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Research Article

Hepatoprotective Activity of *Abelmoschus Esculentus* Stem Bark Extract Using Paracetamol Hepatotoxicity in Rats

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ABSTRACT

Objective: The objective of this research is to see if ethanolic dried stem bark extract of A. esculentus can protect rats' livers from PCM-induced hepatic damage.

Methods: The intraperitoneal administration of PCM (1 ml/kg) resulted in hepatic damage. Hepatic damage was assessed using serum marker enzymes, histological examinations on the liver, and wet liver weight. The oral administration of ethanolicstem bark extract of *A. esculentus* (200 and 400 mg/kg) treated group in comparison with standard drug silymarin (10mg/kg) treated group.

Results: The ethanolic extract of stem bark, A. esculentus (200 and 400 mg/kg) significantly lowered serum levels of total protein, SGPT, ALP, SGOT, and total bilirubin throughout pre- and post-treatment (total seven days). By comparison with the PCM treated group (untreated group). The histopathological study too confirms the liver protective effect of the ethanolic stem bark extracts of *A. esculentus*. The revealed phytochemical analysis shows that plant confirms the presence of terpenoids, phenols, tannins, steroid, saponins, and flavonoids.

Conclusion: In paracetamol-induced liver damage in rats, the ethanol stem bark extract of A. esculentus (200 and 400 mg/kg) appears to show liver protective action.

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INTRODUCTION

The liver is the human body's largest solid organ. It's found below the diaphragm layer in the right-hand abdominal (upper section) cavity [1]. The liver is involved in secretion (bile juice secretion), metabolism (carbohydrate, lipid, and protein metabolism), storage (glycogen storage), and excretion of waste and hazardous substances.[2] [3]. In present-day hepatic disease is the most serious issue for health care professionals due to a higher rate of death [4][5]. There is various type of liver disease which affect the liver such as alcoholic liver disease, viral hepatitis, non-alcoholic fatty liver disease, autoimmune liver disease, metabolic liver disease, gallstones, and drug-induced liver injury, etc.[6]. Various toxic chemical and pharmaceutical drugs able to damage the liver. There are various types of xenobiotic which exhibit hepatotoxic potentials such as carbon tetrachloride, tetracycline, acetaminophen, and ethanol [7][8]. Hepatotoxicity is the more common cause of drug-mediated liver injury. PCM is considered a more hepatotoxic agent. Which are widely assigned for estimation hepatoprotective effect of drug and plant extract in experimental rats [9]. It is essential to search for new drugs heaving great pharmacological effects and less adverse toxic effects. For fulfilment of this purpose, the researcher searches new herbal medicinal plants due to their safety and efficacy. Approximately 25% of the current medication is derived from herbal plant resources [10]. There is various plant chemical constituent which posses hepatoprotective activity such as polysaccharides, flavonoid glycoside, glycoside, coumarin scopoletin, isoquercetin, quercetin 3-o robinobioside, uridine, riboflavin, stigmasterol, gammasitosterol, adenosine, myricetin[11]. *A.esculentus L.* Moench (family- Malvaceae) is a more common and important vegetable widely spread from Africa to America, Asia, and Southern Europe, and it generally knows as Ladies' finger [12]. Pod of *A. esculentus* is an important vegetable and stem bark is also cultivated because of their medicinal and nutritional property. It helps against the treatment of fever, catarrhal attacks, leucorrhoea, genito-urinary disorders, spermatorrhoea, gonorrhea, and chronic dysentery [13][14]. Researchers estimate that A. esculentus possesses hepatoprotective, laxative, antihyperlipidemic, antiulcer, antidiabetic, anticancer, anti-fatigue activities, anti-inflammatory, neuroprotective, analgesic activities [15]. Various research estimate that A. esculentus helps to restore hepatic function so the current research, conducted to evaluate the liver protective potential of *A. esculentus* in rats.

MATERIALS AND METHODS

Drugs and chemicals:

Paracetamol (Qualikems Fine Chem Pvt.Ltd.),Silymarin (Chemica - Biochemica - Reagents.), chloroform (THOMAS BAKER (CHEMICALS) Pvt.Ltd.).

Plants collection:

The stem of *Abelmoschus esculentus* containing stem bark fruit, flower was collected from mohaddipur, District kaushambi, Uttar Pradesh, India in the month of October 2019. The plant materials were authenticated in the department of environmental and forest at Botanical Survey of India, prayagraj and one voucher specimens were deposited in the botanical survey of India, prayagraj, India for future reference No-BSI/CRC/T/2018-19/252 [16][17].

Preparation of plant extract

The stem bark of *A. esculentus* is also known as the ladies finger was cleaned and shade dried for up to ten days. After ten days it was crushed into small pieces and the coarse powder was prepared by using an electric grinder and then passed through a sieve no 10. Finally, sieve passed powdered material (200 g) was sub-jected to soxhilation using the Soxhlet apparatus at a temperature of 50°C–60°C by using 70% ethanol for nine hours for the extraction process. Then the powder extracts (*A. esculentus*) were filtered utilizing Whatman filter paper no one and solvent were evaporated and the concentrating was extract utilizing a rotary evaporator. then the concentrated extract

was allowed to freeze dry. after freeze-dry it was stored at 2 to 80c till for the use and phytochemical screening was also done for screening of carbohydrates, fixed oils, flavonoids, glycosides, and mucilage [18].

Animals

Male adult Wistar albino rats (6–8 weeks), weighing up to 150–200gm were assigned for the experimental procedure. After dividing into 5 groups (n=6), animals were acclimatized for up to 7 days under standard environment conditions regulated by the regulatory authority (CPCSEA). All rats were placed in polypropylene cages containig suitable bedding material, maintaining the temperature at 25 ± 2ºC, humidity (35-55%) and under 12 h light/dark cycle as per regulatory guideline. All rats were received standard rodent food pellet diet and water ad libitum was allowed. Ethical clearance for continuing experiments on animals was authorized by the Institutional Animal Ethics Committee (IAEC) [19].

Acute toxicity studies:

The acute toxicity approach was used to determine the best and most effective oral doses for a test animal. For acute toxicity, the OECD guideline 423 was used. Female rats (n=3) were given stem bark extract into distilled water by oral route at various doses (5, 50, 300, 2000 mg/kg). The animals were examined clinically for toxicity symptoms (behaviour and neurological) and animals were observed individually after 30 min, 24 h, and daily observation for 14 days. The mortality occurring was noted and LD50 was calculated [20].

PCM-induced liver toxicity:

All rats were divided randomly into 5 groups (1 group = 6 rats) the experimental plan was given below.

Group 1: Normal control: Rat were orally administered distilled water. 2 to 5 groups administered PCM at (1 g/kg b. wt. By orally. Route).

Group 2: As disease control. Animals were administered PCM at (1 g/kg b. wt. By orally. Route).

Group 3: Standard control. All animals were administered with silymarin (10 mg/kg. b. wt. orally).

Group 4: As test control 1. Animals were administered with ethanolic extract of *A. esculentus* stem bark(200mg/kg orally).

Group 5: As test control 2. Animals were administered with ethanolic extract of *A. esculentus* stem bark (400 mg/kg orally).

Ethanol stem bark extract administration was started 5 days before PCM dosing and run on until the last day (on the 7th day) of the study. The blood sample of all rats was collected by puncturing the heart after 48 h of PCM, and a blood sample was clotted and the blood serum was isolated. After isolation, the rats were sacrificed under mild ether anaesthesia. The blood serum and liver of the experimental animal were separated and further proceeded for biochemical analysis and histopathological evaluation [21][22].

Assessment of Hepatoprotective Activity:

The last day (at 7thday), the blood sample was isolated by puncture the heart, and then serum (blood) was isolated and centrifuged (3000 rpm for 15 min). The isolated blood serum was examined for biochemical parameters liketotal protein, serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP), serum glutamate oxaloacetate transaminase (SGOT), and total bilirubin[23][24].

Histopathological investigation:

Tissue section of the liver were immerged into formalin solution for one day. thinnest of the liver tissue Section were analyze by histopathological investigation to assess the liver protective effect of *A. esculentus* stem bark (ethanolic extract)[25].

Statistical Analysis:

Values are presented as arithmetic means ±SEM. Arithmetic Data were examined statistically by using one way ANOVA followed by the multiple comparisons test. P

values < 0.05 were considered as significant differences.

Result and discussion

Result:

Phytochemical screenings:

Preliminary phytochemical screening *A .esculentus* leave to confirm the presence of flavanol, glycoside, and protein.

Acute toxicity study:

No toxicity was found of 2000 mg/kg during a study in rats.

Table 1. Preliminary phytochemical screening of A. esculentus *stems bark.*

S.N.	Extract	Phenolic compound	flavonoid	alkoloids	Tannin	Protein	saponin	Fats and oil
1	Petroleum ether	+	-	-	-	-	-	++
2	Chloroform	-	-	-	+	-	-	++
3	Methanol	+	+	++	+	+	-	-
4	Ethanol	++	++	+	+	++	-	-
5	Water	+	+	+	++	-	-	-

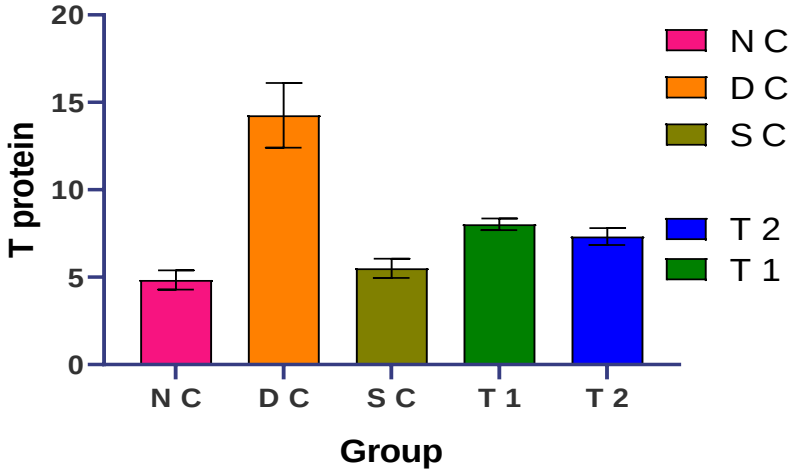
Effect on biochemical parameter:

Effect of serum hepatic marker in untreated disease rats and rat treated with silymarin ethanolic extract of *A. esculentus* is given [Table 3]. The effect of serum liver marker enzymes such as total protein, SGPT, ALP, SGOT, and total bilirubin should significant ($p<0.0001$) increases in PCM administered rat (group 2) as compared to normal rat (group 1). The (group 3) treated rats with silymarin at the dose 10 mg/kg and ethanolic stem bark extract of *A.esculentus* 200mg/kg (group 4) an ethanolic stem bark extract of *A.esculentus* 400 mg/kg (group 5) show significant ($p<0.05$, $p<0.01$ and $p<0.0001$). Reduction in total bilirubin, SGPT, ALP, SGOT, and Total protein as compared to PCM treated rat (group -2), EEAE 400 was comparable to silymarin.

Table 2. Effect of ethanolic extract of **A. esculentus** *stem bark on some serum biochemical parameters of PCM-induced hepatotoxicity in rats*

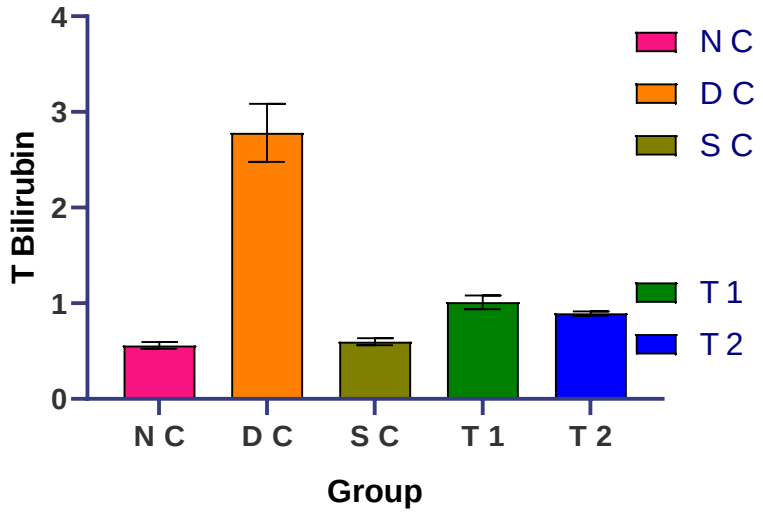
Treatment	Dose mg/kg	T bilirubin (mg/dl)	SGPT (U/L)	SGOT (U/L)	ALP (U/L)	T protein (mg/dl)
Normal	vehicle	0.56 ±0.03 ****	36.83 ±2.08 ****	57.88 ±2.55 ****	100.54 ±4.23 ****	4.83 ±0.55 ****
Disease control (ccl4)	1	2.78 ±0.30	134.20 ±4.12	178.69 ±3.47	153.97 ±5.49	14.26 ±1.84
Silimarin	10	0.59 ±0.03 ****	56.09 ±3.02 ****	74.94 ±2.55 ****	113.09 ±4.28 ***	5.50 ±0.55 ****
EEHE	200	1.01 ±0.07 **** ns	80.22 ±6.02 **** ##	89.90 ±6.94 **** ns	134.18 ±7.67 ns	8.02 ±0.33 *** ns
EEHE	400	0.89 ±0.02 **** ns	64.77 ±5.35 **** ns	76.20 ±1.92 **** ns	125.70 ±3.18 **ns	7.33 ±0.48 *** ns
All value as Mean ±SEM ****$P<0.001$ versus CCL4						

ns versusstandadard drug silymarin
EEAE: ethanolic extract of A. Esculentus. ****$P<0.001$ versus PCM group. $p<0.05, $$p<0.01, $$$p<0.001, $$$$p<0.0001 versus PCM group. #p<0.05, ##p<0.01, ####p<0.0001 versus Silimarin. .!p<0.05, !p<0.0001 versus EEHE 200. All statical analysis was done by one way ANOVA followed by Tukey's multiple comparison test.



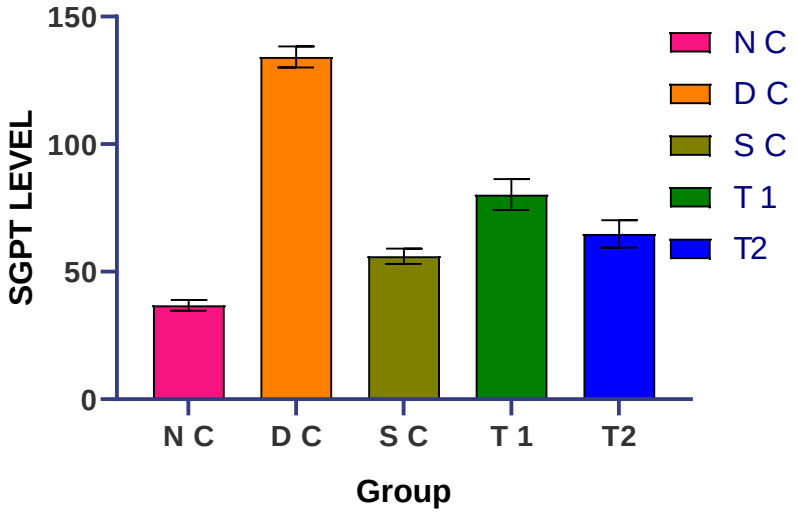
**(*Figure 1*: *Effect of ethanolic leave extract of* A. Esculentus *on Total protein against PCM-induced liver toxicity in rats.NC: normal control, DC: disease control, SD: Standard drug (silymarin); T1: Ethanolic extract of* A. esculentus *(200mg* A. Esculentus *crude extract), T2: Ethanolic extract of* A. esculentus *(400 mg* A. Esculentus *crude extract))*

Values are means ± SEM. (N=6), *P*<0.001; significant difference when compared with the control;* P*<0.01; significant difference when compared with the PCM group.*

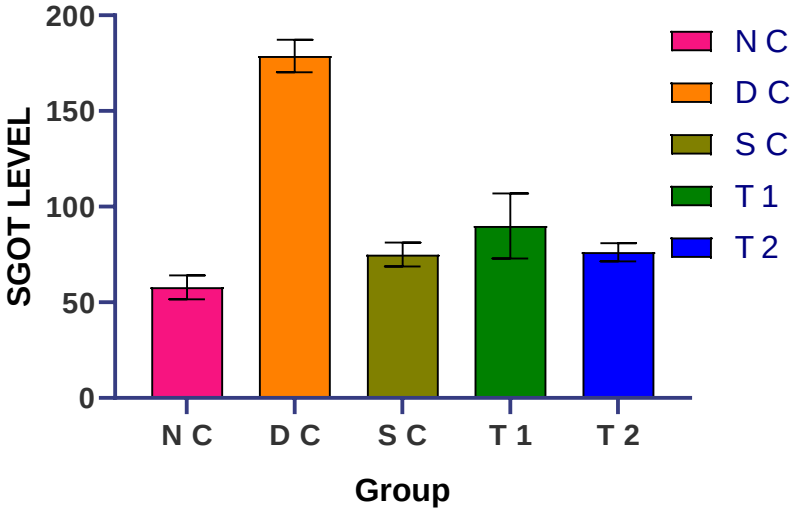


***(Figure 2**: Effect of ethanolic leave extract of A. esculentuson Total bilirubin against PCM-induced liver toxicity in rats. NC: normal control, DC: disease control, SD: Standard drug (silymarin); T1: Ethanolic extract of* A. esculentus *(200mg A. Esculentus crude extract), T2: Ethanolic extract of* A. esculentus *(400 mg A. Esculentus crude extract).*

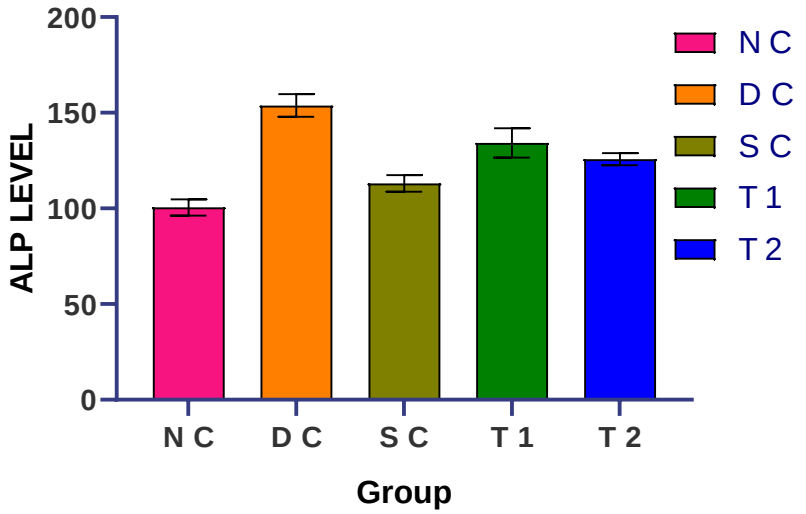
*Values are means ± SEM.(N=6), *P<0.001; significant difference when compared with the control; P<0.01; significant difference when compared with the PCM group)*



***(Figure 3**: Effect of ethanolic leave extract of A. Esculentuson SGPT: serum glutamate pyruvate transaminase against PCM-induced liver toxicity in rats.NC: normal control, DC: disease control, SD: Standard drug (silymarin); T1: Ethanolic extract of A.Esculentus (200mg A. esculentus crude extract), T2: Ethanolic extract of A. esculentus (400 mg A. Esculentus crude extract).*
*Values are means ± SEM.(N=6), *P<0.001; significant difference when compared with the control; P < 0.01; significant difference when compared with the PCM group).*



*(**Figure 4**: Effect of ethanolic leave extract of A. esculentuson SGOT: serum glutamate oxaloacetate transaminaseagainst PCM-induced liver toxicity in rats.NC: normal control, DC: disease control, SD: Standard drug (silymarin); T1: Ethanolic extract of* A. esculentus *(200mg A. Esculentus crude extract), T2: Ethanolic extract of* A. esculentus *(400 mg A. Esculentus crude extract).*
*Values are means ± SEM.(N=6), *P < 0.001; significant difference when compared with the control; P < 0.01; significant difference when compared with the PCM group)*



*(**Figure 5**: Effect of ethanolic leave extract of A. Esculentus on ALP: Alkaline phosphatase against PCM-induced liver toxicity in rats.NC: normal control, DC: disease control, SD: Standard drug (silymarin); T1: Ethanolic extract of A. esculentus (200mg A. Esculentus crude extract), T2: Ethanolic extract of A. esculentus (400 mg A. Esculentus crude extract).*
*Values are means ± SEM.(N=6), *P < 0.001; significant difference when comparedwith the control; P < 0.01; significant difference when compared with the PCM group.)*

Histopathological analysis:

The Histopathological analysis produces supportive evidence for the biochemical parameter. The microscopic image of normal rat (group 1) show normal hepatic cell. The microscopic structure of the liver of PCM induced diabetic rat (group 2) show severe necrosis area. liver section of the silymarin treated group (group 3) showing an absence of necrosis area shoe the necrosis healing potential of standard drug silymarin. ethanolic leave fraction of *A. esculentus* (200and 400 mg/kg) show dose potency-dependent activity of ethanolic extract of *A. esculentus* 400 show marked hepatic cell regeneration activity as compared to ethanolic extract of *A. esculentus* 200mg/kg.

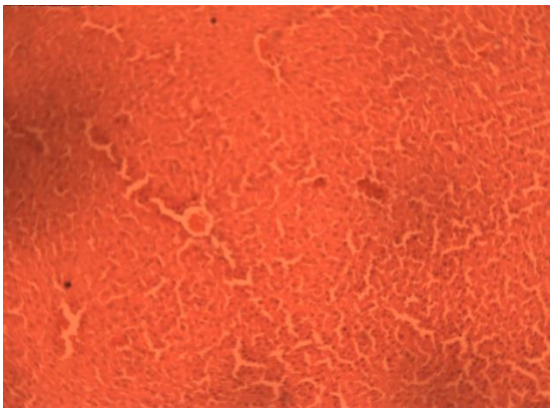


Figure:1 normal control (10 x)

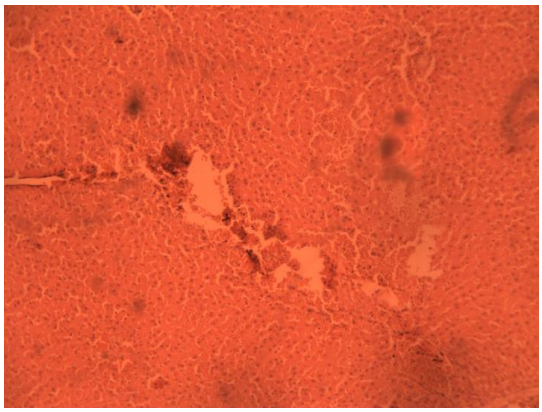


Figure:2 disease control (10 x)

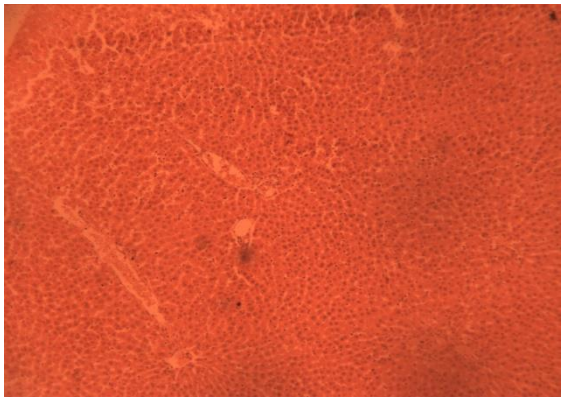


Figure:3 standard control (10 x)

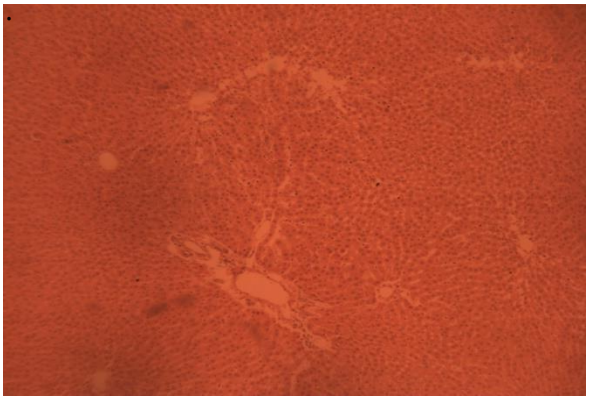


Figure:4 ethanolic extract 200mg/kg (10)

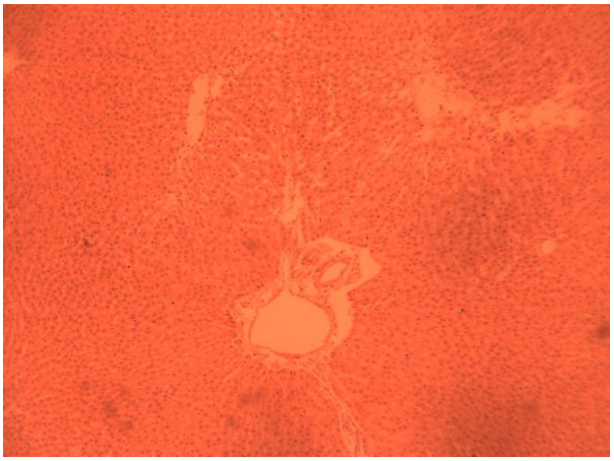


Figure: 5 ethanolic extract 400 mg/kg (10 x)

DISCUSSION:

The PCM is one of the toxic chemicals used to cause liver damage in rat to evaluate the effects of synthetic and herbal drugs. Administration of PCM results in an elevation gain in the level of hepaticmarker enzymes and a reduction in the activity of antioxidants in the body. The rise of serum liver enzymes (Total bilirubin, SGOT, ALP, SGPT, and total protein) indicates the harmful action of PCM on a hepatic cell of experimental animals Herbal extracts contain phytochemicals like flavonoids and phenolic compounds and triterpenes, shown the antioxidant and hepatoprotective effects of *A.esculentus* stem bark. The results indicated that *A.esculentus*stem bark show explicit hepatoprotective activity against PCM induced liver toxicity. Among these stem bark, extracts of *A.esculentus* showed more prospective activity at a dose of 200 mg/kg [26].

Oral administration of PCM shows increases serum hepatic marker like AST, ALP, ALT, total protein, and total bilirubin which are primary enzyme markers for a hepatic function test. The increase in the level of these markers because of a systemic eruption of the liver cells. Treatment with EEAE (200and 400 mg/kg) as well as silymarin (10 mg/kg) in PCM treated rat reduces the level above serum biomarkers, which was dose-dependently. The above histological result of an experimental animal also shows that the EEAE has a protective ability against liver damage. A decrease in body weight and liver weight gain in PCM treated rat was identified in the disease control animals, which indicates that the severity of the liver damage and oral treatment with EEAE (200and 400 mg/kg) as well as silymarin show weight gain and reduces liver weight as normal From the above result, it is concluded that the EEAE (200and 400 mg/kg)

have liver protective potential which was comparable to silymarin [18].

CONCLUSION:

Our findings significantly show that the alterations in histopathological parameters induced by PCM in hepatic tissue were reserved by the silymarin and ethanolic extract of *A .esculentus* correlating with its ability to decrease the activity of serum enzymes hepatic marker. From our result, we suggest that *A. esculentus* can be used as alternative preventive and protective herbal drugs against hepatic injury. This protective activity of *A.esculentus*is due to the presence of various important phytochemicals that is responsible for the prevention of hepatic damage.

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