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SCREENING AND EVALUATION OF PHARMACOGNOSTIC, PHYTOCHEMICAL AND HEPATOPROTECTIVE ACTIVITY OF JUNIPERUS COMMUNIS LINN. STEMS

Department of Pharmacy, B.B.D.N.I.T&M., Lucknow, U. P., India

*Corresponding author worldofherbs@rediffmail.com

Detailed microscopy of Juniperus communis Linn. stems (J. communis) 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. Preliminary phytochemical analysis indicated the presence of glycosides, alkaloids, flavonoids, steroids, triterpenoids and tannins. Further, qualitative TLC fingerprint profiles of petroleum ether, chloroform and ethanol extracts of J. communis were also developed to facilitate proper identification of the plant material. J. communis was evaluated for hepatoprotective activity using carbon tetrachloride (CCl_4)-induced hepatic damage in rats. It was concluded that stems possess hepatoprotective activity.

Juniperus communis Linn., carbon tetrachloride, phytochemical analysis, hepatoprotective activity

Juniperus communis Linn. (Fam. Cupressaceae) is also known as Aaraar, Haubera, Abhal (Hindi), Havusha (Bengali), Betar, Petthri (Punjab and Kashmir) [1]. Hapusha (Sanskrit), Juniper (English) [2]. It is found in the Himalayas from Kumaon westwards at altitudes of 5000-14000 ft and in Subarctic Europe, Asia, North Africa and North America [3].

All parts of the tree contain volatile oil. Terminal twigs and needles yield bright yellow oil with characteristic odor of juniper oil; it contains d– -pinene, camphene and cadinene. Juniper needles are rich in ascorbic acid (88 mg/ 100 g), resin, wax and esters [1]. The stem is bitter, purgative, styptic, diuretic, emmenogouge, aphrodisiac, tonic, enriches the blood, useful in stomatitis, bronchitis, piles, labor pain and in liver complaints [3].

As is the case with most of the herbal drugs, no work has been carried out for standardizing the stems of this potentially useful plant. Thus, it was considered worthwhile to work on J. communis with a view to establish its pharmacognostic standards. The plant was also subjected to hepatoprotective activity.

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Plant material

J. communis Linn. stems were collected from reputed supplier from Delhi in the month of February and authenticated by Forest Research Institute (F.R.I.), Dehradun. A voucher specimen (No: D.O./Bot./85- 1/SB) was deposited at F.R.I. Dehradun for future reference.

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.

Microscopic studies of J. communis Linn. stems

Qualitative and quantitative studies on plant were carried out using Will-Optik (Wezler-Nbn-Germany) compound microscope. Observations were made using ×10 eye piece and ×10 or ×45 objectives.

The dried stems were boiled with water until soft. Free hand sections were cut, transferred on slides, cleared by warming with chloral hydrate (Reidal Research Laboratory Chemicals) and mounted in glycerine (Ranbaxy Laboratory Chemicals) aqueous solution (50% v/v). Lignified tissues were identified by staining with pholoroglucinol and hydrochloric acid. Powdered J. communis (# 60) was cleared with chloral hydrate and mounted in glycerine. Micrometric determinations viz., length and width of vessels and fibres were also determined using eye and stage micrometer (Erma, Japan). For micrometric determinations, stems were cleared using Schultz's maceration fluid. Schultz's maceration fluid was prepared by adding sufficient potassium chlorate to nitric acid solution (50% v/v) to maintain a steady but gentle effervescence while heating on a water bath. A fragment of the tissue was placed in above macerating fluid. The potassium chlorate was added time to time till tissues softened and disintegrated. The treated tissues were taken on a slide, teased with a mounted needle and repeatedly washed with water to free the acid. Length and width of the vessels were recorded using a calibrated eye piece micrometer. Dimensions of 50 xylem vessels and fibres were recorded.

Moisture content

The moisture content was determined by loss on drying method on the dry weight basis following the procedure given in Indian Pharmacopoeia [4]. The experiment was done in triplicate.

Ash/Extractive values

Alcohol- and water-soluble extractive values, total ash, acid insoluble ash and water soluble ash of dried powdered stems of the plant were determined following the procedures given in the Indian Pharmacopoeia [4]. Ash was prepared in a muffle furnace (Narang Scientific Works, New Delhi).

TLC fingerprint profiles

TLC glass plates (5 cm x 15 cm), 0.25 mm thick were prepared using silica gel-G (E- Merck) and were activated at 110^{o}C for 30 minutes. Petroleum ether (S.D. Fine chemicals Pvt. Ltd.), chloroform (S.D. Fine chemicals Pvt. Ltd.) and ethanol (E- Merck), all of LR grade, distilled under normal atmospheric pressure were employed for extraction of the plant material. All the solvents employed as mobile phase for thin layer chromatography were of AR grade.

Dried stems of J. communis (2 g each) were packed in a filter paper sachet and placed separately inside three 500 ml round bottom flask fitted with condenser, and macerated for 15 min. The material was refluxed (1 h each) separately with petroleum ether, chloroform, and ethanol on boiling water bath. Solvents from the respective extracts were recovered under reduced pressure (Buchi 461 Rotary Vacuum Evaporator, Switzerland). The dried petroleum ether, chloroform and ethanol extracts were dissolved

in 3 ml in the volumetric flasks. 10 µl of the standard solution of each extract was loaded on TLC glass plates using 2 µl capillary tubes (CAMAG). The thin layer chromatograms were visualized on spraying with 0.5% anisaldehyde followed by heating at 110^{o}C for 10 minutes. The final chromatograms were developed on pre-coated plastic TLC sheets (Merck pre-coated plastic sheets, Silica gel G, 0.2 mm).

Phytochemical screening

Dried, coarsely powdered stems of J. communis (200 g) were successively soxhlet extracted with petroleum ether (60-80^{o}C), chloroform and ethanol. The marc was air dried, and water extract was obtained by boiling it with distilled water for 2 h. It was filtered, concentrated, and dried in an oven at 40-50^{o}C. All the four extracts were dissolved in their respective solvents and were screened for different classes of phytoconstituents [5].

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.

Experimental procedure

Wistar albino rats were divided into seven 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 VII was 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 to VII, received plant extract in addition to CCl_4 treatment as given below-

Group III- silymarin 100 mg/kg
Group IV- petroleum ether extract
Group V- chloroform extract
Group VI- ethanolic extract
Group VII- aqueous extract

Biochemical estimations

The plant extracts were given orally 500 mg/kg bw dissolved in 0.2 ml NSS, one hour after the administration of CCl_4, for nine days. On 10th 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) [6], total bilirubin (TB) [7] and alkaline phosphatase (ALP) [8].

Histopathological investigations

On 10th 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.

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 $p<0.01$.

T. S. of stem

The T.S. of stems showed annual rings (xylem growth), medullary rays, phloem, pits with outer layers of cortex, epidermis on staining with phloroglucinol: HCl (1:1).

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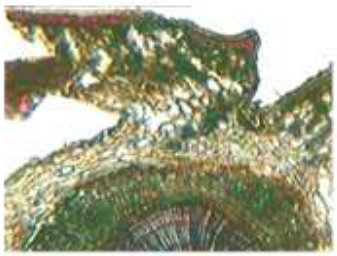
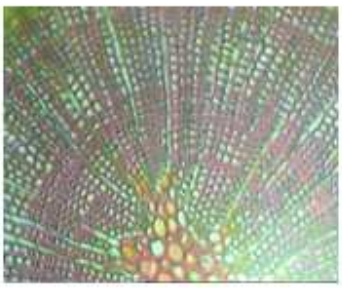
Figure 1 T.S. of J. communis stems



(a)

(b)

Photomicrographs of (a) and (b) showing epidermis, cortex, annual rings, medullary rays, pith (×40)



(c)

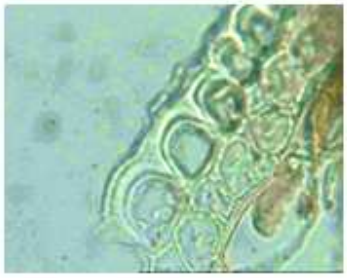
(d)

Photomicrographs of (c) and (d) showing cortex, annual rings, medullary rays, phloem fibres, pith (×100)

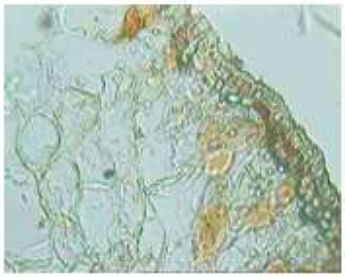
(e)

(f)

Photomicrographs of (e) and (f) showing epidermis and sclerides (×100)

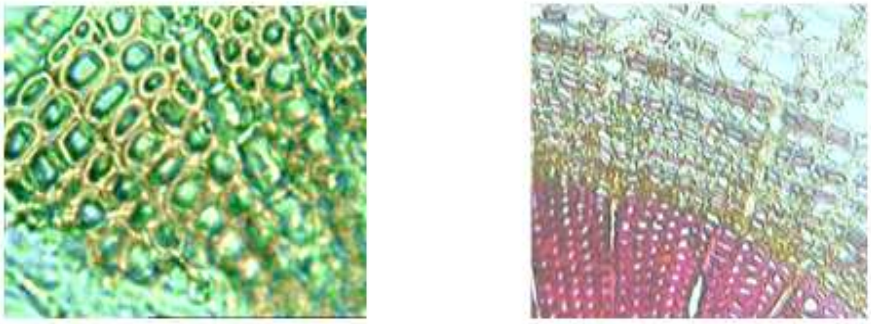


(g)



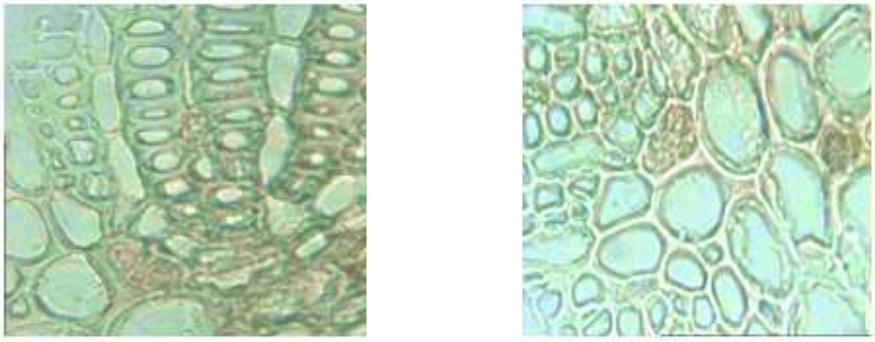
(h)

Photomicrographs of (g) and (h) showing epidermis (×400) and cortex (×100)



(i) (j)

Photomicrographs of (i) and (j) showing phloem fibers unstained and stained (×100)



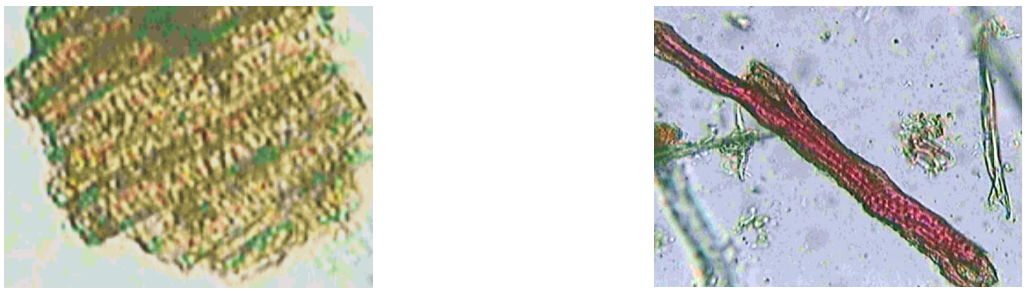
(k) (l)

Photomicrographs of (k) and (l) showing medullary rays, phloem fibers and pith (×400)

Powder microscopy

J. communis powder (#60) was cleared with chloral hydrate, mounted in glycerine and observed under the microscope. The drug showed the following microscopical characters in powder study.

Figure 2 Powder microscopy of J. communis stems



(a) (b)

Photomicrographs of (a) and (b) showing xylem vesse ls, (b) with Phloroglucinol: HCl (1:1) stained (×10 0)

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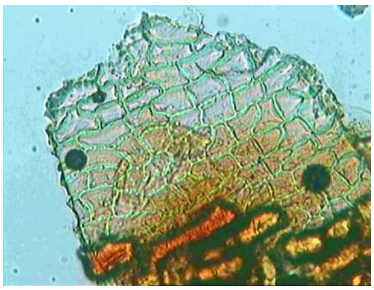
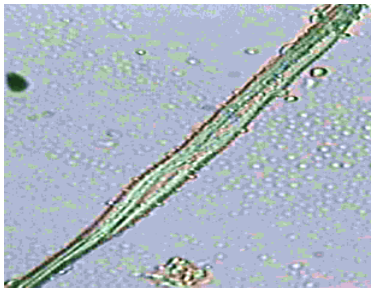
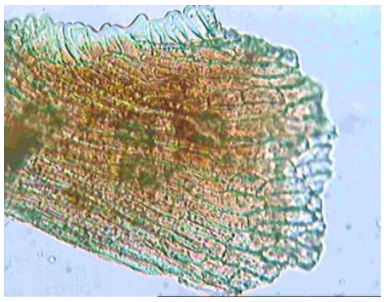


(c)

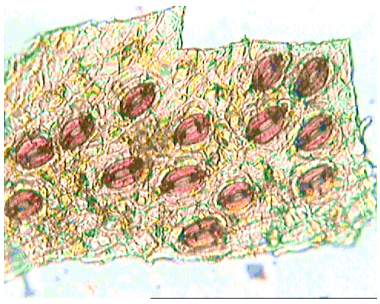


(d)

Photomicrographs of (c) and (d) showing epidermal c ells and phloem fibre (×100)

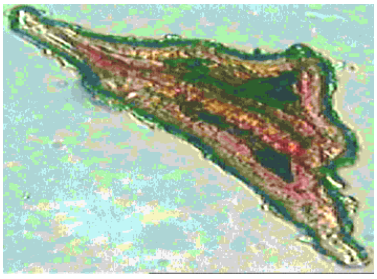


(e)



(f)

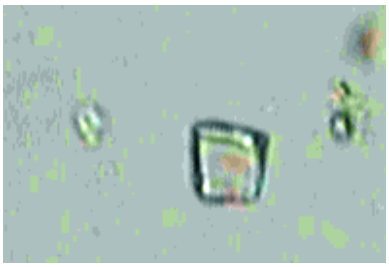
Photomicrographs of (e) cortical cells (×100) (f) s tomata (×100)



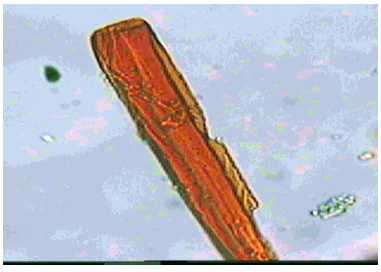
(g)

(h)

Photomicrographs of (g) and (h) showing sclerides, Phloroglucinol: HCl stained (×100)



(i)



(j)

Photomicrographs of (i) and (j) showing calcium oxa late crystals and resin, Phloroglucinol: HCl staine d (×100)

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The eyepiece micrometer was calibrated using a stage micrometer, and magnification factor was calculated. Mean length and width of the xylem vessels were observed to be 234.9 µm and 25.16 µm respectively, while the length of fibres was recorded 908.8 µm.

The drug was subjected to physico-chemical evaluation and following data was obtained-

Table 1
Mean values for various physico-chemical parameters of J. communis

Parameters	Sample Mean [n]
Moisture content	9 % w/w
Ethanol soluble extractives*	11.6 % w/w
Water soluble extractives*	3.92 % w/w
Total ash*	9.66 % w/w
Water soluble ash*	1.02 % w/w
Acid insoluble ash*	6.66 % w/w

n = 3: * dry weight basis

Table 2
Yield of various extracts of J. communis

Extract	Yield (% w/w)
Petroleum ether	5.60
Chloroform	10.18
Ethanol	9.02
Water	2.80

Volatile oil content of J. communis was found to be 1.50 % v/w.

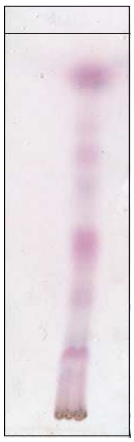

Fig. 3a

Fig. 3b

Fig. 3c

Figure 3a, 3b and 3c TLC fingerprint profile of pet roleum ether, chloroform and ethanolic extract of J. communis

The TLC profile of petroleum ether extract was best resolved in Hexane : dichloromethane : chloroform : : 1 : 7 : 2 (Figure 3a). The chloroform extract was best resolved in Benzene : Chloroform :

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Methanol : : 9 : 0.5 : 0.5 (Figure 3b). The ethanol extract exhibited optimum resolution in Hexane : Ethyl acetate : Acetone : : 8.5 : 0.5 : 1.5 (Figure 3c). Visualization of the spots was done using 0.5% anisaldehyde- sulphuric acid.

Table 3 shows the result of phytochemical screening-

Table 3

Phytochemical screening of various extracts of J. communis

S.No.	Phytoconstituents	Petroleum ether extracts	Chloroform extracts	Methanol extracts	Aqueous extracts
1	Alkaloids	-	+	+	+
2	Glycosides	-	-	+	-
3	Tannins and Phenolic compounds	-	-	+	+
4	Flavonoids	-	+	+	+
5	Steroids/ Triterpenoides	+/-	+/+	+/+	+/+
6	Proteins and Amino acids	-	-	-	-
7	Carbohydrates	-	-	-	-

+: present, - : absent

The plant extracts were given orally 500 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 10^{th} day. Blood samples were collected and serum was separated. The liver was excised and fixed in 10% buffered formalin for histopathological assessment.

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 extract treatment (Group IV to VII) significantly reduced SGOT, SGPT, TB and ALP values as compared to CCl_4- treated animals (Group II) and afforded hepatoprotection (Table 4). This was supported by histopathological studies.

Table 4

Protection afforded by J. communis 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	26.60±0.08	12.0±0.71	0.81±0.03	204.6±2.11
CCl_4 treated	II	78.0±1.41*	52.0±1.84*	1.52±0.01*	324±2.35*
Silymarin	III	22.4 ±0.87	14.6±1.21	0.58±0.03	183.4±2.25
Petroleum ether	IV	30.8±1.07*	23.6±0.81*	0.89±0.13*	208.8±1.28*
Chloroform	V	33.2±1.07*	27.8±0.86*	0.90±0.01*	241.2±1.07*
Ethanol	VI	27.8±0.74**	20.6±1.50**	0.43±0.01**	191.8±1.46**
Aqueous	VII	24.6±1.21**	15.0±1.72**	0.80±0.02**	222.2±0.86**

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

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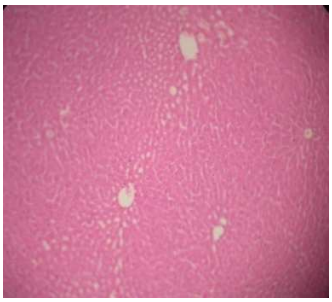

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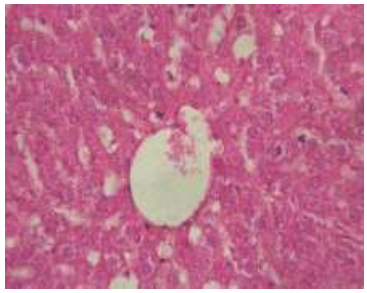
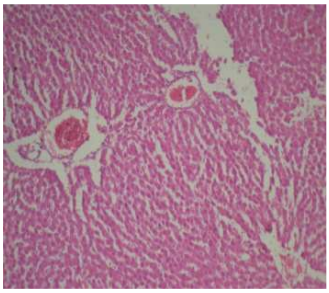


(b)

Rat liver (control) (a) 10X; (b) 40X



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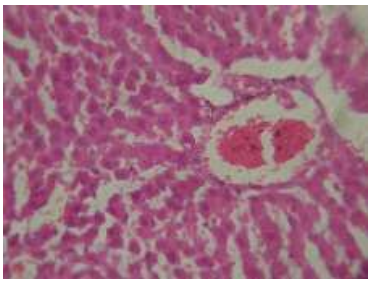
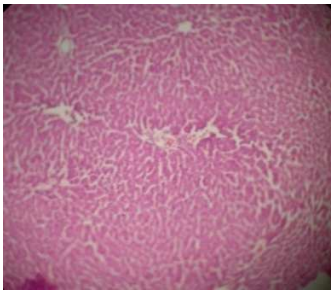


(b)

CCl_4 –induced hepatic damage in rats (a) 10X; (b) 40X



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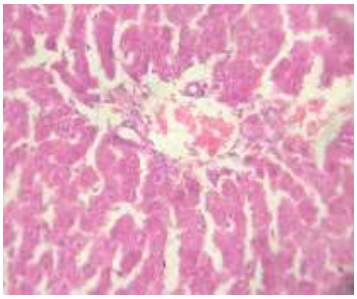

(b)

Effect of Silymarin in rats treated with CCl_4 (a) 10X; (b) 40X

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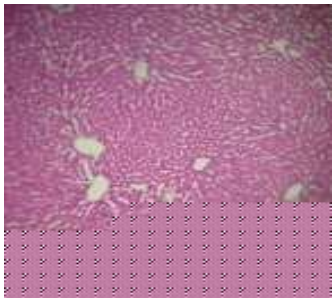

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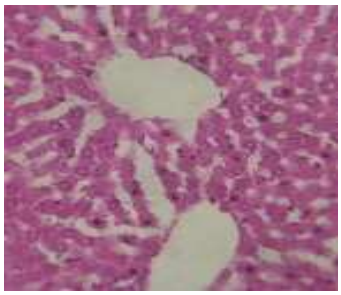
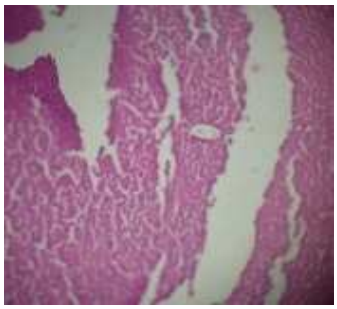


(b)

Hepatoprotective effect of ethanol extract in rats (a) 10X; (b) 40X



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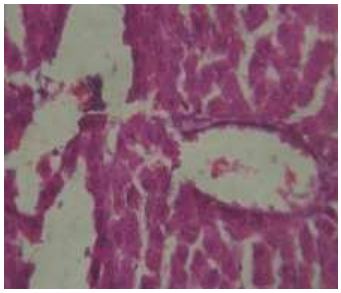

(b)

Hepatoprotective effect of aqueous ext ract in rats (a) 10X; (b) 40X

Figure 4 Photomicrographs of liver showing histopa thology of control, CCl_4–induced hepatic damage group, silymarin, ethanol and aqueous extrac t treated groups

The abnormal high level of SGOT, SGPT, ALP and bilirubin observed in this study are the consequence of CCl_4- induced liver dysfunction and denotes the damage to the hepatic cells. Treatment with extracts of J. communis reduced the enhanced levels of serum SGPT, SGOT, ALP and bilirubin, which seem to offer protection and maintain the functional integrity of hepatic cells. Both, ethanol and aqueous extracts, appear to provide better protection than the petroleum ether and chloroform (Table 4).

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