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Research Article

The anti-toxic activities of *Calotropisprocera* extract against liver injury in chronic ethanol toxicity rat model

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ABSTRACT

Objective: This study investigated the hepatoprotective role and antioxidant activities of the ethanolic extract of *Calotropisprocera* leaves using chronic ethanol toxicity rat model.

Method: One hundred and twenty male albino rats (weighing 150 - 160 gm) were divided into 4 groups each containing 30 rats as follows: (G1): Served as control, rats received 1 ml/100g body weight per day deionized water by gavage technique. (G2): (*Eth group):* Rats were received 1 ml/100g body weight per day 15% (v/v) Ethanol. (G3): (*Cp+Eth) group:* Rats were received 1 ml/100g body weight per day 15% (v/v) Ethanol and treated by *Cp* leaf extract that was dissolved in normal saline and administered orally to rats at dose 500 mg/kg. (G4) (*Cp group):* Rats were treated by *Cp* leaf extract that was dissolved in normal saline and administered orally to rats at dose 500 mg/kg. On the 31st day of treatment, blood was collected for assessment of serum biochemical parameters and harvested liver samples were assessed for antioxidants activity.

Results: The hepatotoxicants significantly (P <0.001) increased the levels of alanine transaminase (ALT), aspartate transaminase (AST), gamma-glutamyltransferase (GGT), malondialdehyde (MDA),H_2O_2,nitric oxideand reduced the levels of catalase (CAT), and reduced glutathione (GSH) compared to control. *Calotropisprocera*significantly reversed (P <0.001) the elevation in the level of ALT, AST, ALP, and bilirubin caused by the hepatotoxicants. The extract (500 mg/kg) significantly reversed (P < 0.001) the diminution in the level of in vivo antioxidants and increased the level of MDA produced by ethanol. Calotropisprocera(500 mg/kg) elicited significant reduction (P < 0.001) in the level of MDA compared to the alcohol group. Calotropisproceraalso reversed the deleterious effects of the hepatotoxicants.

Conclusion: The ethanolic extract of Calotropisprocera leaves possesses hepatoprotective activity forenhancement of in-vivo antioxidants as a possible mechanism of action.

Kew words: *Calotropisprocera* – Ethanol toxicity- hepatoprotective- antioxidants

INTRODUCTION

Medicinal plants represent considerable source in the treatment of various human diseases. *Calotropisprocera (cp)*is a member of the family *Asclepiadaceae* and has various pharmacological actions, hepatoprotective, action and anti-inflammatory actions[1]in addition to a wide range of actions including analgesic properties[2], immune responses[3], antimicrobial[4], antioxidant activity [5].

Alcohol consumption combined by prevalent and devastating diseases may lead to obesity and further increase a person's risk of accumulation of fat molecules, enlarged liver (i.e., hepatomegaly,

gastrointestinal damage, pancreatitis,alcohol liver disease, cirrhosis,and cancer (hepatocellular carcinoma)[6]. In many cases,heavy alcohol consumption can cause death. Alcoholcontributesto serious health and socioeconomic problems worldwide with high incidence in counters such as Spain and France[7-8].

Alcohol and its metabolites lead to decrease absorbing, digesting, and using essential nutrients (e.g., proteins, carbohydrates and vitamins particularly vitamin A) which may lead to liver disease. In addition alcohol catabolism, in the liver by the enzyme alcohol dehydrogenase or by microsomal ethanol–oxidizing system generates toxic and highly reactive oxygen products which may interact with lipid and causing lipid peroxidation and liver cell damage[9].

Chronic consumption of alcohol leads to increasereleasof reactive oxygen species (ROS), including hydroxyl radical ($OH^{\bullet}$), superoxide anion radical ($O_2^{-\bullet}$), and hydrogen peroxide (H_2O_2). Reactive oxygen species are very toxic to all types of cells because they can react with most cellular macromolecules, including proteins, lipids, and DNA.ROS may induce oxidation of proteins lead to conformational changes in the proteins' three-dimensional structure as well as aggregation, fragmentation or cross-linking of the proteins[10]. The release of ROS induced by heavy consumption alcohol is considered asone of the important factors in the development liver injury. Antioxidants system include various enzymatic and non-enzymatic mechanisms that protectingour cells against ROS. Antioxidant enzymes include superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), and nonenzymatic antioxidants system can be enhanced by antioxidant intake and include vitamin A, vitamin E, ascorbate, glutathione (GSH), and ubiquinone[11].

The aim of the current study wasto show the role of aqueous leaf extract of (*Cp)* extract on the development of liver injury and determine its antioxidant role in chronic ethanol toxicity.

MATERIALS AND METHODS

Preparation of the extract: *C. procera*plant was collected from Al-Dawadmi, KSA, in April 2018, the plant was authenticated by (Department of Botany), at the college of Science, University of Shaqra, KSA. The leaves of the plant were dried under shade at ambient temperature and aqueous extract was prepared by using a soxhlet apparatus[12].The extract was centrifuged for 10 min at 4^0C at 4000 rpm and filtered by using Whitman 1 filter paper (size 11 µm). The solvent was removed completely under reduced pressure. The extract was dissolved in normal saline and administered orally to rats at dose 500 mg/kg.

Chemicals: Ethanol with high purity grade (99.9%) was purchased from Merck company (Germany). Biochemical measurement kits were purchased from Sigma chemical company.

Animals: 120 male albino rats (weighing 150 - 160 gm) were obtained from the College of Science, Qassim University, KSA. The animals were accommodated in standard conditions of ventilation and temperature (25 ± 2^0C), humidity (60-70%) and light/dark condition (12/12). Animals had access to a standard pellet diet. Animals were divided into 4 groups each containing 30 rats as follows: (G1): Served as control, rats received 1ml/100g body weight per day deionized water by gavage technique. (G2): (*Eth group):* Rats were received 1 ml/100g body weight per day 15% (v/v) Ethanol. (G3): (*Cp+Eth) group:* Rats were received 1 ml/100g body weight per day 15% (v/v) Ethanol and treated by *Cp*leaf extract that was dissolved in normal saline and administered orally to rats at dose 500 mg/kg. (G4) (*Cp group):*Ratswere treated by *Cp*leaf extract that was dissolved in normal saline and administered orally to rats at dose 500 mg/kg. The experimental lasted 30 days procedures involving animals and their care were performed in accordance with the guidelines of the Institution Animal Ethics Committee and the

National Institutes of Health (NIH) for the care and use of animals.

Parameters: The present experiment was continued for one month. At weekly intervals beginning one week after treatment, each rat was weighed. At the end of the experiment, animals were sacrificed and blood was collected into clean tubes. The clear serum samples were separated by centrifugation at 3000 r.p.m. for 10 min, then serum was kept at -80 ^{0}C and used later in biochemical assays.

The livers were removed, weighted and liver weight/body weight ratio was determination. For biochemical measurements, liver pieces on ice immediately homogenized in ice-cold phosphate buffer (pH 8). For all measurements, tissue-protein estimation was performed using Bradford's method[13]. Malondialdehyde (MDA) was assayed according to the method reported byYoshioka et al. (14), hydrogen peroxide was assayed according to Aebi et al.(15). Nitric oxide was determinate by the colorimetric method according to Montgomery and Dymock(16).

Statistical analysis

Data analysis was done by using SPSS version 21.0 for Windows (Statistical Package for the Social Sciences Inc, Chicago, Illinois). Means were compared by independent-samples, t-test. All data are expressed as the mean ± the standard error (SEM).

RESULTS

A- Changes in the total Body Weight and Lw/Bwratio

In all ethanol treated groups (groups 2 and 3), animals showed normal increase in body weight during the first four weeks, after that, a marked decrease in its body weights compared to the control group was observed especially in group 4. In addition, animals treated with *Calotropisprocera*leaf extract in group 4(*Cp group)*showed less decrease in their body weight than group 2 and 3.

Table 1: The body weight (gm) in different groups during the period of the experiment

Groups / Parmeters	Group 1	Group 2	Group 3	Group 4
Body weight (gm)	**211 ± 17.8***	**189 ± 8.1***	**197 ± 13.07**	**204 ± 9.23**
Liver weight (gm)	**3.3 ± 0.41***	**4.55 ± 0.30***	**4.07 ± 0.27***	**3.97 ± 0.54***
Lw/Bw ratio	**3.05±0.70***	**5.13±0.50***	**4.23±0.0.53***	**3.82±0.38***

Data presented as mean±SE, n= 30 for all groups,
* values are statistically different.
G1: Control group G2: *Ethanol* treated group G3: Ethanol +*C. procera*treated group G4: *C. procera*treated group

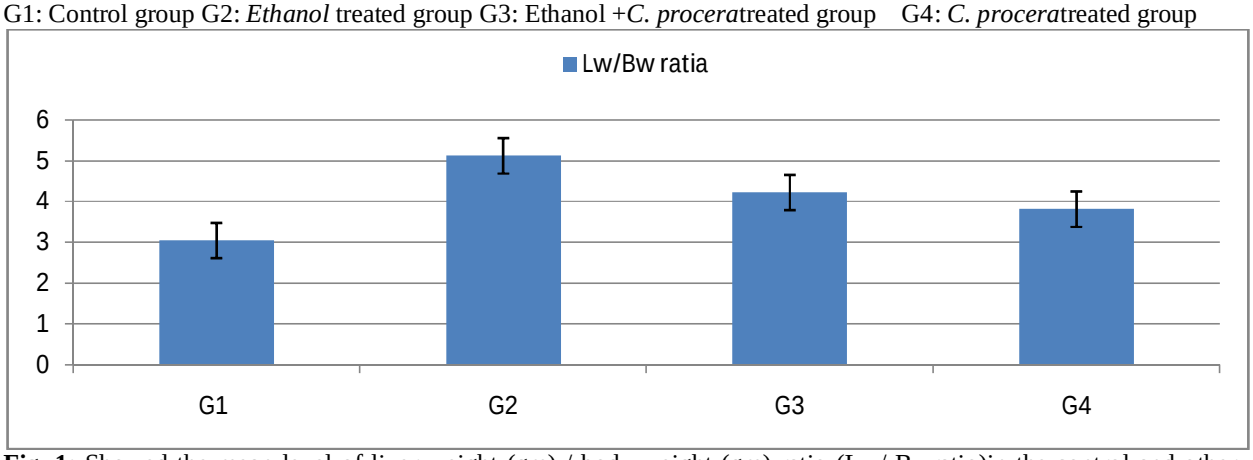

Fig. 1: Showed the mean level of liver weight (gm) / body weight (gm) ratio (Lw/ Bwratio)in the control and other treated groups. Data presented as mean±SEM, n= 30 for all groups
G1: Control group, G2: (*Ethanol*) treated group, G3: (Ethanol +*C. procera*) treated group, G4: (*C. procera*)treated group

Table 2: The mean level of liver enzymes (AST, ALT,and GGT) in the different groups.

Groups	AST (U/L)	ALT(U/L)	GGT (U/L)
G1	32.5±3.9	35.5 ± 4.2 (a)	31.4±0.2
G2	97.4±17.6	225.6 ± 18.6 (e)	71.2±0.1
G3	63.5±13.8	185.2 ± 12.24 (d)	51.1±0.3
G4	41.6±7.2	45.2 ± 7.54 (c)	41.4±0.1
p-value	<0.001	<0.001	<0.001

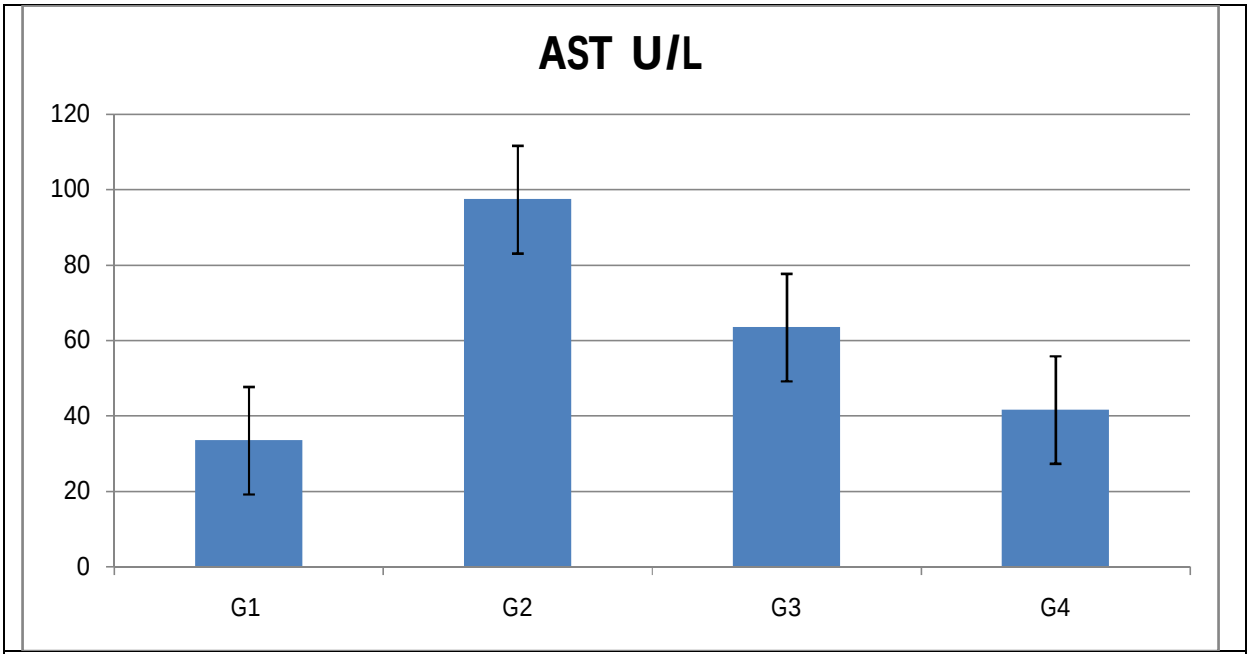

Fig.2: The mean level of liver enzymes (AST) in the different groups. G1: Control group, G2 : (*Ethanol*) treated group, G3: (Ethanol +*C. procera*) treated group, G4 : (*C. procera*) treated group

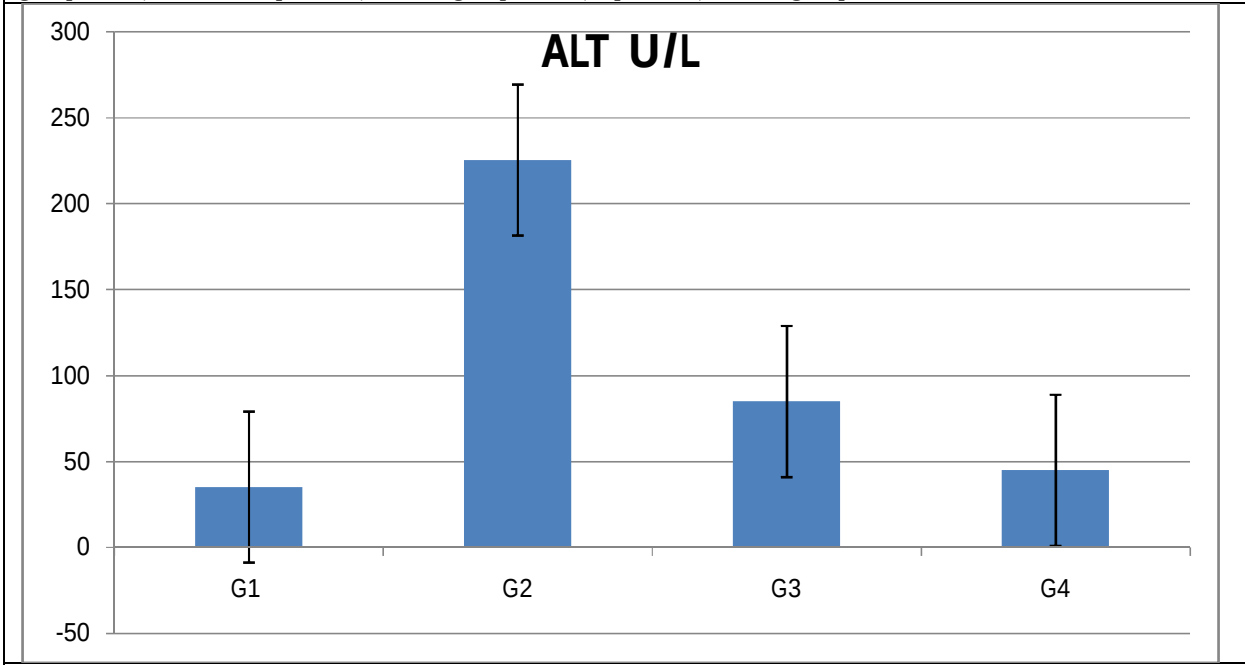

Fig.3: The mean level of liver enzymes (ALT) in the different groups. G1: Control group, G2 : (*Ethanol*) treated group, G3: (Ethanol +*C. procera*) treated group, G4 : (*C. procera*) treated group

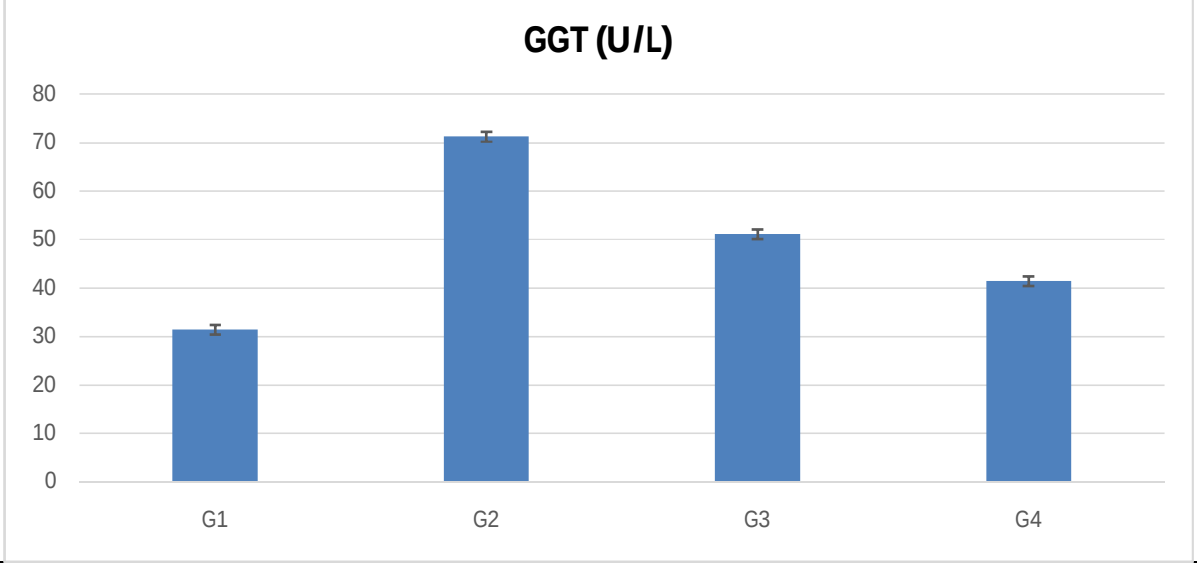

Fig.4: The mean level of liver enzymes (GGT) in the different groups. G1: Control group, G2 : (*Ethanol*) treated group, G3: (Ethanol +*C. procera*) treated group, G4 : (*C. procera*) treated group

The results showed a significant increase in the liver weight in the ethanol-treated group compared to normal rats. Values of mean relative liver weight (LW/BW) percent showed a significant difference between treated groups compared to the control group (Table 1).

B - Biochemical parameters

Liver Enzymes

The results showed that the mean level of liver enzymes ALT, AST and GGT were significantly increased in Ethanol treated group than control,*Cp*and (*Cp+Eth)*treated groups respectively (table 2, fig.2, 3 and 4).

Antioxidant activity

1- Hydrogen Peroxide levels

The mean levels of hydrogen peroxide in G2, G3, and G4 treated groups were (35.13 ± 0.42), (27.47 ± 0.24)and (21.36 ± 0.25) µmol/l respectively which showed a significant increase when compared to the normal control mean level (15.26 ± 0.12)p <0.001(table 3, fig. 5).

2-Nitric Oxide levels

The mean levels of nitric oxide in G2, G3,and G4 treated groups were (59.51 ± 5.09), (29.60 ± 3.97) and (16.48 ± 0.75) nmol/ml respectively which showed a significant increase when compared to the normal control mean level (10.63 ± 2.22) p<0.001 (table 3, fig. 6).

3- Catalase levels

The mean level of nitric oxide in G2, G3,and G4 treated groups were (11.6 ± 0.03), (15.5 ± 0.05), and (20.1 ± 0.02) µmol/min/mgrespectively which showed a significant decrease when compared to the normal control mean level (23.5 ± 0.04) p<0.001 (table 3, fig. 7).

4- Reduced Glutathione levels

The mean level of nitric oxide in G2, G3,and G4 treated groups were (16.8 ± 0.32), (21.6 ± 0.42),and (25.5 ± 0.25) µmol/min/mg respectively which showed a significant increase when compared to the normal control mean level (28.6 ± 0.52) p<0.001 (table 3, fig. 8).

5- Malondialdehyde levels

The mean level of malondialdehyde levelsin G2, G3,and G4 treated groups were (120 ± 1.42), (85.5 ± 1.11), and (62.4 ± 1.25) nmol/grespectively which showed a significant increase when compared to the normal control mean level (55.12 ± 1.2) p <0.001(table 3, fig.9).

Table 3: The mean level of hydrogen peroxide (µmol/l), nitric oxide levels (nmol/ml), catalase (U/ml), reduced glutathione (mg/dl) and malondialdehyde(nmol/ml) levels in the different groups.

Groups	G1	G2	G3	G4	p-value
Hydrogen Peroxide (µmol/l)	15.26 ± 0.12 (a)	35.13 ± 0.42 (e)	27.47 ± 0.24 (d)	21.36 ± 0.25 (c)	<0.001
Nitric Oxide levels (nmol/ml)	10.63 ± 2.22 (a)	59.51 ± 5.09 (e)	29.60 ± 3.97 (d)	16.48 ± 2.75 (b)	<0.001
Catalase (µmol/min/mg)	23.5 ± 0.04 (a)	11.6 ± 0.03(e)	15.5 ± 0.05 (d)	20.1 ± 0.02 (b)	<0.001
Reduced Glutathione (µmol/min/mg)	28.6 ± 0.52 (a)	16.8 ± 0.32 (e)	21.6 ± 0.42 (d)	25.5 ± 0.25 (b)	<0.001
Malondialdhyde levels (nmol/g)	55.12 ± 1.2 (a)	120 ± 1.42 (e)	85.5 ± 1.11 (d)	62.4 ± 1.25 (c)	<0.001

Data presented as mean±SEM, n= 30 for all groups

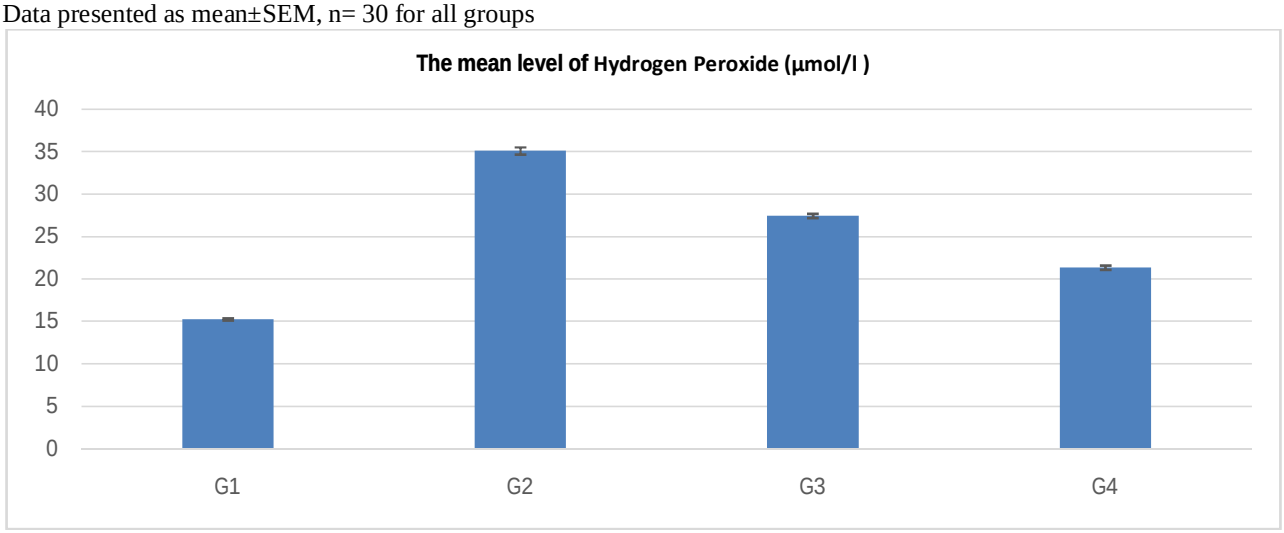

Fig.5: The mean level of hydrogen peroxide (µmol/l) in the different groups.Data presented as mean±SEM, n= 30 for all groups.
G1: Control group, G2: (*Ethanol*) treated group, G3: (Ethanol +*C. procera*) treated group, G4: (*C. procera*) treated group

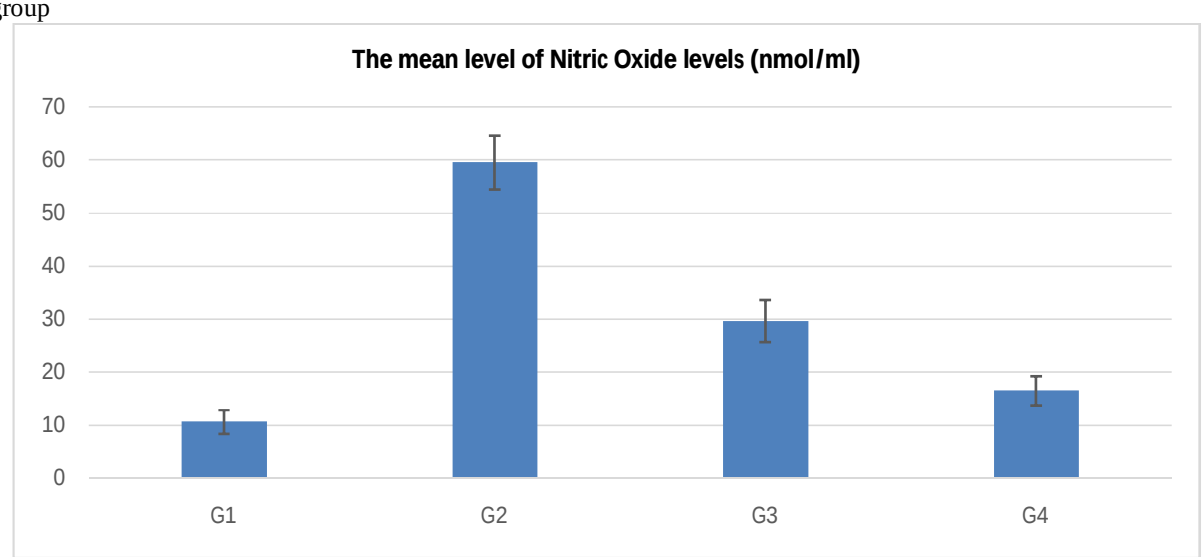

Fig. 6: The mean level of nitric oxide (nmol/ml) in different groups. Data presented as mean±SEM, n= 30 for all groups: G1: Control group, G2: (*Ethanol*) treated group, G3: (Ethanol +*C. procera*) treated group, G4: (*C. procera*) treated group

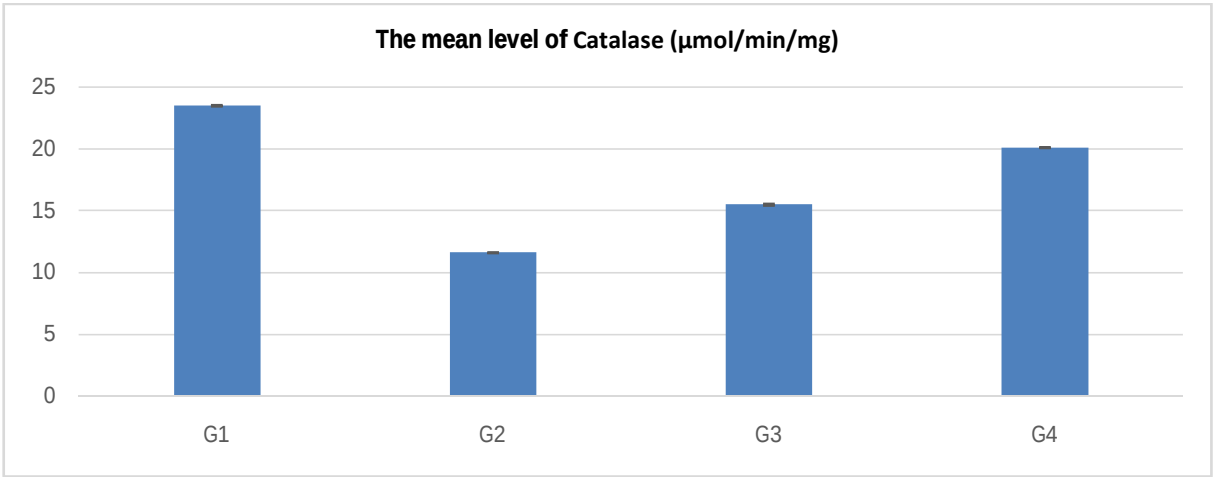

Fig. 7: The mean level of catalase (µmol/min/mg) in different groups. Data presented as mean±SEM, n= 30 for all groups: G1: Control group, G2: (*Ethanol*) treated group, G3: (Ethanol +*C. procera*) treated group, G4: (*C. procera*) treated group

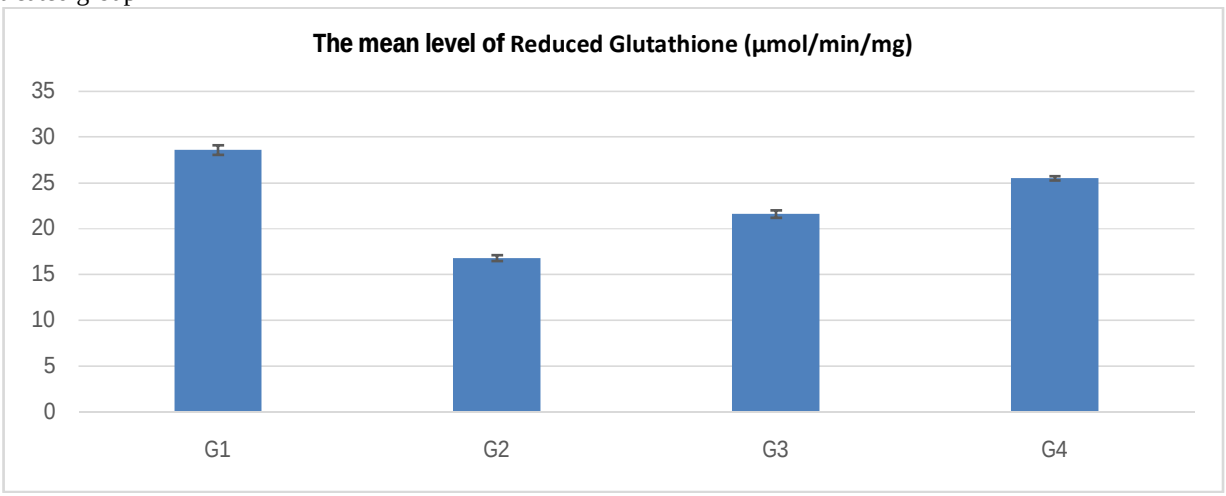

Fig. 8: The mean level of reduced glutathione (µmol/min/mg) in different groups. Data presented as mean±SEM, n= 30 for all groups
G1: Control group, G2: (*Ethanol*) treated group, G3: (Ethanol +*C. procera*) treated group, G4: (*C. procera*) treated group

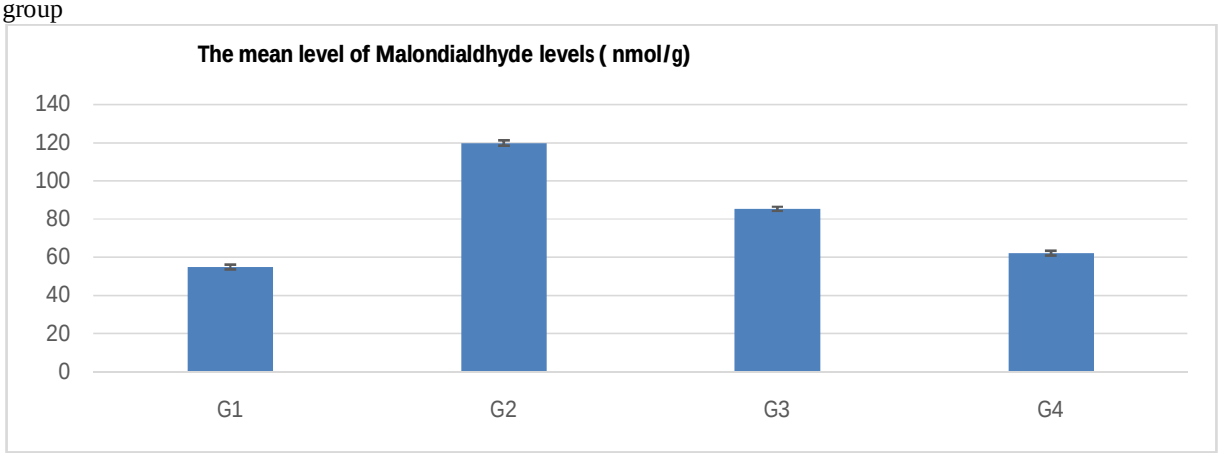

Fig. 9: The mean level of malondialdehyde (µmol/g) in the different groups.Data presented as mean±SEM, n= 30 for all groups
G1: Control group, G2: (*Ethanol*) treated group, G3: (Ethanol +*C. procera*) treated group, G4: (*C. procera*) treated group

DISCUSSION

The results showed that the mean level of liver enzymes ALT, AST and GGT were significantly increased in Ethanol treated group than control, Cp and (Cp+Eth) treated groups respectively. Chronic excessive alcohol consumption more than (40–80) gram per day for males and more than (20–40) gram per day for females could induce serious liver injury. The liver is a complex and large organ play a central role in carbohydrate, fat and protein metabolism. Liver involved in many of biosynthetic functions. AST levels increase in about 60% of toxic liver injury. Ethanol exerts many toxic effects that induced liver damage leading to increasing the activity of its enzymes. Elevation in liver enzymes suggests hepato parenchymal injury (ALT and AST) or Hepatobiliary diseases (GGT)[17]. Cp extract may improve the liver withstand capacity to damaged that occur due to Ethanol toxicity that indicated by elevation of liver enzymes. Our results in agreement with other results that studied the effect of ethanol on rat and showed an increased level of ALT, ALP,and AST, as well as elevated TG after 7 days of continual feeding with a high concentration (50% v/v) of ethanol[18-19].

In addition, the results showed a significant increase in the mean level of hydrogen peroxide andnitricoxide levelswhile the mean level ofreduced glutathione and CAT was significantly decreased than normal and Ethanol + C. procera treated groups respectively (Table 3, Fig.5-8). The antioxidant defense mechanism including many enzymes as glutathione S-transferase (GST), superoxide dismutase and catalase which constitute an enzymatic family that protect against exogenous and endogenous oxidative stress and detoxify many toxins induced liver damage[20], a varietyofelectrophilic compounds, oxidized lipid and reactive oxygen species that cause damage to intracellular molecules. GST also involved in metabolizing of Ethanol[20]. Excessive production of free radicals in the organism indicated an imbalance between the concentrations of these toxic products and the antioxidant defensessystem and combined by the accumulation of ROS and polyunsaturated fatty acids leading to increasing the oxidative stress and toxicity to the hepatic cells[21].

The mean level of MDA was significantly increased in the ethanol-treated group when compared to the normal control mean level (table 3, fig.9).Malondialdehyde considers as a lipid peroxidation marker and represents the final products of polyunsaturated fatty acids peroxidation in the cells it is increased during free radicals over-production. Ethanols are dangerous haven a high risk to cause human hepatocellular carcinoma (HCC) [22]. The results indicated the ability of *Calotropisprocera*leaf extract with its rich natural anti-toxic substance to protect the liver from severtoxic action of ethanol and minimize its hazard.

In our study, we find that *Calotropisprocera*leaf extract exhibit activity against ethanoltoxicity. The ethanolic extracts of the different parts especially flower andbuds have been reported to possess an antimalarial activity[23].The chloroform extract of the *Calotropisprocera*root has been shown to exhibit hepatoprotective effects against carbon tetrachloride that can induce liver damage[24].

Previous studies report that, *Calotropisprocera* aqueous root extract posses antioxidant and membrane protective activities against free radical and metal ion-mediated oxidative damage and its capability to combat free radical-mediated damage[25]. The present results demonstrated that Ethanol was associated with a significant increase in the concentration of H_2O_2, one form of active oxygen species. *Calotropisprocera*leaf extract could be regarded as a source of future antioxidant compounds of natural origin. Several trials found that supplementation with antioxidants can reduce the risk of cancer or inhibit the further development of cancer precursors and has produced neither benefit nor harm[26].Mathur et al,(27) tested four different types of *Calotropisprocera*root extracts including methanol, hexane, aqueous and ethylacetate

extract and its possible mechanism against Hep2 cancer cells via apoptosis and cell cycle disruption based mechanisms.

The results have linked alcohol high consumption with increased lipid peroxidation biomarker MDA and combined with increased of GSH level and indicated on abnormally high levels of ROS and oxidative damage initiated by reactive oxygen species and this may explain the toxic findings that occurred in livertissues, this is in agreement with findings reported previously and showed that ethanolhigh consumption may interfere with the normal functioning of the liver cells by alters energy metabolism in the Liver[28] similarly, ethanol metabolism may alter the ratio of NAD^+ to reduced NAD (NADH) and promotes gene silencing or activation, leading to diseased phenotype[29]. In addition, ethanol may lead to oxidative stress, mitochondrial directed apoptosis of the liver cells as well as inhibiting the ability ofliver cells to synthesisprotein, RNA and DNA[30]. *Calotropisprocera* leaf extracts may act as an antitoxic agent and this supported by other studies that indicated that showed antitoxin activity of *Calotropis gigentea* against Najanaja-cobra) venom[31]. The aqueous flower extract has been shown to possess anti-inflammatory activities[32].

CONCLUSION

In conclusion, our current investigation was found that the beneficial role of *Calotropisporcera* in the therapeutic management of liver toxicity. The research also supports the antioxidant activity of *Calotropisprocera.* Further studies are needed for the confirmation of the mechanism of action of *Calotropisprocera*inthe liver and find out the active constituents responsible for this property. This plant could be employed as an adjuvant in the treatment of liver disease patient to alleviate or suppress some process of this disease.

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AUTHOR CONTRIBUTIONS

Dr. Ali conducted the experiment and prepared the manuscript. Dr. Zafar Al-shahri designed the experiment and contributed in the experimental part of the work.

CONFLICT OF INTERESTS

The authors confirm that this article content has no conflict of interest.

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