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EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF HYDROETHANOLIC EXTRACT OF WHOLE PLANT OF *CLEMATIS BUCHANANIANA* DC AGAINST PARACETAMOL INDUCED LIVER INJURY IN WISTAR ALBINO RATS.

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ABSTRACT

Objective: To investigate the hepatoprotective activity of hydroethanolic extract of whole plant of *Clematis buchananiana* against Paracetamol-induced liver injury in wistar albino rats. Belong to family Ranuculaceae. The degree of protection was measured by estimation of serum biochemical parameters, histopathology study.

Method: The albino wistar rats (120-180gm) were divided into 6 group 5 animals in each, Group I: Received distilled water (5ml/kg. p.o) once daily, and served as normal control. Group II: Received paracetamol suspension (640 mg/kg suspended in 1% methyl cellulose; orally as toxin control. Group III: Received standard drug Silymarin (25 mg/kg. p.o.) + paracetamol suspension (640 mg/kg suspended in 1% methyl cellulose; orally once daily Group IV, V, VI administered HEECB at different doses300, 400, 500 mg/kg orally + paracetamol suspension (640 mg/kg suspended in 1% methyl cellulose; for 21 days. And collect blood from experimental animals by retrorbital puncture for estimation of biochemical parameters and other parameter also evaluate like physical histological changes in livers of rats.

Results: Experimental finding reveal that Paracetamol produce significant change in physical (increase liver weight) biochemical (increase alkaline phosphate, serum glutamic oxalacetic transaminase, serum glutamic pyuruvic transaminase, total protein, total bilirubin, direct bilirubin and decrease the level of total protein and albumin) histological (damage to hepatocyte) and in liver parameters. Pretreatment with extract significantly minimization of physical, biochemical, histological and functional change induced by Paracetamol in liver.

Conclusion: Experimental data and analysis of different parameter declare that hydroethanolic extract of *Clematis buchananiana* could be a useful hepatoprotective agent and it has significant hepatoprotection potential it is possible due to their active constituent alkaloids. However further study still needed to be causes on exposure of extract to human beings.

KEYWORDS : *Clematis Buchananiana*, Paracetamol, Hepatoprotective, Alkaline Phosphate, Serum Glutamic Oxalacetic Transaminase, Serum Glutamic Pyuruvic Transaminase.

1. INTRODUCTION

Liver is one of the largest glands in human body and the chief site for intense metabolism and excretion. So it has a surprising role in the maintenance, performance and regulating homeostasis of the body.[1] It is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction, histologically the liver is composed of several components like Hepatocytes which are the major functional unit of the liver and perform a numerous array of metabolic, secretory, and endocrine functions.[2] Hepatocytes are specialized hexagonal epithelial cells that make up about 80% of the volume of the liver. Complex three-dimensional arrangements of hepatocytes is known as hepatic laminae between neighboring hepatocytes Grooves are present and provide spaces for canaliculi, into which the hepatocytes secrete bile. Bile in appearance is a yellow, brownish, or olive-green secretion of hepatocytes, serves as both an excretory product and a digestive secretion too. [3] Paracetamol induces a number of deleterious metabolic changes in the liver. Its excessive use for a long-time leads to development of steatosis, alcoholic hepatitis and cirrhosis resulting in weight and volume changes.1 At least 80% of heavy drinkers had been reported to develop steatosis, 10-35% alcoholic hepatitis, and approximately 10% liver cirrhosis.2 Recent studies in animal models suggest that liver injury in chronic alcoholics is due to oxidative stress that leads to fibrosis and impaired liver functions and increased apoptosis. [4]. The present study describes the hepatotoxic effects of Paracetamol in albino rats.[4]

Acetaminophen (N-acetyl-p-aminophenol; APAP) is widely used as an analgesic and antipyretic drug worldwide. It produces alanine derivatives by hydrolysis, which is directly converted into hydroxylamine. N-Acetyl-p-benzoquinoneimine (NAPQI), is an intermediate product of acetaminophen produced in the presence of cytochrome-p450 that causes hepatic damage[5] and tubular necrosis in the kidney [5] in both humans and experimental animals. In this situation, a large amount of APAP is metabolized by the presence of P450s, which leads to reduced GSH levels by NAPQI conjugation and covalent binding of NAPQI. Acetaminophen's clinical and biochemical side effects are well known and it is therefore used as a reference compound to assess the strengths and weaknesses of genomic and proteomic technologies as toxicological tools.[6]

Clematis buchananiana is a woody vine with branches shallowly 8-10-grooved, densely yellowish velvety. Leaves are compound, carried on 4.5-9 cm long stalks [7].Nadkarni (1976). Leaflet blades are broadly ovate, ovate, or elliptic, 4-11 cm long, 4-10 cm wide, papery, undivided or 3-lobed, rarely sparsely pubescent, Clematis is found in the Himalayas, from Kashmir to NE, Assam, N. Burma, Indo-China, W. China, and Uttarakhand India at altitudes of 1800-3300 meter. [8] A paste of the roots is used as a poultice to treat swellings caused by inflammation [9] (Naman et al. 2006). This plant is used in the treatment of peptic ulcers and is also inhaled to get rid of coughs and colds, A paste of the stem or root bark is kept pressed against the teeth for about 15 minutes to relieve toothache (Rudra and Rajendra 2015). It is also warmed and placed inside the nose when treating sinusitis [10].(Bhakta and Lalita 2011), traditionally several parts of the plants in different forms are used for treating different ailments like Microbial infection[11]. (Abhishek et al. 2017). Epilepsy [12] inflammatory antioxidant as well as jaundice and other liver disorders certainly this plant is being used as treat neurodegenerative disorder.

MATERIAL & METHODS:

Silymarin was obtained from Yarrow chem products, Mumbai, India. The kits for biochemical estimation were purchased from Transasia Bio Medical, HP, India. The solvent and other chemical was used of analytical grade. The whole plant *C. buchananiana* was collected during September to October in 2015 from the wild region of Rudraprayag, Uttarakhand, India, and authenticated by botanist Professor S. K. Srivastava, Botanical Survey of India, Dehradun, Uttarakhand, India with a Specimen number for plant is 118186. The whole plant was shade dried at room temperature and extracted with 40:60 distilled water with ethanol 95% v/v for 24 hrs using hot soxhlet apparatus and extracts were dried at 50°C on water bath and the % yield of 12.8%. Phytochemical screening of whole plant extracted with hydroethanolic solvents revealed the presence of various secondary plant metabolites (Table 1).

Animal:

Wistar albino rats (120-180) of either sex obtained from, Department of pharmaceutical sciences Bhimtal campus Kumaun University, Uttarakhand. On the animal house, experimental rats were acclimatized for 10 days, under a room temperature of 24±2^{0}c relative humitidity 45-55^{0}c with 12:12 hrs light and dark cycle. The animals had free accesses to food (Ashirivad food industry Mohali, Chandigarh) and water ad

lebitum. The animals had habituated to laboratory condition for 48 hrs prior to the experimental protocol to minimize the non specific stress. The institutional animal ethics committee of Department of pharmaceutical sciences Bhimtal campus Kumaun University Rudrapur, Uttarakhand, India, approved the experimental protocol in accordance with the guideline provided by committee for purpose of control and supervision of experimental on animals (CPCSEA) with the registration no KUDOPS/38/16/03/2016.

Acute toxicity Test:
Acute toxicity studies, Healthy Wistar albino rats of either sex weighing 120-180 g maintained under standard laboratory conditions were used for acute oral toxicity test according to Organization for Economic Cooperation and Development guidelines 423. Animals were observed individually at least once during first 30 min after dosing, periodically during first 24 h (with special attention during the first 4 h) and daily thereafter for period of 3 days (OECD, 423). Observations were done daily for changes in skin and fur, eyes, mucus membrane (nasal), respiratory rate, circulatory signs (heart rate), autonomic effect (salivation, lacrimation, perspiration, urinary incontinence and defecation) and central nervous system (drowsiness, gait, tremors and convulsion) changes. The whole plant of *C. buchananiana* at a dose of 2 gm/kg body weight was given to 6 animals and was continuously observed for 14 days for mortality and general behavior. No deaths were observed till the end of this study. The plant extract was considered to be safe up to a dose of 2 gm/kg body weight. From these results, test drug dose of 300, 400 &500 mg/kg body weight was chosen for the efficacious studies. [13]

2.4 Experimental design:
The albino wistar rats (120-180gm) were divided into 6 groups, each group contain 5 animals. Group I: Received distilled water (5ml/kg. p.o) once daily, and served as normal control. Group II: Received paracetamol suspension (640 mg/kg suspended in 1% methyl cellulose; orally and Served as toxin control, Group III: Received Standard drug Silymarin (25 mg/kg. p.o.) + paracetamol suspension (640 mg/kg suspended in 1% methyl cellulose; orally once daily. Group IV, V, VI administered HEECB at the doses of 300, 400, 500 mg/kg orally + paracetamol suspension (640 mg/kg suspended in 1% methyl cellulose; orally repetitively for 21 day.

On 21 day after administration of test as well as standard drug, suspension (640 mg/kg suspended in 1% methyl cellulose; orally within 30 minute, and all animals were anesthetized using Thiopentone sodium 4 mg/kg ip injection. The blood samples were collected from retro orbital puncture and allowed to stand for 30 min at 37^0C then serum was separated from it with the help of centrifugation for further estimation of serum biochemical parameters. The animals were sacrificed under mild diethyl ether anesthesia and livers get isolated, washed with ice cold saline and weighed and send for further histopathlogical examination.

Histopathological examination:
Histopathology was carried by modified methods of "Luna" (Luna, 1999) [16]. In brief the autopsied livers were washed with normal saline and material were fixed in 10% buffered neutral formalin for 2 days followed by bovine solution for 6 hrs and paraffin embedded livers get sectioned of 5µ thickness by using microtome than processed in absolute alcohol- xylene, served and stand with haematoxyline and eosin blue. The slides were examined under a light microscope for any histological damages/protection. [14]

2.5 Statistical Analysis:
In animal study, the data are expressed as mean ±SD. For statistical analysis data was subjected to analysis of variance (ANOVA) by using Graph Pad Instat. Values are considered statistically significant at $P<0.001(n=5)$.

2. RESULT & DISCUSSION

3.1 *In Vivo* Hepatoprotective Activity:

3.2 Body Weight:
The body weight estimation in the control and Paracetamol induced rats were shown in Fig 1. The body weight was reduced in Paracetamol treated rats when compared to normal control rats. Treatment with hydroethanolic extract of *C. buchananiana* 300, 400, 500 mg/kg p.o and Silymarin at 25mg/kg dose to Paracetamol induced toxic rats cause an increase in the body weight during the experimental period.

3.3 Liver Weight
The liver weight in Paracetamol control group was increased significantly ($p<0.01$) in comparison with control group. Pretreatment with hydroethanolic extract of *C. buchananiana* and Silymarin showed significantly reduction in liver weight as compared to Paracetamol treated rats as shown in Fig.2.

3.4 Estimation of biochemical parameter:
The serum levels of SGOT, SGPT, ALP, Total bilirubin, direct bilirubin total protein and albumin were significantly increased in Paracetamol treated group in comparison normal control group, while the level of total protein albumin were reduced significantly**.** Pre-treatment with hydroethanolic extract of *C. buchananiana* and Silymarin significantly *($p<0.001$) get reduced the elevated serum enzyme such as SGOT, SGPT, ALP, total bilirubin and direct bilirubin as well as increased the level of total protein, albumin when compared with Paracetamol control group. The results indicates that pretreatment with HEECB (300, 400, 500) and Silymarin significantly prevented the biochemical changes may induced by Paracetamol. HEECB produce the greater hepatoprotective effect by normalizing the elevated serum enzymes level in Paracetamol induce liver damage in rats.

TABLE NO. 1: Effect of hydroethanolic extract of C. buchananiana on SGPT, SGOT, and ALP in Paracetamol induce hepatotoxicity on the 21 day.

Treatment Group	Dose mg/kg	SGPT (IU/L)	SGOT (IU/L)	ALP (IU/L)
Normal control	5 ml/kg	69.58±59.21	93.36±42.19	116.43±16.03
Toxin Control (Paracetamol control)	640 mg/kg suspended in 1% methyl cellulose; orally	312.59±16.16	371.87±41.87	388.75±56.78
Silymarin+ 640 mg/kg suspended in 1% methyl cellulose; orally	25mg/kg	96.17±41.14*	114.72±29.51*	216.02±91.47*
HEECB+640 mg/kg suspended in 1% methyl cellulose; orally	300mg/kg	202.16±54.32*	246.44±53.88*	268.21±13.07*
HEECB+640 mg/kg suspended in 1% methyl cellulose; orally	400mg/kg	114.26±34.12*	171.04±36.81*	219.04±19.04*
HEECB+640 mg/kg suspended in 1% methyl cellulose; orally	500mg/kg	81.035±40.51*	122.96±53.13*	216.19±27.58*

Data are expressed as mean ± SD (n = 5). One-way ANOVA followed by Tukey's post hoc:b * P< 0.001 compared with group II.

Table No. 2: Effect of hydroethanolic extract of *C. buchananiana* on total bilirubin, direct bilirubin, total protein, and albumin in Paracetamol induce hepatotoxicity on 21 day.

Treatment Group	Dose mg/kg	Total bilirubin mg/dl	Direct bilirubin mg/dl	Total protein (g/dl)	Albumin (g/dl)
Normal control	5 ml/kg	0.48±0.09	0.19±0.04	8.16±0.05	4.57±0.03
Toxin Control (40% Paracetamol (v/v, 2.0 ml/l00g body wt)	20ml/kg	2.30±0.12	0.84±0.081	5.38±0.10	2.39±0.01
Silymarin+ 40% Paracetamol (v/v, 2.0 ml/l00g body wt	25mg/kg	0.65±0.13*	0.26±0.76*	7.81±0.06*	4.35±0.07*

HEECB+640 mg/kg suspended in 1% methyl cellulose; orally	300mg/kg	1.73±0.25*	0.52±0.11*	7.32±0.03*	3.19±0.06*
HEECB+640 mg/kg suspended in 1% methyl cellulose; orally	400mg/kg	1.46±0.18**	0.37±0.12**	7.32±0.01**	3.69±0.10*
HEECB+640 mg/kg suspended in 1% methyl cellulose; orally	500mg/kg	0.87±0.16*	0.29±0.03*	7.56±0.02*	3.81±0.03*

Data are expressed as mean ± SD (n = 5). One-way ANOVA followed by Tukey's post hoc: * P< 0.001 compared with group II.

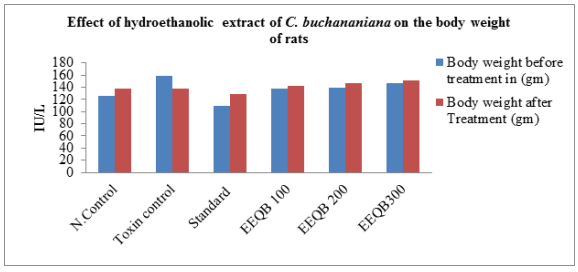


FIG:-1 Effect of hydroethanolic extract of *C. buchananiana* on the body weight (Before and after treatment) in Paracetamol induces hepatotoxicity.

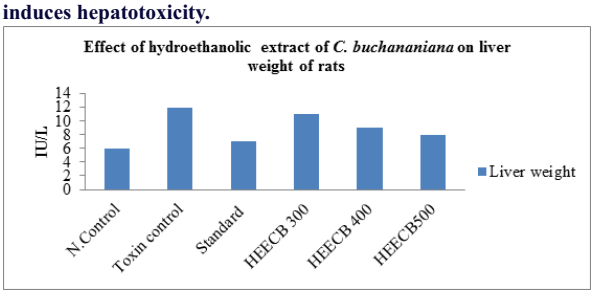


FIG:-2 Effect of hydroethanolic extract of *C. buchananiana* on liver weight in Paracetamol induced hepatotoxicity.

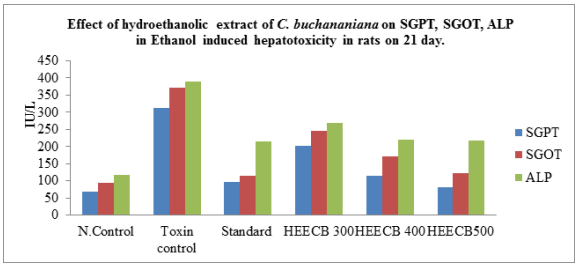


FIG:-3 Effect of hydroethanolic extract of *C. buchananiana* SGPT, SGOT, ALP in Paracetamol induced hepatotoxicity in rats on 21 day.

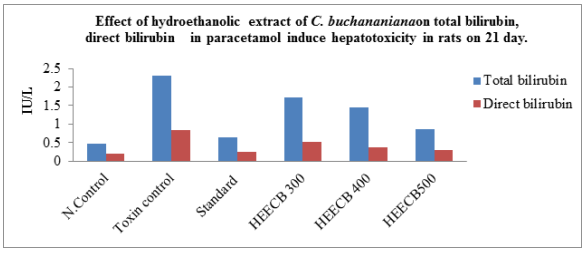


FIG:-4 Effect of hydroethanolic extract of *C. buchananianaon* total bilirubin, direct bilirubin in Paracetamol induce hepatotoxicity in rats on 21 day.

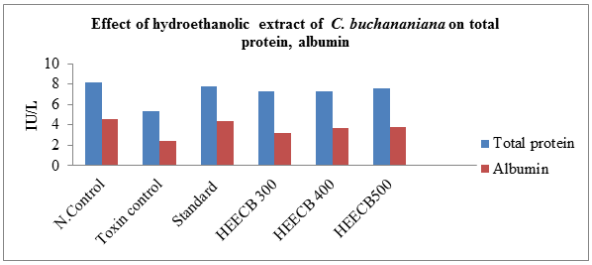
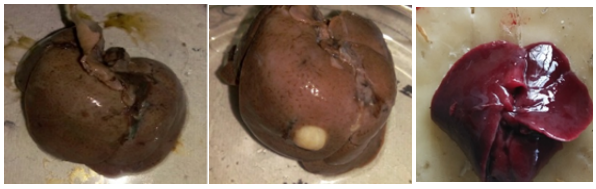
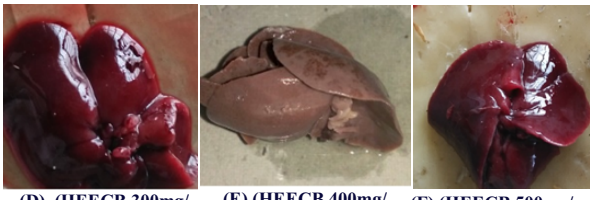


FIG:-5 Effect of hydroethanolic extract of theC. buchananianaon total protein and albumin in Paracetamol induced hepatotoxicity in rats on 21 day

Fig:-6 Visual appearance of necrosis in rats liver.



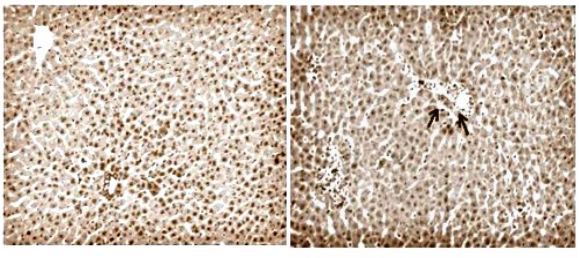
(A) (Normal Control) (B) (Paracetamol Control) (C) (Standard)



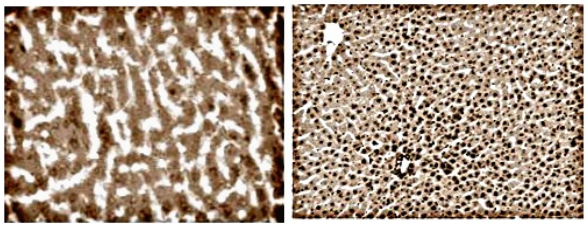
(D) (HEECB 300mg/ Kg) (E) (HEECB 400mg/ Kg) (F) (HEECB 500mg/ Kg)

3.5 Histopathological examination

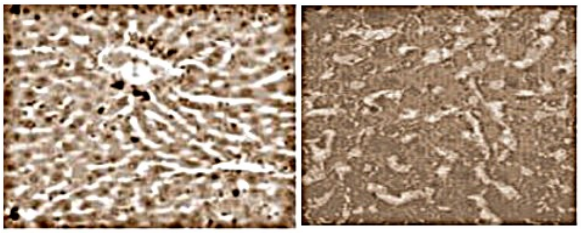
In fig no 8 (A) is a liver of normal control group, **(B)** is liver of Paracetamol control group, **(C)** is liver of standard group (Silymarin 25mg/kg) **(D)** is liver of test sample of HEECB (300mg/kg) **(E)** is liver of test sample of HEECB (400mg/kg) **(F)** is liver of test sample of HEECB (500mg/kg) On the behalf of visual necrosis the test dose 400,500 mg/kg is more significant and good Hepatoprotective.



(A) (B)



(C) (D)



(E) (F)

FIG: -7 Histopathological changes of Hepatic tissue.

3.6 Inferences of hepatocellular necrosis/protection

Slide (A) Section of the liver tissue of normal control (vehicle) animal showing normal histology and portal triad. Showing normal portal vein, hepatic artery and bile duct in this study (H×E 100x).

Slide (B) Section of the liver tissue of animal treated with Paracetamol showing a central vein necrosis and heavy necrosis in fatty acid vacuoles (H×E 100x).

Slide (C) Section of the liver tissue of animal treated standard drug Silymarin treated animal showing normal histology and portal triad showing normal portal vein, hepatic artery and bile duct (H×E 100x).

Slide (D) Section of the liver tissue of animal treated with hydroethanolic extract of *C. buchananiana* (HEECB) at dose 300 mg /kg treated animal showing .Normal arrangement of hepatocyte around the portal vein hepatic artery shows few necrosis and fatty vacuoles necrosis (H×E 100x).

Slide (E) Section of the liver tissue of animal treated with hydroethanolic extract of *C. buchananiana* (HEECB) at dose 400 mg /kg treated animal showing .Normal arrangement of hepatocyte. Absence of necrosis in portal veins and bile duct but some fatty vacuoles necrosis (H×E 100x).

Slide (F) Section of the liver tissue of animal treated with hydroethanolic extract of *C. buchananiana* (HEECB) at dose 400 mg /kg treated animal showing .Normal arrangement of hepatocyte. Absence of necrosis in portal veins, bile duct and absence of fatty vacuoles necrosis (H×E 100x).

1.7 DISCUSSION:

Liver is largest organ and it is target for toxicity because of its role in clearing and metabolizing chemicals through the process called detoxification. Drug induced liver disorders occurred frequently can be life threatening and mimic all forms of liver diseases Paracetamol being a drug capable of causing liver disorders if overdoses are consumed. The covalent binding of N-acetyl-P benzoquinone imine, an oxidation product of paracetamol, sulphydryl groups of protein resulting in cell necrosis and lipid peroxidation induced by decrease in glutathione in the liver as the cause of hepatotoxicity have been reported earlier .[14]

In the current investigations we observed that Paracetamol produced hepatotoxic effects in rats, as manifested by significant increase in the serum levels of alanine amino-transferase (ALT) and gamma glutamyl transaminase (GGT) in group B, when compared to those in group A (p value being < 0.05); this was presumably due to production of reactive oxygen species, inducing protein oxidation and lipid per oxidation which resulted in hepatocyte injury. These observations were comparable to those reported by Enomoto, 10 who observed the effect of Pioglitazone in prevention of Paracetamol induced liver injury in rats [15] Quaternary alkaloids from extracts of *C. buchananiana* were reported. In the hepatotoxicity activity we find that at 21 day, the effect on different biochemical parameter in Paracetamol induced hepatotoxicity in rats was estimated as shown in table no 3, and 4. The serum levels of SGPT, SG0T and ALP were significantly increased as 16%, 24% and 27% respectively ($p<0.05$, $p<0.01$) in Paracetamol treated group when compared with normal control group. Total bilirubin and direct bilirubin were also increased as (19.02% and 26% respectively) ($p<0.01$) in Paracetamol treated group as compared to control group while the level of total protein albumin were reduced significantly by 35% and 33% respectively ($p<0.01$) in the Paracetamol control group as compared with normal control group.[16]

At the 21 day Pre-treatment with hydroethanolic extract of *C. buchananiana* at dose of 300, 400, and 500 mg/kg and Silymarin significantly get reduced the elevated serum enzyme such as SGPT 39%, 60%, 77% and 63% respectively, in SGOT 38%, 53%, 66% and 63% respectively in ALP 39%, 45%, 43% and 48% respectively. The percentage reduction in total bilirubin were significantly reduced as 11%, 27%, 58% and 72% respectively while reduction in direct bilirubin were 30%, 50%, 60% and 65% respectively the percentage increments of total protein, were 72%, 75%, 73% and 68% respectively, in albumin percentage increment were 74%, 64%, 62% and 54% respectively when compared with Paracetamol control group. The results indicates that pretreatment with HEECB (300, 400, 500) and Silymarin significantly prevented the biochemical changes may induced by Paracetamol. HEECB produce significant hepatoprotective effect by normalizing the elevated serum enzymes level in Paracetamol induced liver damage in rats.

CONCLUSION: These studies have reflected that *C. buchananiana* contains bioactive compound with the potential of being good hepatoprotective agents. On the basis of the available data in this report, it can be suggested that the histological architecture of liver sections of the rats treated with the extract showed more or less normal patterns, with a mild degree of fatty change, necrosis and lymphocyte infiltration. However further study still needed to be causes on exposure of extract to human beings.

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Conflict of interest: Author has no conflict.

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