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Evaluation of Pharmacognostical parameters and Hepatoprotective activity in *Bryonia alba* Linn.

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ABSTRACT

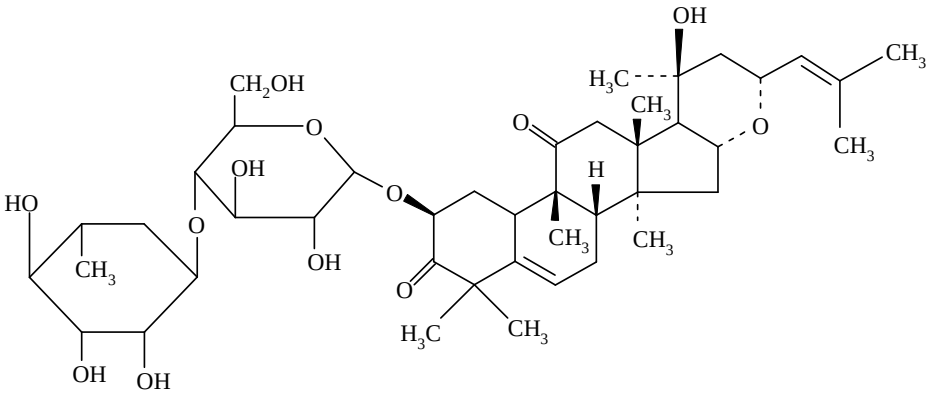
In Homoeopathic formulations the Bryonia alba (B. alba) Linn. (Cucurbitaceae), commonly known as "White Bryony", is considered as antirheumatic, antiphlogistic and anti-anxiety. An attempt has been made to highlight the pharmacognostic studies and to screen the phytoconstituents of this useful medicinal plant in Indian and European varieties both. Detailed microscopy of B. alba root has been worked up along with the determination of various physico-chemical parameters viz. moisture content, ethanol and water soluble extractives and total, water soluble and acid insoluble ash values. Further, qualitative TLC fingerprint profiles of petroleum ether, chloroform and ethanol extracts of B. alba roots were also developed to facilitate proper identification of the plant material. Preliminary phytochemical analysis indicated the presence of alkaloids, saponins, steroids, triterpenoids, carbohydrates and proteins. The hepatoprotective activity was evaluated in the chloroform and ethanol extracts against CCl_4 induced hepatotoxicity.

Key words: *B. alba*, pharmacognostic studies, preliminary phytochemical analysis.

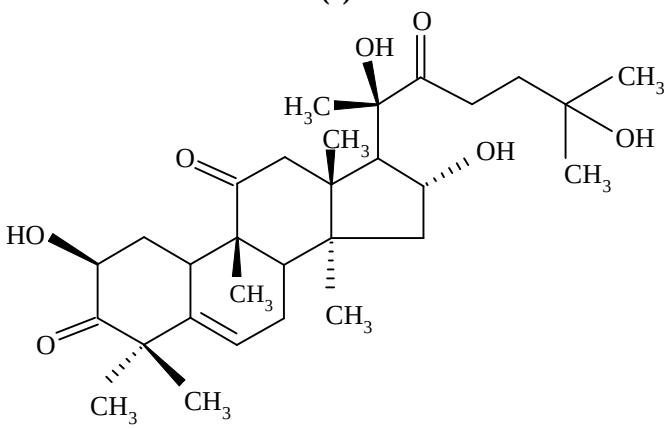
INTRODUCTION

Bryonia, one of the smallest genus in the family cucurbitaceae, comprises about 12 species distributed throughout the Europe and West Asia [1]. *B. alba* (White bryony) is found in Central Eruope, and is distinguished from *B. dioica* (Red bryony) by its black fruits and monoecious flowers [2]. *B. alba* has been traditionally used in the treatment of frontal pain, cough, peritonitis, inflammation of serous tissues, typhoid, pneumonia, jaundice, rheumatism and as heart tonic [3]. *B. alba* has also been used in the treatment of facial neuralgia of 6 years standing. The plant has

also been used to treat brain disorders with serous exudation. *Bryonia* contains alkaloid bryonicine [4]; flavonoids [5], [6], [7] saponarin, vitexin, isovitexin, 5, 7, 4'-trihydroxy flavone 8-C-glucopyranoside, lutonarin, isoorientin; glycosides [8], 22-deoxocucurbitosides A and B, 22-deoxocucurbitacin D (1); triterpenoids [9], [10] cucurbitacin L, 23, 24-dihydrocucurbitacin B, 23, 24,-dihydrocucurbitacin D (2), arvenin IV; lipids [11], [12], [13]; proteins [14].



(1)



(2)

Further, a perusal of available literature reveals that the *B. alba* possesses hypoglycemic activity [12], [15], [16] and anti-atherosclerotic effects [17].

As is the case with most of the herbal drugs, no work has been carried out for standardizing the root of this potentially useful plant. Thus, it was considered worthwhile to work on *B. alba* with a view to establish its pharmacognostic standards.

EXPERIMENTAL SECTION

2.1 Plant material

B. alba roots (European sample and Indian sample) were procured from Rati Ram Nursery, Village Khurrampur, district Saharanpur, Uttar Pradesh (India) in June and were dried in shade. Identity of the plant was confirmed through Forest Research Institute, Dehradun.

2.2 Experimental animals

Inbred wistar albino rats (either sex) were used for the evaluation of hepatoprotective activity. All the animals were kept under standard environmental condition. Animals were given standard diet of Hindustan Liver Limited and water *ad libitum*. All procedures were complied with the norms of institutional animal ethics committee under CPCSEA guidelines.

2.3 Solvents

Petroleum ether (60^{o}-80^{o}C), chloroform (Ranbaxy Laboratory Chemicals) and ethanol (S.D. Fine Chemicals Pvt.), all are LR grades, distilled under normal atmospheric pressure were employed for extraction of the plant material. All solvents employed as mobile phase for thin layer chromatography were of analytical grade. Absolute alcohol (Bengal Chemicals) was used in permanent staining of plant sections.

2.4 Microscopic techniques

All microscopic studies were carried out following the procedures laid down by khandelwal **[18]**. Qualitative and quantitative studies on plant samples were carried out using a Will-Optik (Wetzler-Nbn-Germany) compound microscope. Qualitative and quantitative observations were made using 12.5 x eyepiece and 4×, 25×, 10× or 40 × objectives. The roots of Indian and European *B. alba* were boiled with water until soft. Free hand sections were cut, taken on slides, cleared by warming with chloral hydrate and mounted in glycerine.

2.4.1 Histochemical reagents

Various test reagents viz., phloroglucinol + conc. HCl (1:1), iodine solution were used for staining lignified structures and starch grains respectively. Safranin (0.5% aqueous solution) / fast green and clove oil were used for making permanent stains. All chemicals were procured from S.D. fine chemicals, Mumbai.

2.4.2 Macerating fluid

Nitric acid solution (50%) + potassium chlorate (S.D. Fine Chemicals) was used for disintegration of plant tissues and separation of cells.

2.4.3 Clearing reagents and mountants

Aqueous chloral hydrate (Reidel Research laboratory chemicals, Hapur) solution and clove oil (S.D. fine chemicals) were used for clearing temporary mounts and permanent mounts respectively. Iodine solution and Lactophenol were used as mountant for studying starch grains, while glycerine (Ranbaxy Laboratory Chemicals) and Canada balsam (Loba chemie, Mumbai) solution were used respectively for mounting temporary and permanent sections.

2.5 Moisture content

The moisture content of the plant material was determined in triplicate by azeotropic distillation method given in the Indian Pharmacopoeia **[19]**.

2.6 Extractive and ash values

Ethanol and water soluble extractive values, and total, water soluble and acid insoluble ash values of dried powdered roots of *B. alba* were determined in triplicate following the procedures given in the Indian pharmacopoeia (1996).

2.7 Thin layer chromatography (TLC)

TLC glass plates (5 cm × 15 cm), 0.25 mm thickness were prepared using silica gel-G (E-merck). The plates were activated at 110^{o} C for 30 minutes. The final chromatograms were developed on Merck pre-coated Aluminum TLC sheets, silica gel G, 0.2 mm.

2.8 Recovery of solvents

Solvents from extracts were recovered under reduced pressure using Buchi 461 Rotary vacuum evaporator and were preserved in a vacuum desiccator containing anhydrous silica gel blue.

2.9 Acute toxicity study

The wistar albino rats of either sex weighing between 120-170 g were selected for acute toxicity study. A dose of 2000 mg/kg was selected based on OECD 423 Guideline, method of CPCSEA. The extract was administered intraperitonially (i.p.). The animals were continuously observed for 24 hr to detect any changes in autonomic or behavioral responses. Mortality in each group was observed for 7 days.

2.10 Experimental procedure

Wistar albino rats were divided into five groups, each comprising five animals. Group- I was control and received a single daily dose of 0.2 ml normal saline solution (NSS) by oral gavage, Groups II to V were treated and received CCl_4 as a 1:1 solution in olive oil at a dose of 1 ml/ kg body weight (bw), orally; Groups III animals treated with the standard drug silymarin (Ranbaxy Lab) at a dose of 100 mg/kg, p.o., served as standard. Group IV and V received plant chloroform and ethanolic extract in addition to CCl_4.

2.11 Biochemical estimations

The plant extracts were given orally 250 mg/kg bw dissolved in 0.2 ml NSS, one hour after the administration of CCl_4, for nine days. On 10^{th} day all the animals were anesthetized under light ether anesthesia and blood was withdrawn by puncturing retro-orbital plexus using fine glass capillary tube and collected in plain sterile centrifuge tubes and allowed to clot. Serum was separated by centrifugation at 7000 rpm for 15 min. at 5^{0}C. The separated serum was used for estimation of serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) **[20]**, total bilirubin (TB) **[21]** and alkaline phosphatase (ALP) **[22]**.

2.12 Histopathological investigations

On 10^{th} day the animals were sacrificed and abdomen was cut open, the liver was dissected out. Liver was rinsed in water and preserved in 10% formalin solution. The samples were given to the pathological laboratory for histopathological examination.

2.13 Statistical analysis

The results were expressed as mean ± SEM and statistically analyzed by ANOVA followed by Dunnett's test, with level of significance set at $p<0.05$ and $p<0.01$.

RESULTS

The transverse section of the root of *B. alba* showed cork, parenchymatous cortex, and vascular bundles (Fig. 2-5). Microscopic examination of the transverse section of the roots as well as the

powdered roots showed that the cortical and xylem fibres were absent. Fig. 6 shows the simple and compound starch grains of *B. alba* of both Indian and European sample. The vessels were observed to have scalariform thickenings (Fig. 7).

Fig. 1a: Indian sample

Fig. 1b: European sample

Fig. 1: *B. alba* root

Fig. 2: T.S. of *B. alba* root (×50)

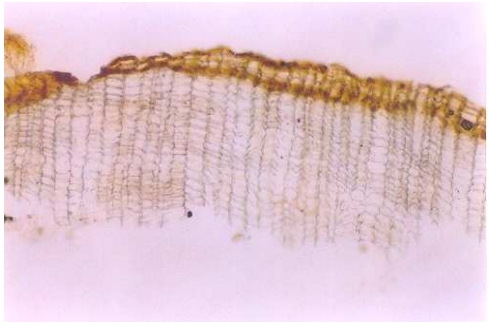
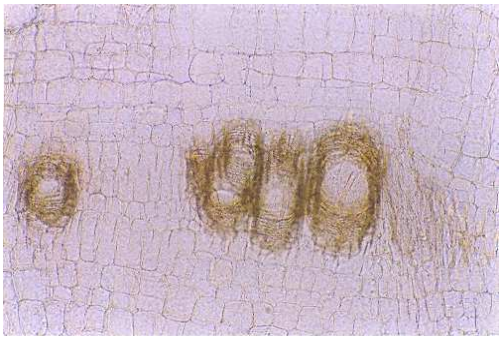


Fig. 3: T.S. of *B. alba* root (×125) showing Cork



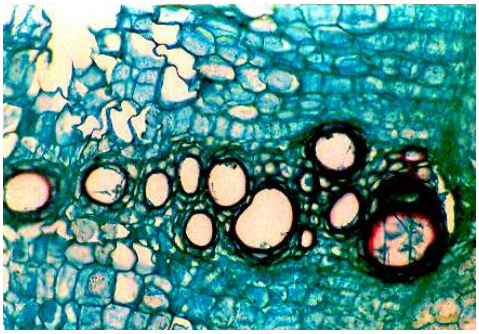
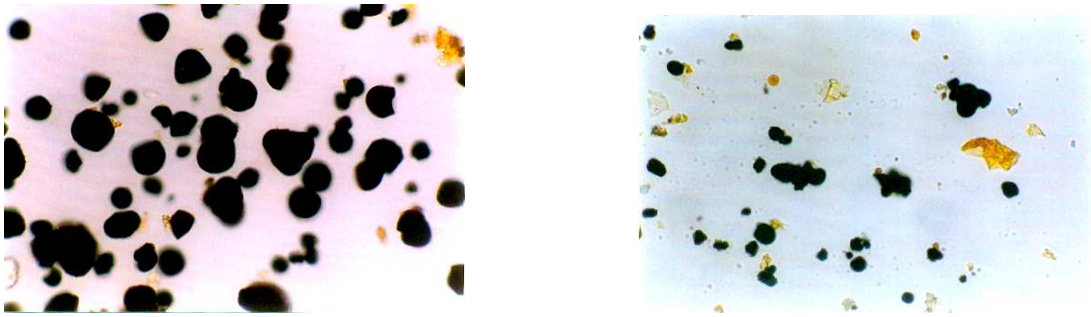


Fig. 4: T.S. of *B. alba* root (×125) showing vascular bundles Fig. 5: Safranin + Fast green stained (×400)

Table 1 Mean values for various physico-chemical parameters of *B. alba* roots

Parameters	Indian sample Mean[n]	European sample Mean[n]
Moisture content	14% v/w	14% v/w
Ethanol Soluble extractives*	0.60% w/w	3.08% w/w
Water Soluble extractives*	10.57 w/w	23.11% w/w
Total ash*	3.66% w/w	4.71% w/w
Water soluble ash*	0.99% w/w	1.34% w/w
Acid insoluble ash*	0.26% w/w	1.31% w/w

*n = 3: * dry weight basis*



(A) **(B)**

Fig. 6: Photomicrographs of simple and compound starch grains of *B. alba* (×400)
A, Indian sample; B, European sample

Fig. 7: Photomicrograph of lignified vessel of *B. alba* (× 400)

The quantitative microscopy of the root powder showed mean values and the range for the diameter of starch grains 14.3-21.16-25.74 µm and 8.58-11.95-14.3 µm for Indian and European samples respectively. Mean length and width of the xylem vessels were observed to be 604.5 µm and 14.63 µm, respectively for Indian sample, while 568.2 µm and 139.8 µm respectively for European sample.

Physico-chemical evaluation of powdered root of *B. alba* viz. moisture content, ash values, extractive values are presented in Table 1. The yield of powdered *B. alba* root of various extracts of Indian and European samples is recorded in Table 2.

Table 2 Yield of various extracts of Indian and European *B. alba* roots

Extract	Yield (% w/w)	
	Indian	European
Petroleum ether	0.75	0.69
Chloroform	0.57	0.39
Ethanol	5.6	5.47
Water	50.5	44.5

Presence and absence of different phytoconstituents were detected. Table 3 shows the result of phytochemical screening.

Table 3 Results of phytochemical screening of various extracts of Indian and European *B. alba* roots

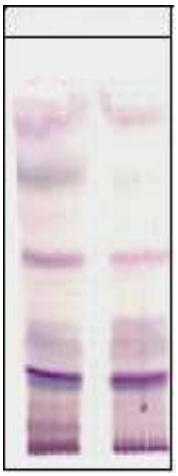
Chemical constituents	Petroleum ether extract		Chloroform extract		Ethanol extract		Aqueous extract	
	Indian	European	Indian	European	Indian	European	Indian	European
Alkaloids	-	-	-	-	+	+	+	+
Steroids/ Triterpenoids	-/-	-/-	+/+	+/+	-/-	-/-	-/-	-/-
Saponins	-	-	-	-	+	+	+	+
Flavonoids	-	-	-	-	-	-	-	-
Carbohydrates	-	-	-	-	+	+	-	+
Proteins	-	-	-	-	+	+	+	+

+: present, - : absent

Further, Fig. 8-10 show qualitative TLC fingerprint profiles of petroleum ether, chloroform and methanol extracts of *B. alba* roots of both the samples.

Table 4 Thin layer chromatograms of various extracts of (a) Indian sample (b) European sample of *B. alba* root

S. No.	Extract	Mobile system	Ratio
1.	Petroleum ether	Pet. ether : Chloroform	9 : 11
2.	Chloroform	Toluene : Ethyl acetate : Glacial acetic acid	18 : 1 : 1
3.	Ethanol	Chloroform : Ethanol	19 : 1



(a) (b)

Fig. 8

(a) (b)

Fig. 9

(a) (b)

Fig. 10

Fig: 8, 9, 10 Thin layer chromatograms of petroleum ether, chloroform extract and ethanol extract (a) Indian sample, (b) European sample

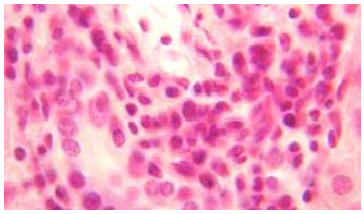
Standard solutions of the extracts were prepared and loaded quantitatively on silica gel TLC plates. Four spots were observed having R_f values 0.08, 0.11, 0.29, 0.32 and six spots with R_f values 0.08, 0.11, 0.19, 0.32, 0.66, 0.72 in TLC of petroleum ether extract of Indian and European samples respectively (Fig. 8). The chloroform extract of both samples showed seven spots having R_f values 0.22, 0.27, 0.35, 0.48, 0.58, 0.68, 0.78 (Fig. 9). The ethanol extract of Indian sample showed eight spots with R_f values 0.15, 0.35, 0.43, 0.50, 0.55, 0.58, 0.63, 0.68, while European sample showed ten spots having R_f values 0.15, 0.18, 0.26 0.35, 0.43, 0.50, 0.55, 0.58, 0.63, 0.68 (Fig. 10).

The plant extracts were given orally 250 mg/kg bw dissolved in 0.2 ml NSS, one hour after the administration of CCl_4, for nine days. All the animals were sacrificed on the 10th day. Blood samples were collected and serum was separated. The liver was excised and fixed in 10% buffered formalin for histopathological assessment.

Table 5: Protection afforded by *B. alba* roots extracts on CCl_4- induced hepatic damage in rats

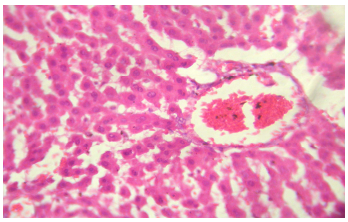
Groups		SGPT (U/L)	SGOT (U/L)	Total billirubin (mg/dl)	ALP (IU/L)
Control	I	20.62±1.52	64.12±1.62	0.18±0.01	218.84±1.51
CCl_4 treated	II	100.24±0.91	126.61±2.28	0.52±0.04	493.35±3.65
Silymarin	III	40.64 ±2.07	74.46±1.31	0.19±0.03	326.4±2.15
Chloroform	IV	46.23±0.97**	114.65±1.50*	0.47±0.02*	390.54±2.52*
Ethanol	V	70.87±2.37**	119.2±0.55*	0.48±0.03*	349.8±1.91**

*Values are expressed as mean ± SEM of 5 animals in each group. *p <0.05, **p <0.01 as compared to Group II*



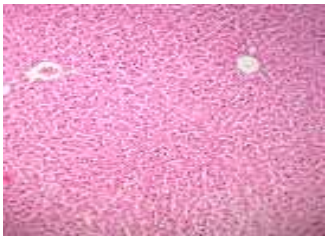
(a)

Rat liver (control)

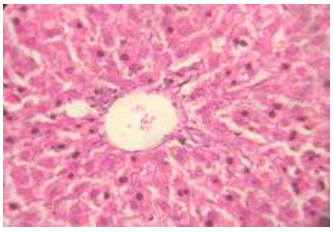


(b)

CCl_4 –induced hepatic damage in rats (a) and (b) 40X

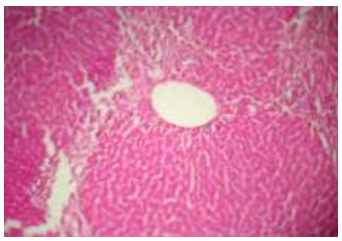


(c)

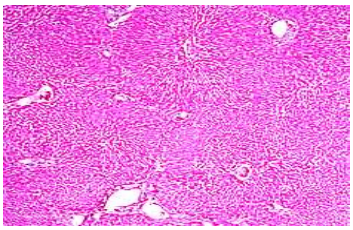


(d)

Effect of silymarin in rats treated with CCl_4 (c) 10X and (d) 40X



(e)



(f)

Hepatoprotective effect of chloroform extract and ethanol extract in rats (e) and (f) 10X

Fig: 11 Photomicrograph of liver showing histopathology of control, CCl_4 –induced hepatic damage group, silymarin, chloroform and ethanol extract treated groups

CCl_4 treatment (Group II) induced hepatic damage as there was a significant increase in SGOT, SGPT, TB and ALP values when compared to control group (Group I). Silymarin treatment (Group III) afforded hepatoprotection against CCl_4-induced damage as the SGOT, SGPT, TB and ALP values were significantly reduced. Plant extracts treatment (Group IV and V)

significantly reduced SGOT, SGPT, TB and ALP values as compared to CCl_4-treated animals (Group II) and afforded hepatoprotection (Table 5). This was supported by histopathological studies (Fig. 11).

DISCUSSION

Morphologic and microscopic characters are indispensable in establishing the identity of a plant. The efforts were made in the current study to delineate the microscopic details of the roots of *B. alba* which are reportedly used in the traditional therapeutics.

Standard protocol for standardizing crude drugs prescribes the determination of moisture content, and various extractive and ash values. Excess moisture is undesirable as it promotes microbial growth, and may be detrimental to the phytoconstituents. Furthermore, excess moisture in a drug acts as an adulterant by unduly adding to the weight of the drug. Moisture content of air dried Indian and European *B. alba* roots was found to be similar. Moisture content of the roots and rhizomes was accounted for calculating values of other physico-chemical parameters on dry weight basis.

Ethanol and water were used to evaluate the extractable constituents in the roots and rhizomes of *B. alba* in terms of extractive values, following the standard pharmacopoeial procedure. Water soluble extractive of Indian sample was found to be about half that of European sample. Ethanol soluble extractive of European sample was found to be about five times that of Indian sample. Determination of ash is useful for detecting adulteration with spurious, exhausted drugs, and excess of sandy and earthy matter. Most of drugs contain calcium oxalate crystals, sometimes in large and variable amounts. The acid-insoluble ash is determined to remove all the variable constituents of the ash using dilute hydrochloric acid. The water soluble ash is used to detect the presence of material exhausted by water. The acid insoluble ash was found to be about twelve times less than the total ash in Indian sample, while about 4 times less with European sample indicating very low content of sandy/earthy matter in the roots of Indian *B. alba*.

Thin layer chromatography is a handy technique for development of separation pattern of plant extracts. TLC fingerprint profiles are useful for the proper identification/authentication of plant material. In order to prepare qualitative TLC fingerprint profiles of petroleum ether, chloroform and methanol extracts of *B. alba* roots, the plant material was subjected to a standardized extraction procedure wherein petroleum ether, chloroform and ethanol extracts were obtained by direct extraction with petroleum ether, chloroform and ethanol.

Attempts were also made to evaluate various classes of phytoconstituents in *B. alba* roots. For this purpose, petroleum ether, chloroform, ethanol and aqueous extracts were subjected to standard phytochemical screening procedures. The roots showed the presence of alkaloids, steroids/triterpenoids, carbohydrates and proteins.

Carbon tetrachloride is one of the most commonly used hepatotoxins in the experimental study of liver diseases. The hepatotoxic effects of CCl_4 are largely due to its active metabolite, trichloromethyl radical **[23]**. These activated radicals bind covalently to the macromolecules and induce peroxidative degradation of membrane lipids of endoplasmic reticulum rich in

polyunsaturated fatty acids. This leads to the formation of lipid peroxides. This lipid peroxidative degradation of biomembranes is one of the principle causes of hepatotoxicity of CCl_4 **[24]**. This is evidenced by an elevation in the serum marker enzymes namely SGPT, SGOT, ALP and total bilirubin.

In the assessment of liver damage by CCl_4 the determination of enzyme levels such as SGPT, SGOT is largely used. Necrosis or membrane damage releases the enzyme into circulation and hence it can be measured in the serum. High levels of SGOT indicates liver damage, such as that caused by viral hepatitis as well as cardiac infarction and muscle injury, SGOT catalyses the conversion of alanine to pyruvate and glutamate and is released in a similar manner. Therefore SGPT is more specific to the liver, and is thus a better parameter for detecting liver injury. Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver **[25]**. Serum ALP and bilirubin levels on other hand are related to the function of hepatic cell. Increase in serum level of ALP is due to increased synthesis, in presence of increasing biliary pressure **[26]**. Administration of CCl_4 caused a significant elevation of enzyme levels such as SGPT, SGOT, ALP and total bilirubin when compared to control. There was a significant restoration of these enzyme levels on administration of the root extract and also by silymarin at a dose of 100 mg/kg. The reversal of increased serum enzymes in CCl_4-induced liver damage by the extract may be due to the prevention of the leakage of intracellular enzymes by its membrane stabilizing activity. This is in agreement with the commonly accepted view that serum levels of transaminases return to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes **[27]**. The efficacy of any hepatoprotective drug is dependent on its capacity of either reducing the harmful effect or restoring the normal hepatic physiology that has been distributed by a hepatotoxin. Both silymarin and the plant extracts decreased CCl_4 induced elevated enzyme levels in tested groups, indicating the protection of structural integrity of hepatocytic cell membrane or regeneration of damaged liver cells.

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REFERENCES

[1] LH Bailey. The Standard Cyclopedia of Horticulture, Vol. 1. The MacMillian Company, New York, **1950**, 453-583.
[2] AB Rendle. The classification of flowering plants, Vol. 2. Cambridge at the University Press, Cambridge, **1959**; 266-227.
[3] HW Felter and JU Lloyd. *Bryonia* (U.S.P.)-*Bryonia*, King's American Dispensatory **1898**, from www.henriettesherbal.com .
[4] RF Raffauf. A Handbook of Alkaloids and Alkaloid-Containing Plants. Smith Kline and French Laboratories, Philadelphia, **1970**; 24-33.
[5] M Krauze and W Cisowski. *Polish Journal of Chemistry*, **1991**, 65, 2289-2290.
[6] M Krauze and W Cisowski. *Polish Journal of Chemistry*, **1992**, 66, 951-957.
[7] M Krauze and W Cisowski. *Polish Journal of Chemistry*, **1995**, 69, 123-129.

[8] AG Panosyan. *Bioorg Khim*, **1985a**, 11, 264-269.
[9] AG Panosyan; MN Nikishchenko; GM Avetisyan. *Khim Prir Soedin*, **1985b**, 5, 679-687.
[10] NI Baek; DW Lee; YH Lee, SH Kim; GV Aprikian. *Natural Product Sciences*, **1995**, 1, 43-49.
[11] AG Panosyan; GM Avetisyan; VA Mnatsakanyan; TO Asatryan; SA Vartanyan; RG Boroyan; SG Batrakov; LD Bergelson. Tezisy Dokl-Sov-Indiiskii Simp Khim Prir Soedin., **1978**, 5, 71.
[12] AG Panosyan; GM Avetisyan; KG Karagezyan; GS Vertanian; GK Bunyatyan. *Dokl Akad Nauk.*, **1981**, 1267-1269.
[13] AG Panosyan; GM Avetissian; VA Mnattsakanian; SG Batrakov; SA Vartanian; ES Gabrielian; EA Amroyan. *Plant Medica*, **1983**, 47, 17-25.
[14] LM Gogilashvili and EP Kemertelidze. *Chemistry of Natural Compounds*, **2000**, 36, 399-401.
[15] GS Vartanyan; GK Parsadanyan; KG Karagezyan. Byull Eksp *Biological Medicines*, **1984**, 97, 295-297.
[16] KG Karageuzyan; GS Vartanyan; MI Agadjanov; AG Panosyan; JRS Hoult. *Planta Medica*, **1998**, 64, 417- 422.
[17] AN Orekhov and AG Panosyan. *Phytomedicine*, **1994**, 12, 123-126.
[18] KR Khandelwal; CK Kokate; AP Pawar; SB Gokhale. Practical Pharmacognosy, 1st Edition, D.K. Furia (ed.), Nirali Prakashan 41, Pune **1995**; 15-132.
[19] Indian pharmacopoeia, **1996**. Ministry of Health and family welfare, Government of India, New Delhi, A48-A54.
[20] S Reitman and SA Frankel. *AM J Clin. Pathol*. **1957,** 28, 56 – 63.
[21] HJ Malloy and KA Evelyn. *J Biol.Chem*. **1937,** 119, 481.
[22] OA Bessey; DH Lowery; MJ Brock. *J. Biol chem*. **1964**, 164, 321–329.
[23] DE Johnson and C Kroening. *Pharmacol Toxicol*. **1998**, 83, 231–239.
[24] N Kaplowitz; TY Aw; FR Simon; A Stolz. *Ann Intern Med*. **1986**; 104, 826–839.
[25] R Drotman and G Lawhan. *Drug Chem Toxicol*. 1978, 1, 163–171.
[26] P Muriel and T Garcipiana. *J Appl Toxicol*. 1992, 12, 439–442.
[27] M Thabrew and P Joice. *Planta Med*. **1987,** 53, 239–241.