An average watermelon contains about 30% of rind, 68% of flesh or pulp, and 2% of seeds (Hannah and Krishnakumari, 2015). According to reports, the therapeutic properties of watermelon are due to its antioxidant compounds (Leong and Shui, 2002; Lewinsohn et al., 2005). The antioxidant properties of watermelon rinds shields from free radical damage because of

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the citrulline they contain. Additionally, citrulline converts into arginine, an amino acid essential for the immune system, heart, and circulatory system. The rind is typically discarded, but it is edible and occasionally used as a vegetable. In China, rind of the fruit is powdered after drying and after incineration is used for aphthous mouth sores. Pickled watermelon rind is consumed in the southern US (Mandel et al., 2005).

Materials and Methods

Reagents

Ethanol was procured from SD Fine Chemicals Ltd. Commercial kits for the assay of liver serum parameters i.e., Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP) were obtained from ERBA healthcare Pvt Ltd. All the other chemicals and reagents used for phytochemical screening of extract and biochemical assays were of analytical grade.

Preparation of aqueous extract of *C. Lanatus* rind

Whole Watermelon (*Citrullus lanatus*) was thoroughly washed to remove soil debris; the rinds were peeled off and diced into small pieces and shed dried. The dried rinds were ground into fine powder. Aqueous Extract was made by soaking 134 g of rind powder in 500 ml of Distilled water. The mixture was extracted by heating at 70° C for 45 minutes. The extract was then cooled, later was filtered using a muslin cloth. The extract was later concentrated under vacuum at 60° C using a rotary evaporator. Later the obtained extract was kept in vacuum drier until thick paste was formed (Nalimu et al., 2022). The extract was dissolved in distilled water for oral administration to rats.

Experimental Animals

Thirty Male Wistar rats (200-250 g) were obtained from Sainath Agencies, (CPCSEA Reg. no: 320/CPCSEA). Animals were housed at CPCSEA approved Animal House of, G. Pulla Reddy College of Pharmacy, Hyderabad. The animals were kept in polypropylene cages (6 in each cage) under standard laboratory conditions (12 hour light and dark cycle) and had free access to commercial pellet diet with water *ad libitum* at 25 ± 2° C with relative humidity at 50 ± 20 %. The study was approved by the Institutional Animal Ethics Committee of G. Pulla Reddy College of Pharmacy (02/29/12/2021/PCL-5). Ethical norms were strictly followed during all the experiments.

Experimental Design

The study was intended for 28 days, using Male Wistar rats weighing 200-250 g. Experimental animals were randomly divided into 5 groups, each group containing 6 animals each as follows, Group-1 served as the Normal Control, Group-2 served as Disease Control, received normal laboratory food with unlimited supply of water for 28 days. On 28th day, disease control received overdose of 20 ml/kg of 40% ethanol, which is responsible for disease induction. The 40% Ethanol administrated at a total accumulative dosage of of 20 ml/kg body weight by four equally divided gavages in 20-min intervals intragastrically. Groups – 3, 4, 5 served as Treatment Groups which received *Citrulus lanatus* rind extract (CLRE) with different doses 100 mg/kg, 200 mg/kg, 400 mg/kg respectively for 27 days and on 28th day, overdose of 20 ml/kg of 40% ethanol was administered orally.

Sample collection and Biochemical studies

On 29th day, the blood was collected from each rat by retro-orbital plexus using ketamine (50 mg/kg, i. p) as anesthetic. Serum was separated by centrifugation for biochemical estimations. Serum levels of ALT, ALP, and AST were measured using commercially available standard kits (ERBA Healthcare Pvt Ltd) in auto analyzer (Star 21 plus). Thereafter, the animals were sacrificed by CO_2 overdose and liver was isolated carefully, cleaned with normal saline and weighed. Half portion of liver was homogenized to estimate oxidative stress parameter i.e., malondialdehyde (MDA) (Buege and Aus,1978) and antioxidant parameters i.e., superoxide dismutase (SOD) (Marklund S and Marklund G,1974) and catalase (CAT) (Hadwan and Abed, 2016) and the other half portion was proceeded for histological study.

Statistical Analysis

The data were analyzed using one way ANOVA followed by Tukey's multiple comparison test in Graph pad prism 9.4.0 version and the results were expressed as mean ± SEM. P value < 0.05 was considered statistically significant.

Results

Percentage yield of CLRE

The percentage yield of extract obtained from Extraction of Rind of *Citrullus lanatus* using water as a solvent was found to be 11.19% w/w.

Phytochemical Screening of CLRE

Phytochemical investigation of rind of *Citrullus lanatus* was carried out qualitatively. Various qualitative tests were used to identify the presence of alkaloids, carbohydrates, glycosides, saponins, proteins and amino acids, flavonoids, phytosterols, phenolic compounds, tannins anthocyanins and coumarins. The phytochemical investigation of CLRE revealed the presence of carbohydrates, reducing sugars, flavonoids, phenolic compounds, phytosterols, proteins and amino acids.

Effect of CLRE on ethanol-induced alterations in liver weights

Results showed the effect of CLRE on liver weights in various groups (Table 1). There was a significant increase in liver weights in disease control group when compared to normal control group. On other hand, treatment with CLRE 400 mg/kg., p. o led to significant decrease in liver weights when compared to disease control group showing recovery action in dose dependent manner.

Effect of CLRE on ethanol-induced changes in serum biomarkers of liver function

The effect of CLRE on liver serum biomarkers (SGPT, SGOT, and ALP) were estimated in this study. Ethanol at a dose of 20 ml/kg of 40% ethanol, p. o for 1 day has significantly ($^{\alpha}$P <0.0001) increased the levels of SGPT, SGOT, ALP in disease control group when compared to normal control group. Treatment with CLRE (100 mg/kg) significantly (**P<0.001) decreased the levels of SGPT whereas treatment of rats with CLRE 200 mg/kg, 400 mg/kg has significantly and dose dependently decreased the levels of SGPT, SGOT, ALP (***P<0.0001) compared to disease control group (Table 2).

Effect of CLRE on ethanol-induced lipid peroxidation in liver

MDA levels of lipid peroxidation in livers were assessed as a biomarker for oxidative stress. In disease control group, ethanol (20 ml/kg of 40% ethanol, p. o for 1 day) showed a significant ($^{\alpha}$ P<0.0001) increase in levels of MDA when compared to normal control group. In treatment groups, TG-2, TG-3 (200 mg/kg, 400 mg/kg of aqueous CLRE for 28 days) has shown a significant (*P<0.05, ***P<0.0001) decrease in lipid peroxidation when compared to disease control group (Table 3).

Effect of CLRE on ethanol-induced SOD AND CAT (anti-oxidants)

The effect of CLRE on anti-oxidants (SOD, CAT) were estimated. In disease control group, Ethanol (20 ml/kg of 40% ethanol, p. o for 1 day) has significantly ($^{\alpha}$P< 0.0001) decreased the levels of SOD, CAT compared to normal control group. In TG-2 group 200 mg/kg of aqueous CLRE for 28 days, has significant (*P< 0.05) increase in level of SOD, and no significant increase in CAT, the TG-3 group (400 mg/kg of aqueous CLRE for 28 days) has significantly increased the levels of SOD, CAT (**P<0.01) when compared to disease control group (Table 3).

Histopathology

Histological evaluation of livers of Normal Control group

Table 1. Effect of Clre on Liver weights

S. No	Groups	Liver weight (g)
1.	Normal control	7.78 ± 0.411
2.	Disease control	10.23 ± 0.044$^{\alpha}$
3.	TG-1 (100 mg/kg)	10.10 ± 0.42
4.	TG-2 (200 mg/kg)	9.528 ± 0.32
5.	TG-3 (400 mg/kg)	7.66 ± 0.24***

Data was analyzed using One-way ANOVA followed by Tukey's Multiple Comparison Test and expressed as Mean ± SEM (n=6). $^{\alpha}$P< 0.0001 Disease control Vs Normal control, ***P<0.0001 TG-3 Vs Disease Control

Table 2. Effect of CLRE on Liver Function Biomarkers

S. No	Groups	Sgpt (u/l)	Sgot (u/l)	Alp (u/l)
1.	Normal control	18.54 ± 2.976	15.61 ± 1.866	105.5 ± 1.804
2.	Disease control	89.73 ± 2.921$^{\alpha}$	58.34 ± 11.25$^{\alpha}$	445.6 ± 38.33$^{\alpha}$
3.	TG-1 (100 mg/kg)	64.97 ± 5.700**	35.86 ± 3.52	420.0 ± 31.96
4.	TG-2 (200 mg/kg)	41.53 ± 3.415***	25.64 ± 4.36**	185.3 ± 23.46***
5.	TG-3 (400 mg/kg)	21.19 ± 2.376***	15.16 ± 1.368***	117. 7 ± 3.43***

Data was analyzed using One-way ANOVA followed by Tukey's Multiple Comparison Test and expressed as Mean ± SEM (n=6). $^{\alpha}$P <0.0001 Disease control Vs Normal control: **P<0.001, ***P<0.0001 TG-1, TG-2, TG-3 Vs Disease Control.

Table 3. Effect of CLRE on Lipid Peroxidation, Sod and Cat

S. No	Groups	Mda (µmol/mg)	Sod (u/mg)	Cat (u/mg)
1.	Normal control	1.105 ± 0.295	16.00 ± 1.173	15.98 ± 3.070
2.	Disease control	4.733 ± 0.949$^{\alpha}$	1.25 ± 0.144$^{\alpha}$	4.985 ± 0.97$^{\alpha}$
3.	TG-1(100 mg/kg)	3.798 ± 0.208	5.75 ± 0.59	6.603 ± 1.168
4.	TG-2 (200 mg/kg)	2.278 ± 0.211*	7.37 ± 1.84*	7.856 ±0.692
5.	TG-3 (400 mg/kg)	1.140 ± 0.396***	9.00 ± 0.97**	16.56 ± 1.58**

Data was analyzed using One-way ANOVA followed by Tukey's Multiple Comparison Test and expressed as Mean ± SEM (n=6). $^{\alpha}$P < 0.0001 Disease control vs normal control. *P< 0.05, **P< 0.001, ***P< 0.0001 TG-1, TG-2, TG-3 vs Disease control.

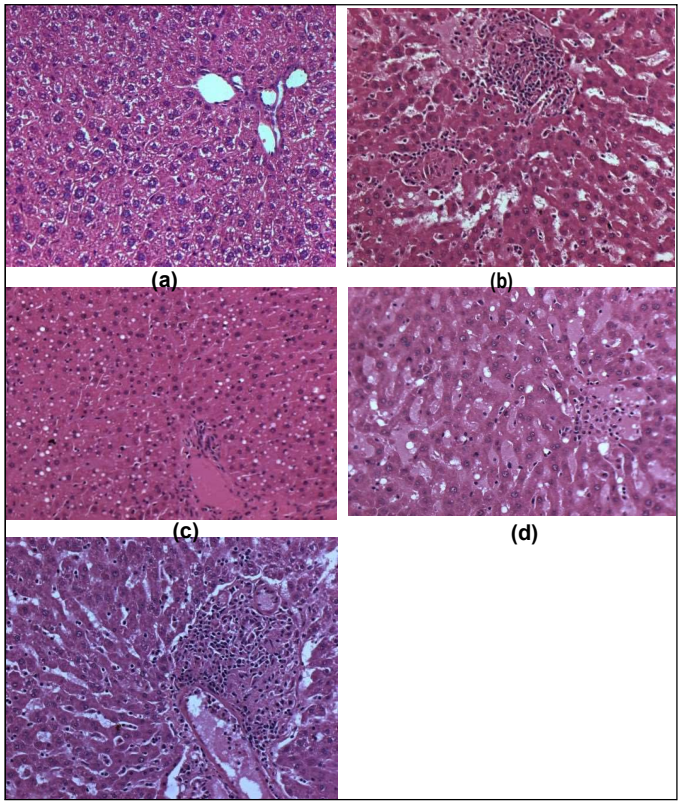

Figure 1. Observation of histopathology of liver (a) Normal Control group, (b) Disease Control group, (c) TG-1(100 mg/kg) group, (d) TG-2(200 mg/kg) group, (e) TG-3(400 mg/kg) group

has shown normal architecture. The morphology of hepatocytes, portal vein, portal region with duct and hepatic artery was normal and devoid of any necrosis and inflammation (Figure 1a). In case of Ethanol treated groups, multi focal necrosis of hepatocytes with infiltration of inflammatory cells were observed in centri lobular and peri-portal region of liver. Moderate sinusoidal dilatation was observed in between hepatocytes. Multi focal peri portal inflammation of hepatocytes with infiltration of inflammatory cells (Figure 1b). Liver tissue examined from CLRE (100 mg/kg p. o) treated rats has shown moderate vacuolar degeneration of hepatocytes, peri portal inflammation with infiltration of inflammatory cells (Figure 1c). In case of CLRE (200 mg/kg p.o) treated rats, dilatation of sinusoidal spaced with accumulation of fluids with inflammatory cells were observed (Figure 1d). Whereas, in CLRE (400 mg/kg p. o) treated rats, normal morphology of hepatocytes in portal, peri portal and centri lobular region was observed (Figure 1e). The highest dose of CLRE could restore the liver damage by showing normal architecture.

Discussion

This study investigated the protective effects of the aqueous CLRE on ethanol-induced hepatotoxicity in male rats by evaluating liver serum biomarkers and oxidative stress. The liver plays a crucial role in the detoxification and excretion of xenobiotic and metabolic wastes. Vast number of studies reported that ethanol causes liver damage (Chandra et al., 2000). The increased levels of aminotransferases (AST, ALT) and alkaline phosphatase (ALP) caused by ethanol exposure is a clear manifestation of cellular leakage and loss of functional integrity of the cell (Saroswat et al., 1993). Previous studies have demonstrated that ethanol exposure alters the structure and operation of the membranes in hepatocytes, increasing AST leakage (Raja Krishnan and Menon, 2001). Research on these enzyme changes may reveal metabolic anomalies and cellular damage in some organs.

Alcohol consumption, both acute and chronic, has been proven to increase the production of reactive oxygen species (ROS). Numerous studies have revealed that excessive ethanol exposure lowers antioxidant levels and raises the production of free radicals in both humans and animals. (Pinto et al., 2014). An imbalance between the generation of free radicals and the cell's capacity to mitigate or repair the harm wrought by these reactive products is known as oxidative stress. There are reports stating that the primary mechanism of ethanol-induced toxicity is oxidative stress (Flora et al., 2012). It has been noted that ethanol produces reactive oxygen species and alters the effectiveness of antioxidants by depleting their intracellular reserves (Dahiru and Obidoa, 2008).

Investigating the use of plant materials as a potent remedy for various ailments has not only validated their efficacy, but has also identified the pharmacological roles of these plant's individual bio constituents. The previous study's findings revealed the presence of phytochemicals such as glycosides, flavonoids, triterpenes, and phenolic compounds as classes of compounds with hepatoprotective activity (Adewusi and Afolayan, 2010). The advent of improved antioxidant defense by CLRE in this study is in line with previous reports that CLR is rich in antioxidants and bioactive molecules such as beta carotene, lycopene, vitamin C, flavonoids, cardiac glycosides, moisture, phenol, protein, lipid, carbohydrates, and fiber (Rahman et al., 2013).

Rats pre-treated with aqueous CLRE prior to ethanol induction resulted in a significant decreased levels of AST, ALT, and ALP in a dose dependent manner compared to disease control group rats. This indicates that pre-treatment with the aqueous CLRE prior to ethanol administration might have protected both plasma membrane and liver cells against membrane damage due to alcohol toxicity and thereby, decreasing the leakage of serum enzyme markers

into the circulation (Vogel, 2002). The significant reduction in liver enzymes after pre-treatment with CLRE suggests that the rind extract is hepatoprotective. Histopathological analysis and the decrease in the serum reduced the severity of necrosis and fatty infiltration observed in molecular architecture also showed that CLRE has hepatoprotective activity against ethanol-induced damage in these rats. As evident from the results, the treatment with CLRE significantly ameliorated the elevated levels of ALT, AST, ALP induced by ethanol in TG-2 (200 mg/kg p. o. of CLRE) and TG-3 groups (400 mg/kg p. o. of CLRE). The observed hepatoprotection by CLRE suggests that the extract tends to prevent liver damage and suppress the leakage of enzymes into the blood stream by preserving hepatocyte membranes.

In this study, the significant increase in MDA levels with apparent decrease in SOD and CAT levels in ethanol-treated rats were consistent with previous reports associated with overproduction of free-radicals in ethanol-induced HTX.

The influence of activated neutrophils and macrophages during inflammation and oxidative stress may explain the increase in lipid peroxidation after ethanol administration. CLRE treatment significantly reduced the ethanol-induced increase in MDA levels in the current study. As a result, significantly lower levels of MDA in treated groups' liver tissues compared to the Disease control group indicate lipid peroxidation attenuation. This was most likely due to CLRE causing less damage from oxygen free radicals.

The dynamic antioxidant enzymes SOD and CAT convert oxygen molecules into non-toxic products. SOD levels decrease as MDA and ROS levels rise. Depletion of CAT, in turn, prevents SOD action. The decrease in antioxidant enzymes is primarily due to increased ROS and lipid peroxidation. CLRE treatment significantly increased SOD and CAT levels after ethanol administration in the TG-2 (200 mg/kg p. o. of CLRE) and TG-3 (400 mg/kg p. o. of CLRE) groups, but not in the TG-1 (100 mg/kg p. o. of CLRE) groups. The results of serum markers and antioxidant parameters of hepatotoxicity were correlated with histopathological examination.

Conclusion

The main endeavor of present study was to investigate whether CLRE has any hepatoprotective activity in ethanol induced hepatotoxicity. According to the findings of the current study, ethanol-induced hepatotoxicity was contributed due to oxidative stress, leading to increasing the levels of liver function biomarkers (ALT, AST, and ALP), lipid peroxidation and reduced antioxidant enzymes. Pretreatment of animals with CLRE (200 mg/kg & 400 mg/kg) dose-dependently and significantly reversed the changes of of serum biomarkers, MDA, SOD and CAT. Thereby, from all the findings of this study it was concluded that CLRE has a potential protective effect against ethanol-induced hepatotoxicity.

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Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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