



Hepatoprotective Activity of *Lagenaria sicerari* Extract against Paracetamol Induced Hepatic Damage in Rats

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Abstract: *Lagenaria sicerari* has been traditionally used in Indian medicine as a result of its curative results of hepatitis, gonorrhea and diabetes, it is probably not proof-founded. However folklore has given us many powerful therapies, based on plant sources. So claims which can be made for the protective efficacy of *Lagenaria sicerari* (family: Cucurbitaceae) to treat hepatic diseases. The present study focused on investigating the role of alcoholic extract of *Lagenaria sicerari*. It appreciably prevented the increased in serum Aspartate amino transferase (AST), Alanine amino transferase (ALT), alkaline phosphatase (ALP) and total serum bilirubin (SB) level in acute liver damage by Acetaminophen and elevated the activities of lipid peroxidation (LPO) and glutathione (GSH) in the liver. Histopathological observation of the liver used to be additionally performed to further support the evidence from the biochemical analysis. The observation that these significant protective effect against acute hepatotoxicity induced by acetaminophen of *Lagenaria sicerari*.

INTRODUCTION

Lagenaria siceraria, (LS) (Family: Cucurbitaceae) is also known as *Lagenaria leucantha* Rusby and *Lagenaria vulgaris* Seringe. Its common names include bottle gourd (Eng.); alabu (Sanskrit); lauki or ghia (Hindi); dudhi or tumbadi (Gujarati); sorakkai (Tamil); chorakkaurdu (Malayalam); and ghiya (Urdu). Geographically it occurs throughout India and is now cultivated worldwide [1-3].

Traditional uses The fruits, leaves, oil, and seeds are edible and used by local people as folk medicines in the treatment of jaundice, diabetes, ulcer, piles, colitis, insanity, hypertension, congestive cardiac failure, and skin diseases. The fruit pulp is used as an emetic, sedative,

purgative, cooling, diuretic, antibilious, and pectoral. The flowers are an antidote to poison. The stem bark and rind of the fruit are diuretic. The seed is vermifuge. Extracts of the plant have shown antibiotic activity. Leaf juice is widely used for baldness [4, 5].

Lagenaria siceraria was researched and reported that different pharmacological like Analgesic and anti-inflammatory activity, Antihyperlipidemic activity, Diuretic activity, Anthelmintic activity, Antihepatotoxic activity, Immunomodulatory activity, Antioxidant activity and Antimicrobial activity [4-7].

Paracetamol (PCM) (N-acetyl-p-aminophenol) is usually used as an analgesic and antipyretic drug. Extensive use of PCM for therapeutic functions leads to severe hepatic damage. Toxic doses of PCM could cause changes in the morphology and function of liver mitochondria [8, 9]. Formation of N-acetyl-pbenzoquinone imine (NAPQI) is the responsible for liver injury through depletion of glutathione (GSH) even as it binds to cellular proteins [10]. PCM induced hepatotoxicity is known to involve liver cytochrome P450 (CYPs) together CYP2E1, CYP3A4 and CYP1A2 and it also inhibits the mitochondrial oxidative phosphorylation, reduction of adenosine triphosphate (ATP) and produces selective mitochondrial oxidant stress [11]. Cellular necrosis of the liver cells raises the lipid peroxidation and depletion of glutathione (GSH) besides elevating the serum biochemical marker levels [12]. The present study exceptionally focused on investigating the function of alcoholic extracts from *Lagenaria siceraria* against PCM-induced hepatic injury of rats. To evaluate the hepatoprotective effect of *Lagenaria siceraria* in the *in-vivo* study, the serum levels of different marker enzymes regarding hepatic integrity, such as Alanine Amino Transferase (ALT), Aspartate Amino Transferase (AST), Alkaline Phosphatase (ALP), and Total Serum Bilirubin (SB) were determined. And also estimation of Glutathione (GSH) and Lipid Peroxidation (LPO) was determined in the form of Malondialdehyde (MDA) protein on the cellular degree in the liver. Furthermore, histological reviews had been carried out to prove the effectiveness of *Lagenaria siceraria* in a preventive and healing function against Acetaminophen induced toxicity of liver histopathology in rats.

MATERIALS AND METHODS

Chemicals

Acetaminophen (Paracetamol) 500 mg tablets obtained from Nirmal Prime, Mumbai, India. Silymarin was purchased from Micro Labs, Tamilnadu, India. Moreover, saline was once bought from the nearby provider GSN Pharmaceutical Private Limited, Hyderabad, Telangana and India. The following biochemical parameters of AST, ALT, ALP and Bilirubin were estimated through specifications kits obtained from Span Diagnostics, Surat, India. Rat's feed was once supplied from Mahaveer Endeavors, Madipally and Hyderabad, India. Other chemicals and reagents for this investigation had been of diagnostic grade.

Plant Materials

Lagenaria siceraria plant material was collected from Medchal malkajgiri District, Telangana . The plant specimen was identified by Prof. Rana Kausar, Dept. Of Botany, Osmania University, Hyderabad, Telangana State. A specimen was deposited in their herbarium. Then after the plants were washed thoroughly to remove adhering soil and earthy matter, later on sliced to thin chips and dried in shade at room temperature and ground to optimal coarse powder.

Extraction:

Lagenaria siceraria Whole plant was collected, shade dried and powdered. Each plant powder was subjected to by soxhelt extraction three times at ambient temperature (50-60°C) with 90 % methanol. During the extraction with solvents, the solvent was changed every 24 h. The solvents from the pooled extracts were removed by rotary evaporator under reduced pressure at 50-60 °C to create crude extracts of alcoholic extracts of *Lagenaria siceraria*. The extracts were subjected to preliminary phytochemical investigation and subjected for the biological activity screening tests.

Preliminary Phytochemical Studies

The extract of *Lagenaria siceraria* were subjected to preliminary phytochemical screening for the detection of various phytochemical constituents such as alkaloids, amino acids, carbohydrates, flavonoids, glycosides, mucilage, proteins, steroids, tannins and terpenoids [13, 14].

Animal model and grouping:

An experimental study was carried out on Wister albino rats of either sex (M/F) rat's age two months. Their body weights ranged from 150 to 200 g. Divided into 6 groups of 6 animals per cage was used. Animals were maintained under standard laboratory aseptic conditions (12-h light/dark cycle, 24hrs). The food in the form of dry pellets and water is provided ad libitum. All the animals were approved by the ethics approval committee of the institute

Experimental setup

- Group I: Vehicle treated rats (1 mg/kg b.wt)
- Group II: Control (PCM 100 mg/kg b.wt)
- Group III: PCM + Silymarin (100 mg/kg b.wt)
- Group IV: Rats treated with LD₅₀ mg/kg of methanol extract (100 mg/kg b.wt)
- Group V : Rats treated with LD₅₀ mg/kg of methanol extract (250 mg/kg b.wt)
- Group VI: Rats treated with LD₅₀ mg/kg of methanol extract (500 mg/kg b.wt)

The paracetamol (PCM) was diluted with saline (vehicle) before oral administration (o.p). To enhance the acute liver damage in animals of groups II, III, IV, V and VI, food were withdrawn 12 h before PCM administration. Animals were sacrificed 24 h after administration of PCM. Blood samples were collected by puncturing the retro-orbital plexus under light ether anesthesia and allowed to coagulate for 30 min at 37°C. Serum was separated by centrifugation at 2500 rpm at 37°C for 15 min and analyzed for various biochemical parameters.

Free radical scavenging activity

The DPPH radical scavenging activities of crude extracts of *Lagenaria siceraria*, were tried by Ohkawa et al. [15]. Briefly, 0.2mL of the sample solutions of various concentrations was added to 1mL of 0.1mM of freshly prepared DPPH (2, 2-diphenyl-1-picrylhydrazyl) solution. The reaction mixture was shaken forcefully and absorbance at 517 nm was determined after 20min at room temperature. Control sample was prepared contain the same volume without test compounds or reference antioxidants, while DMSO (Dimethyl Sulfoxide) was used as blank. The reference antioxidant BHT (butylated hydroxyltoluene) was used as the positive control in all the assays. The radical scavenging activity was measured as a decrease in the absorbance of DPPH[•] and calculated as follows:

$$\text{scavenging effect(\%)} = \left[\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100 \right]$$

Where A_{Control} is the absorbance of the control and A_{sample} is the absorbance of the extract or fractions or standard.

Superoxide Radical-Scavenging Assay

The superoxide radical-scavenging impact was determined by the method of Beutler et al. [16]. The reaction mixture with NBT (Nitro blue tetrazolium), (1mM) in phosphate buffer (0.1 M, pH 7.4), NADH (Nicotinamide adenine dinucleotide-reduced) (1mM) with or without samples, and PMS (Phenazine Methosulphate) (0.1mM) was incubated at room temperature for 10 min and the absorbance was reported at 560 nm. The inhibition percentage was calculated against a control the samples. The scavenging capacity was calculated using the equation as described for DPPH assay.

Assay of FeCl₃ Power

The FRAP assay was determined by the technique of Benzie and Strain with minor adjustments [17, 18]. It depends on the capacity of the sample to reduce the Fe (III)-TPTZ (ferric tripyridyltriazine) complex to ferrous tripyridyltriazine Fe (II)-TPTZ at low pH. Fe (II)-TPTZ has an intensive blue color which can be understand writing at 575nm. The stock solutions consist of 300mM acetate buffer (pH 3.6), 10mM TPTZ (2, 4, 6 tripyridyl S triazine) in 40mM of HCl, and 20mM ferric chloride solution. The new working solution was ready by mixing 25mL of acetate buffer, 2.5mL of TPTZ, and 2.5mL of FeCl₃·6H₂O and the temperature was maintain to 35 °C earlier than use. The various concentrations of extract, fractions, and BHT (10–50 µg/mL) were allowed to react with 2mL of the FRAP solution for 30 min in the dark condition. The absorbance was record at 575 nm. The results are spoken in µMFe (II)/g and were estimated using aqueous FeSO₄·7H₂O (20–100µM) as standard for calibration.

Assessment of Liver Functions

The hepatoprotective impact of extract was assessed by the measure of liver potential, biochemical parameters, for design, Alanine Amino Transferase (ALT) [19], Aspartate Amino Transferase (AST) [20], Alkaline Phosphatase (ALP) [21] and Total Serum Bilirubin (SB) [22],

Lipid Peroxidation (LPO) as Malondialdehyde (MDA) [23] and Glutathione (GSH) [24] as per commonplace protocols.

Histopathological study

Histopathological investigation of the liver was done according to the standish et al [25] method.

Statistical analysis

On each set of data Bartlett's test was conducted to ensure that the variation of the set is homogeneous. In the case of a homogeneous set of results, ANOVA was conducted to assess the treatment effects and using Origin Pro 7.6 statistical software, Dunnett's test was used as applicable. This was transformed using correct transformation in the case of heterogeneous data. The variance was measured at a meaning level of 5 percent and the values expressed as mean \pm SEM, and the statistically significant $P < 0.05$ was considered.

RESULT

Extraction:

Fresh *Lagenaria siceraria* (LS) plants (250gms) were collected under shade and dried. Each plant powder was subjected to by soxhelt extraction three times at ambient temperature (50-60°C) with 90 % methanol. During the extraction with solvents, the solvent was changed every 24 h. The solvents from the pooled extracts were removed by rotary evaporator under reduced pressure at 50-60 °C to create crude extracts of alcoholic extracts of *Lagenaria siceraria* (CELS). The percentage yields (17.5%) of the extractives of the plant CELS. The extracts were subjected to preliminary phytochemical investigation and subjected for the biological activity screening tests.

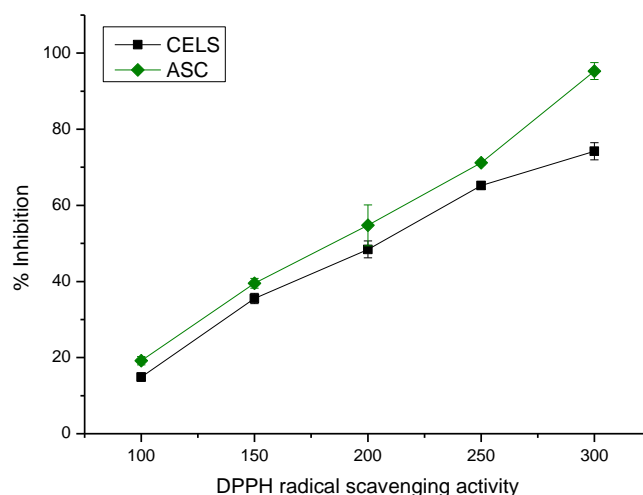
Preliminary phytochemical studies:

The phytochemical constituents present in the extract of CELS were determined as if we done. The outcome of preliminary phytochemical showing was Favonoids, phenols, Terpenoid and Steroids are presented.

Free radical scavenging activity

DPPH Radical Evaluation

The substances are called antioxidants when they can reduce the stable radical (purple) DPPH to the non-radical form DPPH-H (yellow) and thus serve as radical scavengers because of their hydrogen donation capabilities. Figure 1 presents the results of DPPH scavenging activity for all the test samples. With an increase in sample concentration (100-500 $\mu\text{g mL}^{-1}$), scavenging activity of CELS extract and ascorbic acid (ASC) grew. CELS and ASC found the IC₅₀ values at 131.45 $\mu\text{g mL}^{-1}$ ($Y = 0.2967x - 11.492$) and 88.26 $\mu\text{g mL}^{-1}$ ($Y = 0.3677x - 17.546$). From these data obtained, the CELS have been considered an effective free-radical inhibitor as well as the primary antioxidants, which can limit free-radical damage in the body.

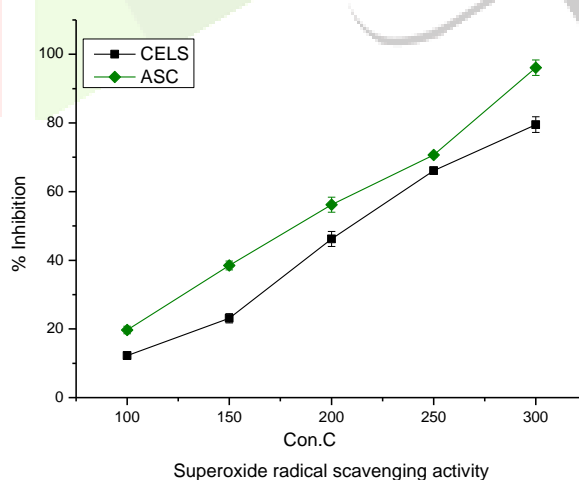


(ASC): Standard antioxidant; data represented as means \pm SD ($n = 3$).

Figure 1: DPPH radical scavenging activity of CELS

Superoxide Radical-Scavenging Assay

The development of ROS such as hydroxyl radical, H_2O_2 , and singlet O_2 in the living system was due primarily to the presence of superoxide anion radicals, either directly or generally by enzyme or metal progression catalyzed. Therefore the relative interceptive capacity of the extracts and fractions to scavenge the radical superoxide was anticipated to be evaluated. It was noted from the data presented in Figure -2 that the CELS and ASC showed the highest radical superoxide scavenging activity, IC_{50} values were found to be ASC $87.11 \mu g mL^{-1}$ ($Y=0.37x- 17.77$). However, it was found that CELS $68.78 \mu g mL^{-1}$ ($Y=0.3551x- 25.574$) scavenging activities are much closer to that of ASC, which is considered to be a strong radical superoxide scavenger.



(ASC): Standard antioxidant; data represented as means \pm SD ($n = 3$).

Figure 2: Superoxide radical scavenging activity of CELS

Antioxidant Power Reduction Assay (FRAP)

The ferric reduction / antioxidant power (FRAP assay) is widely used in dietary polyphenols for assessing the antioxidant component. The reduction properties are usually related to the presence of compounds that exert their action through the donation of a hydrogen atom breaking the free-radical chain. Figure -3 shows the results of plant extracts reduction potential relative to ASC, a well-known antioxidant data. CELS extract and ASC IC₅₀ values showed 76.81 $\mu\text{g mL}^{-1}$ ($Y=0.3411x-24.014$) and 91.74 $\mu\text{g mL}^{-1}$ ($Y=0.3541x-17.512$) both of these.

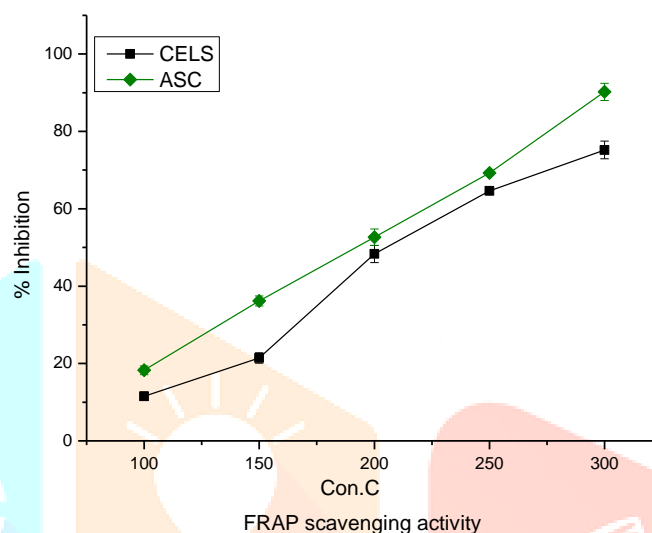


Figure 3: FRAP scavenging activity of CELS

Hepatoprotective activity: PCM Induced Liver Toxicity

As part of the study, crude methanolic extraction of CELS safeguarded the auxiliary uprightness of the hepatocellular film in a subordinate measurement manner as clear from the assurance given by Silymarin (100 mg kg^{-1} b.wt; po), a well-known hepatoprotective specialist. PCM is realized to affect liver damage through the activity of its dangerous metabolite, N-acetyl-P-benzoquinoneimine, delivered by cytochrome activity P-450. This metabolite causes glutathione (GSH) exhaustion which prompts the passage of cells. It is clear that the concentrate of CELS will decrease all the hissed levels of AST, ALT, ALP and Total serum bilirubin towards the ordinary standard means that plasma layer adjustment and also hepatotoxin-induced repair of hepatic tissue harms.

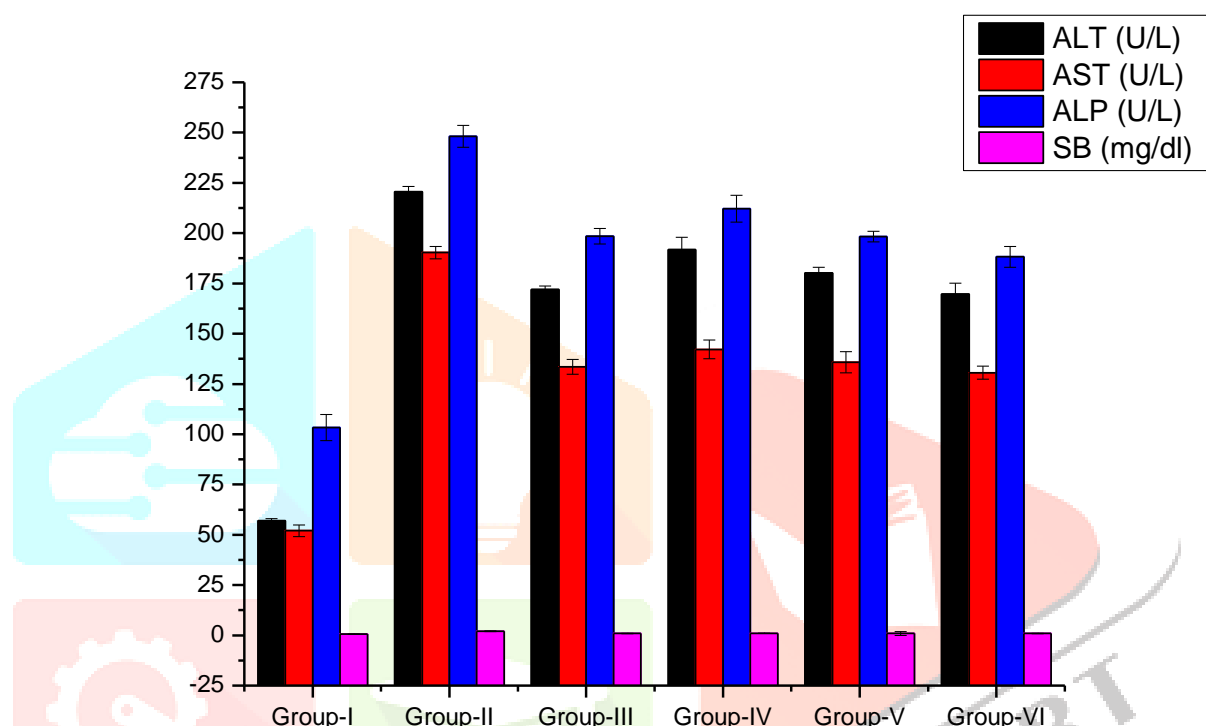
Given Table-1 (Figure -4), the similar viability of the concentrates tried results for their hepatoprotective movement.

Percentage of inhibition = $100 \times (\text{value of toxic control} - \text{value of test sample}) / (\text{value of toxic control} - \text{value of control})$.

Table 1: Effect of CELS on ALT, AST, ALP and SB in PCM induced liver toxicity in rats

Treatment	ALT (U/L)	AST (U/L)	ALP (U/L)	SB (mg/dl)
Group-I	57.00 ±1.06	52.00 ±2.89	103.33±6.52	0.55±0.02
Group-II	220.66±2.52 ^a	190.33±3.02 ^a	248.16±5.38 ^a	2.03±0.17 ^a
Group-III	172.00 ±1.75 ^{***}	133.50±3.68 ^{***}	198.5±3.83 ^{***}	0.93±0.07 ^{***}
Group-IV	191.83±6.17 ^{***}	142.16±4.66 ^{***}	212.16±6.73 [*]	0.99±0.056 ^{***}
Group-V	180.25 ± 2.75	135.78 ± 5.22	198.26 ± 2.65	0.95 ± 0.98
Group-VI	169.72 ± 5.33	130.56 ± 3.25	188.25 ± 5.20	0.92 ± 0.07

Each value represents the mean ± SEM. n =6 number of animals in each group. ^aP<0.001 vs vehicle control, *P<0.05, **P<0.01, *** P<0.001, Compared to respective PCM treated control groups

**Figure 4: Effect of CELS on ALT, AST, ALP and SB in PCM induced liver toxicity in rats**

The *In-vitro* Lipid Peroxidation Inhibition Activity in Rat Liver Homogenate:

There has been a critical increase in MDA substance and decrease in PCM Inebriated animals' GSH activities. Pre-treatment with silymarin (100 mg / kg b.wt) and CELS (100, 250 and 500 mg / kg b.wt) effectively kept the expansion at MDA levels and transmitted them close to the typical level, while GSH levels were increased overall (P<0.01), along these lines giving assurance against toxicity to paracetamol. Results are shown in Table -2 (Figure -5)

Table 2: Effect of CELS on LPO and GSH, PCM induced hepatic damage in rats

Treatment	LPO (nM MDA/mg protein)	GSH (µg/mg protein)
Group-I	0.93±0.03	6.17±0.24
Group-II	5.18±0.18 ^a	2.26±0.30 ^a
Group-III	1.25±0.22 ^{***}	6.01±0.10 ^{***}
Group-IV	4.11±0.22 ^{***}	2.91±0.10 ^{***}
Group-V	2.11±0.11 ^{***}	4.01±0.17 ^{***}
Group-VI	2.64±0.23 ^{***}	4.94±0.06 ^{***}

Each value represents the mean \pm SEM. $n = 6$ number of animals in each group. ^a $P < 0.001$ vs vehicle control, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, Compared to respective PCM treated control groups

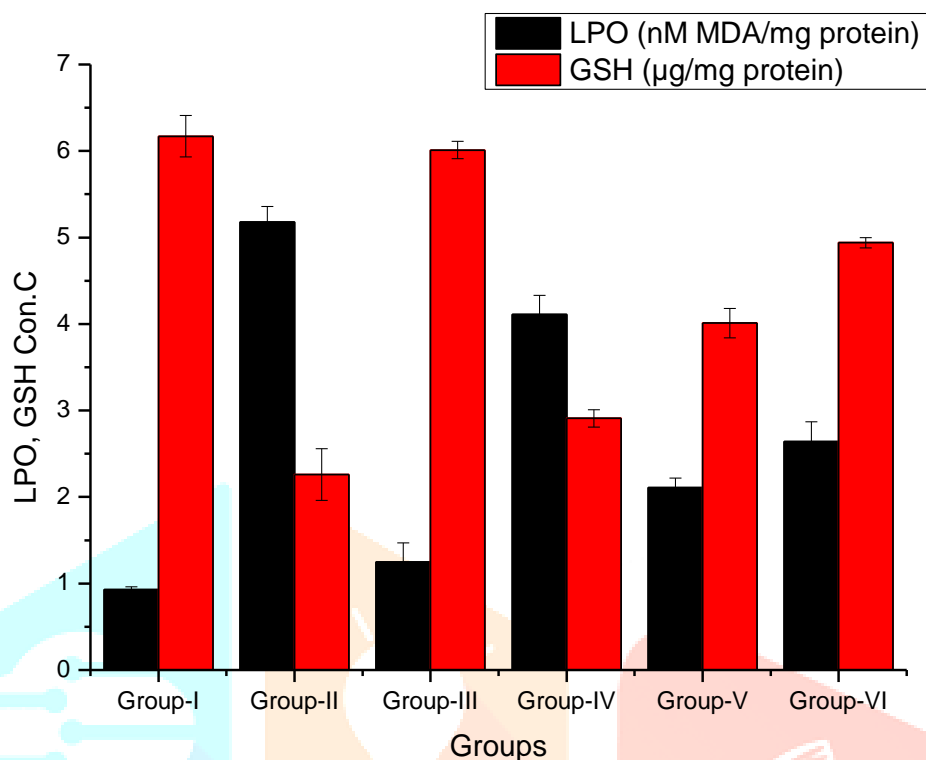


Figure 5:

Effect of CELS on LPO and GSH, PCM induced hepatic damage in rats

Histopathological examination of rat livers

The test animals were later collected on the ninth day, puncturing the retro-orbital plexus under mellow ether anesthesia, then sacrificing animals and collecting liver tissues. Histopathological observation of the liver was performed in this study to further support proof of the biochemical examination. The model collection revealed the most extreme harm of all groups; microscopic view of Silymarin's liver tissue and alcoholic extraction of CELS on ALT, AST, ALP, and SB in PCM affected liver lethality in rats. Histological changes in the liver tissue from collections that were treated at 100, 250 and 500 mg kg⁻¹ (Figure -6 to 11) as well as possible.

Microscopic view of liver tissue of alcoholic extract of CELS on ALT, AST, ALP and SB in PCM induced liver toxicity in rats

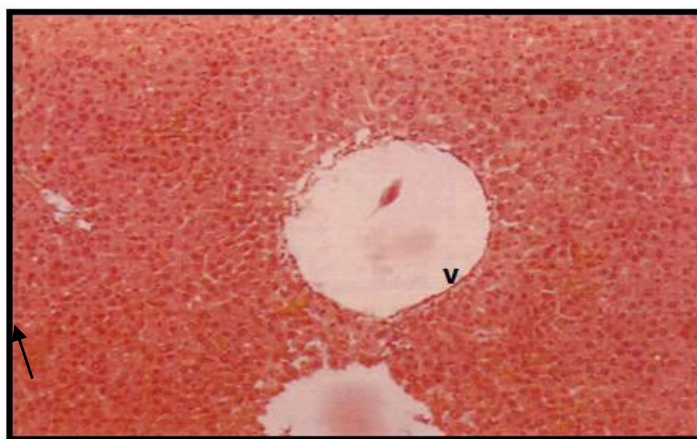


Figure 6: Liver tissues of control animal showing normal histology

Normal liver tissue section with portal triad showing portal vein (V), portal artery (arrow) and liver ducts (arrow head). Stain H and E, grossing 100X (Group I)

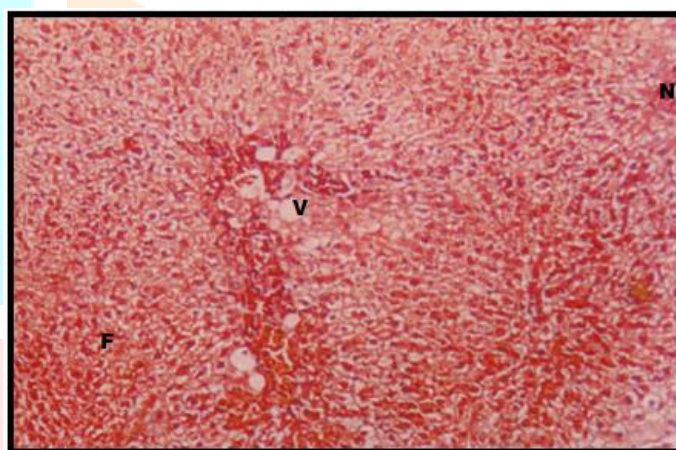


Figure 7: Liver tissue of animal treated with PCM showing necrosis

Live tissue section of the animal treated with PCM showing necrosis (N), fatty vacuole (F) and central vein (v). Stain H and E, magnification 100x (Group II)

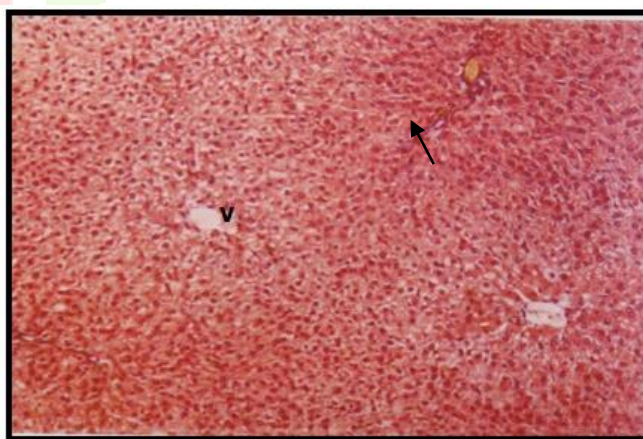


Figure 8: Liver tissue of PCM + Silymarin treated animals showing normal hepatocytes

Normal liver tissue section with portal triad showing portal vein (V), portal artery (arrow) and hepatic ducts (arrow head). Stain H and E, 100X magnification (Group III)

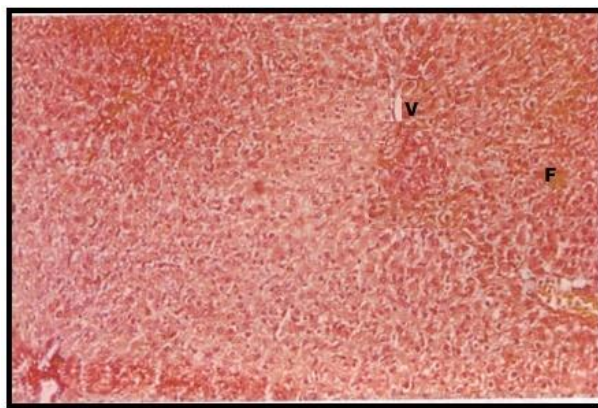


Figure 9 : Liver tissue of PCM + 100 mg/kg b.wt, po CELS showing normal arrangement of hepatocytes.

Liver tissue section of PCM + 100 mg / kg b.wt, po CELS treated animals showing normal pattern of hepatocytes around the portal vein (V), lack of necrosis and moderate accumulation of fatty vacuoles (F). Stain H and E, 100X magnification (Group IV)

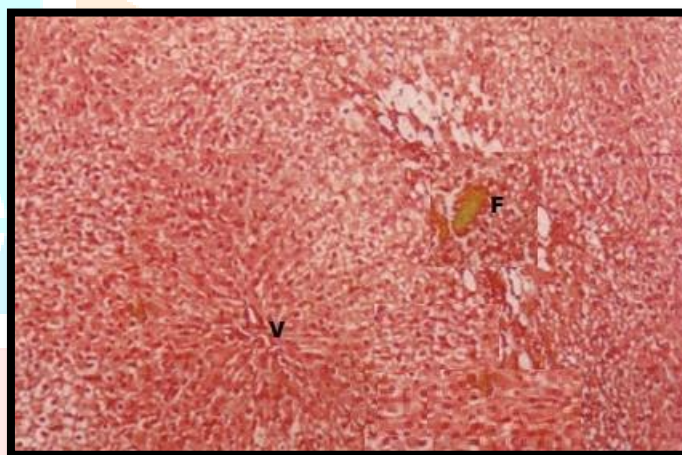


Figure 10: Liver tissue of PCM + 250 mg/kg b.wt, po CELS showing normal arrangement of hepatocytes.

Liver tissue section of PCM + 250 mg / kg b.wt, po CELS treated animals showing normal pattern of hepatocytes around the portal vein (V), lack of necrosis and moderate accumulation of fatty vacuoles (F). Stain H and E, 100X magnification (Group-V)

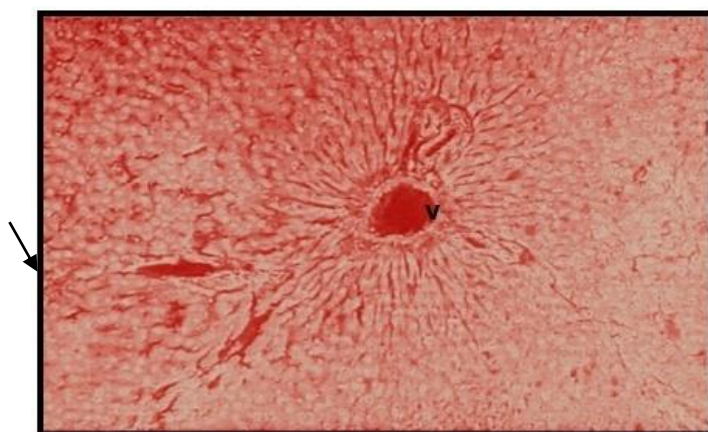


Figure 11: Liver tissue of PCM + 500 mg/kg b.wt, po CELS showing normal arrangement of hepatocytes.

Liver tissue section of PCM + 500 mg / kg b.wt, po CELS treated animals showing normal hepatocyte arrangement around the portal vein (V), the portal artery (arrow) and the hepatic ducts (arrow head). Stain H and E, 100 X magnifications (Group-VI).

DISCUSSION

The *Lagenaria sicerari* extract has been reported to contain different types of terpenoids, the phytochemical screening. A number of compounds belonging to the class of polyphenol have been suggested to possess antioxidant activity. [32] Pre-treatment of animals with CELS and silymarin prevented the Acetaminophen induced rise in serum level of transaminases and total serum bilirubin, confirming the protective effects of CELS against PCM induced hepatic damage. The hepatoprotective activity of CELS (500 mg/kg) was compared with the activity of standard silymarin (100 mg/kg). However, there was no effect on rise in serum alkaline phosphatase levels by the test extract and silymarin. Extensive liver damage by Acetaminophen itself decreases its rate of metabolism and other substrates for hepatic microsomal enzymes [26, 27]. Induction of cytochrome P450 or depletion of hepatic glutathione is a prerequisite for PCM -induced toxicity [28]. The CELS reduced the elevated stages of all the biochemical parameters through PCM. PCM induced liver necrosis was once inhibited significantly by using CELS which confirms the protective action of CELS against experimentally induced liver damage in rats. ALT, AST, ALP and SB are the most sensitive tests employed in the diagnosis of hepatic disease. It can be concluded from this investigation that CELS possess hepatoprotective activity. Further, detailed studies are warranted to confirm the utility profile of this drug.

CONCLUSION

The results of the present study clearly demonstrate that the various biochemical (Serum AST, ALT, ALP and SB) histopathological transformations produced by PCM within the serum and tissue were reserved significantly by the pretreatment of extracts of *Lagenaria sicerari* and Silymarin. This study confirms its use as hepatoprotective as per the ethno pharmacological claims.

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