ejpmr, 2021,8(7), 687-693

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

PHYTOCHEMICAL INVESTIGATIONS AND PHARMACOLOGICAL EVALUATION OF LAWSONIA INERMIS L.BARK FOR HEPATOPROTECTIVE ACTIVITY

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Article Received on 19/05/2021 **Article Revised on 08/06/2021** **Article Accepted on 29/06/2021**

ABSTRACT

Bark of Lawsonia inermis L. is an evergreen tree, belonging to family Sapotaceae, cultivated in gardens as an ornamental tree. It is traditionally used as cardiotonic, anthelmintic, in the treatment of leprosy and various ailments. The present study includes phytochemical and pharmacological evaluation of bark. Here the powdered bark was subjected to successive soxhlet extraction using different solvents of increasing polarity. The successive methanol and aqueous extracts were subjected for *In-vivo* hepatoprotective evaluation. The methanolic extract was found more effective.So it was further subjected for preliminary phytochemical and chromatographic examination. Attempt was made to isolate the compounds using isocratic elution technique. The compounds isolated were characterized by UV, FT-IR, ^{1}H-NMR, ^{13}C- NMR, and Mass spectroscopy.

INTRODUCTION

Herbal medication, occasionally referred to as herbal medicine or Botanical medicine, is the usage of herbs for their therapeutic or medicinal value. Herbs are a plants or parts of a plants that are valued for its medicinal, savory, or the delicious qualities. Plants give a variety of chemicals that affect the human body.

Plants biosynthesize the chemicals that are useful for the preservation of health and the health of humans and other animals. Many of them are secondary and are at least 12,000 have been assigned to a host is to reach at least 10% of the total. In many cases, these substances (particularly the start to be seen) to serve as the defense mechanisms of plants, insects, and herbivores. Many of the herbs and the type is being used as sources to provide a positive connection to the medicines.

An excellent herb to be considered as in a chemical plant, which consists of a number of chemical compounds such as glycosides, saponins, resins, gum-resins, sesquiterpene lactones, and oils, (primary and permanent). Nowadays, there is an increasing interest to the organic mixture of the herbal medicine. Some biologically-active components were isolated and studied for pharmacological activity.

MATERIAL AND METHODS

Data arrangement and verification

Bark of Lawsonia inermis L. taken in local area, Meerut were authenticated by Dr. B. D. Khuddar, Botany Department Head, CCS universityme Meerut. A sample of the voucher was put in the pharmacognosy workshop of the BANK of Translam College of Pharmacy, for further use.

B. Morphological Characters

Morphology is the studies of the shape of an entity, and morphography is the picture of the shape from the material, it is recognized what is there in one form or another. Morphological and organoleptic landscapes, i.e. color, smell, taste, shape and size are experiential and assessed in botany.

Study of Microscopical Characters

a. Transverse piece of the Bark

Microtomic sections of the cortex are observed during small and large magnification of the microscope.

b. Powder microscopy

Dried bark of Lawsomia inermis l. was ground into coarse powder and heated through chloral hydrate for 5-10 minutes, and then stained with chloroglucin and others, a ratio of 1:1, as observed at high power (40x) for various diagnostic signs, such as connect cells with crystal tool, medullary sun rays with attached tool and bark cells using grain.

c. Proximate Values

Below, the values are defined directly for the bark in the form Lawsomia inermis L.

a. Extractive values

Extractive important tool is a certain amount of soluble salt, which make up in size from medicinal plants, raw materials and their extraction with various solvents.

Extraction of raw medicinal products, a certain amount of solvent gives a solution of various phytoconstituents.

Alcohol-soluble extractive value

4 gm of shadedried Lawsomia inermis L. bark residue is soaked in 100ml of 95% ethanol in to a closed bottle, shaken regularly for the main 6 hours and kept standing for 18 hours. After that, it is quickly filtered, taking precautions against the loss of ethanol. 25 ml of filtrate is evaporated to dryness in flat-bottomed fine oil, dried at a temperature of 105^0 C, and tested on a scale. The percentage of extraction that is soluble in ethanol is calculated, which is exactly what the dried bark powder represents.

Water-soluble extracts assessment

4 gm of shadedried Lawsomia inermis bark residue, L. is soaked through 100ml of H_2O in a closed bottle, shaken frequently for the 1^{st} 6 hours, and permissible to stand for 18 hours. After that, this is quickly filtered. 25 ml of filtrate is evaporated to dryness in flat-bottomed fine oil, dried at a temperature of 105^o C, and tested on a scale. Specific extractive parameters were calculated if we take into account that the bark powder was dried in the shade.

Petroleum Ether soluble extracts assessment

4 g of shade-dried Lawsomia inermis L. bark powder was soaked in 100 ml of petroleum ether in a closed flask, shaken frequently for the first 6 hours and kept standing for 18 hours. After that, it was quickly filtered. Evaporate 25 ml of filtrate to dry tarred right in the afternoon, fine oil, dried at a temperature of 105^0 C, and pull. Specific extractive parameters were calculated if we take into account that the bark powder was dried in the shade.

Moisture content

A correctly assessed amount of the shadedried crudely residue *Lawsomia inermis* L. bark residue is occupied to a tared crystal jug and the original mass is taken. The crude drug is boiled at 105^0C to the oven and weight. This process is repetitive till a constant mass is found.

Ash assessment

Total ash

2 g, an exactly weighted amount of dried in the shade, vulgarly chopped bark of Lawsomia inermis L., placed in a grated silica container and burned at a temp not higher than 450^0 C until it was free of carbon dioxide, in the first version, and weighed. The % of whole residue is calculated taking into account the amount of dry bark residue.

Acid insoluble ash

The resulting whole residue is heated for five min in 25 ml of dilute HCl. Unsolvable substances collected on an ash-free fiber filter paper are washed through warm H2O and burned, in the first version, then weighed. The percentage of acid-insoluble ash was determined from the shade of dried bark powder,

Water-soluble ash

The resulting whole residue is heated for five min in 25ml of purified H2O, in the first version, calm and collected in an insoluble substance on residue filter paper, wash away through warm H2O and lit for 15 mins at a temp not exceeding 450^0 C. Subtracted weight of insoluble ash. The proportion of water-soluble ash made, depending on the shade of dry bark residue.

Sulphated ash

Silica crucible, which heated to vitamin c, ten mins, cool and turned on the scale. 1 gm of airdried dust, bark is located in a silica crucible and moisturized through sulfuric acid, it is carefully spilled, over moisturized through sulfuric acid and burns at a temperature of around 800^0 C. Cooling and the gasket are applied again after 15 minutes, and then weighed. The proportion of sulfated ash was calculated with to air-dried bark powder.

RESULTS AND DISCUSSION

Table No: 1. Proximate values of *Lawsonia inemis* L bark.

Sl.No.	Parameter	Determined Value % w/w
A	Extractive values	
1	Alcohol soluble extractes value	22.00
2	Water soluble extractes value	28.00
3	Pet ether soluble extractes value	1.00
B	Moisture content	12.75
C	Ash Values	
1	Total ash	6.5
2	Water soluble ash	1.00
3	Acid insoluble ash	1.5
4	Sulphated ash	4.5

Phytochemical Investigations

Table No 2: Percentage yield and physical characteristics of various extracts of *Lawsonia inemis* L bark.

Successive Extraction

Extract	% Dry wt in gms.	Colour	Odour	Consistency
Chloroform	2.85	Brownish black	Characteristic	Sticky mass
Methanol	23.9	Brown	Characteristic	Powder
Aqueous	3.35	Reddish brown	Characteristic	Powder

Methanolic extract was further fractionated with ethyl acetate.

Fraction	% Dry wt in gms.	Colour	Odour	Consistency
Ethyl acetate	4.00	Reddish brown	Characteristic	Powder

Table No 3: Preliminary phytochemical tests of various extracts of *Lawsonia inemis* L bark.

Nature	Successive Extraction.			
	CHCl3	MeOH	AQ	EA
Alkaloids	Positive	--ve	--ve	Positive
Steroids	Positive	--ve	--ve	Positive
Carbohydrates	Positive	--ve	--ve	Positive
Phenolic compounds	Positive	+ve	+ve	Positive
Flavonoids	Positive	+ve	--ve	Positive
Glycoside	Positive	--ve	--ve	Positive
Triterpenoid	Positive	--ve	--ve	Positive
Tannins	Positive	+ve	+ve	Positive

Pharmacological Investigations

Table No. 4: Effect of Methanol and Aqueous extracts on SGPT levels in hepatotoxic rats.

Animals	Normal	CCl_4	Standard	Methanol	Aqueous
1	47.42	164.42	68.15	78.65	125.43
2	50.12	154.35	84.35	94.16	122.41
3	53.46	146.32	61.35	80.46	112.41
4	48.16	156.35	60.43	74.31	126.48
5	54.68	134.32	57.64	62.46	135.31
6	55.12	164.56	75.12	58.46	125.24
Mean	51.49	153.4	67.84***	74.75***	124.5**
SEM	3.369	11.57	10.26	12.97	7.385
SD	1.376	4.725	4.190	5.295	3.015

*****P < 0.0001, **P < 0.001, when compared to CCl_4 control.**

Table No. 5: Effect of Methanol and Aqueous extracts on SGOT levels in hepatotoxic rats.

Animals	Normal	CCl_4	Standard	Methanol	Aqueous
1	64.67	165.31	87.23	106.36	124.14
2	55.57	176.26	91.65	85.64	134.56
3	57.74	174.26	86.15	98.68	104.23
4	62.17	184.21	78.69	101.45	126.25
5	58.43	176.25	82.13	112.81	117.31
6	56.12	165.14	94.58	88.47	121.24
Mean	59.12	173.6	86.74 ***	98.90 ***	121.3***
SEM	3.580	7.313	5.869	10.40	10.16
SD	1.462	2.986	2.396	4.244	4.146

*****P < 0.0001, when compared to CCl_4 control.**

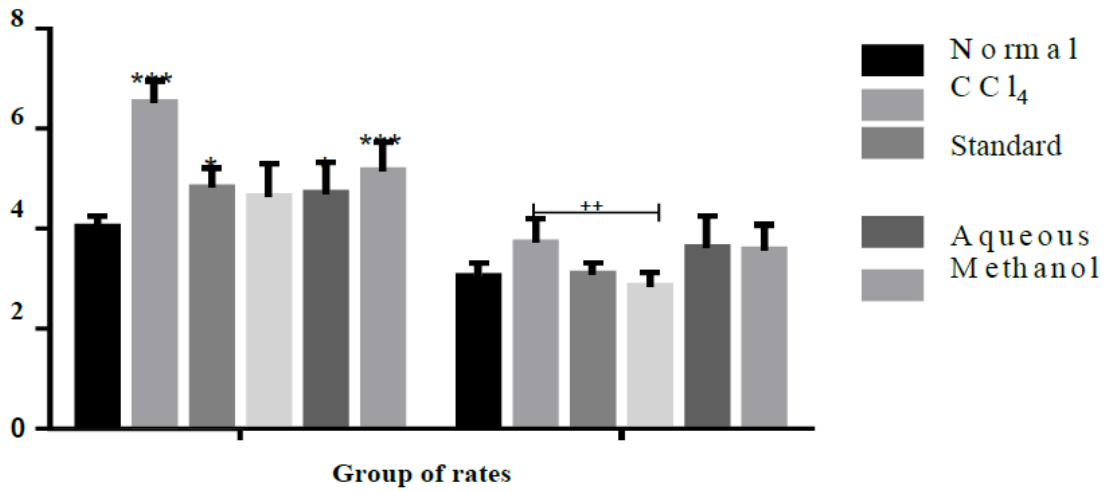


Table No. 6: Effect of Methanol and Aqueous extracts on SALP levels in hepatotoxic rats.

Animals	Normal	CCl_4	Standard	Methanol	Aqueous
1	146.15	346.14	167.01	198.16	214.64
2	138.23	421.35	156.46	194.35	248.34
3	158.16	384.68	178.16	187.64	201.46
4	162.14	425.47	164.58	201.65	224.64
5	159.46	412.06	182.15	227.16	253.18
6	147.25	304.31	177.16	175.14	204.13
Mean	151.9	382.3	170.9***	197.4***	224.4**
SEM	9.410	48.31	9.819	17.35	22.06
SD	3.842	19.72	4.009	7.081	9.007

*****P < 0.0001, **P < 0.001 when compared to CCl_4 control.**

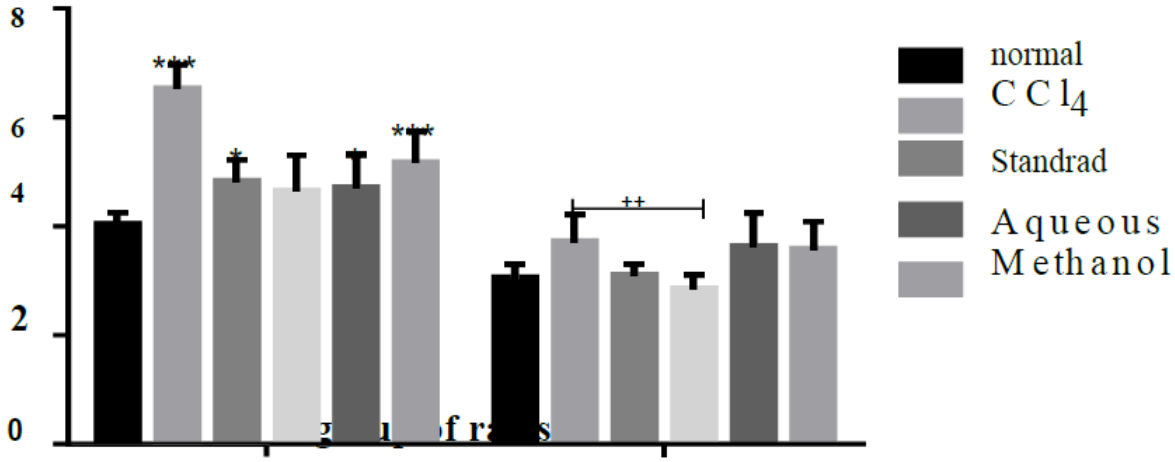


Table No. 7: Effect of Methanol and Aqueous extracts on Total Bilirubin levels in hepatotoxic rats.

Animals	Normal	CCl_4	Standard	Methanol	Aqueous
1	0.07	3.12	1.12	1.43	2.45
2	0.14	3.46	0.77	2.01	3.01
3	0.21	4.64	0.84	2.12	2.14
4	0.15	5.31	1.05	0.85	2.16
5	0.16	3.42	1.13	1.56	3.05
6	0.18	4.71	1.41	2.13	3.41
Mean	0.1517	4.110	1.053	1.683	2.703
SEM	0.04708	0.8899	0.2295***	0.5041***	0.5273***
SD	0.01922	0.3633	0.09369	0.2058	0.2153

*****P < 0.0001, when compared to CCl_4 control.**

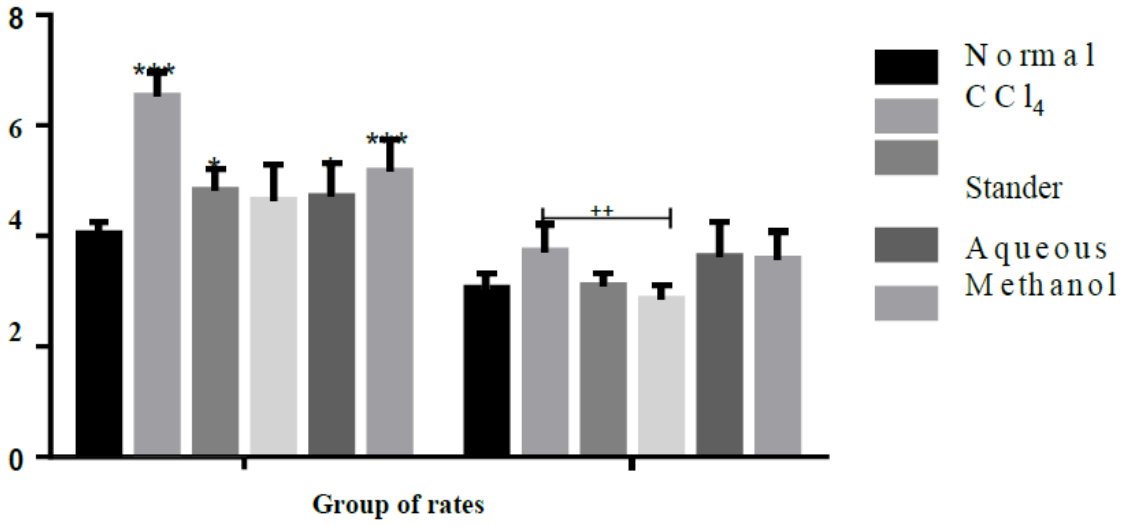
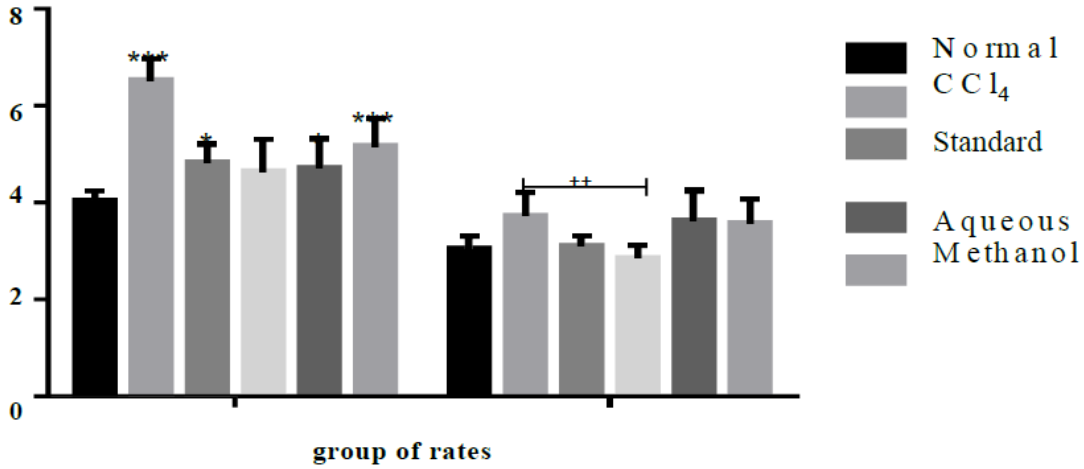


Table No. 8: Effect of Methanol and Aqueous extracts on Direct Bilirubin levels in hepatotoxic rats.

Animals	Normal	CCl_4	Standard	Methanol	Aqueous
1	0.084	0.41	0.06	0.16	0.27
2	0.051	0.74	0.10	0.24	0.34
3	0.074	0.64	0.14	0.34	0.36
4	0.064	0.35	0.08	0.42	0.37
5	0.056	0.42	0.21	0.31	0.21
6	0.070	0.57	0.23	0.17	0.32
Mean	0.0665	0.5217	0.1367***	0.2733***	0.3117**
SEM	0.01210	0.1525	0.07005	0.1019	0.06113
SD	0.004938	0.06226	0.02860	0.04161	0.02496

*****P < 0.0001, **P < 0.001when compared to CCl_4 control.**



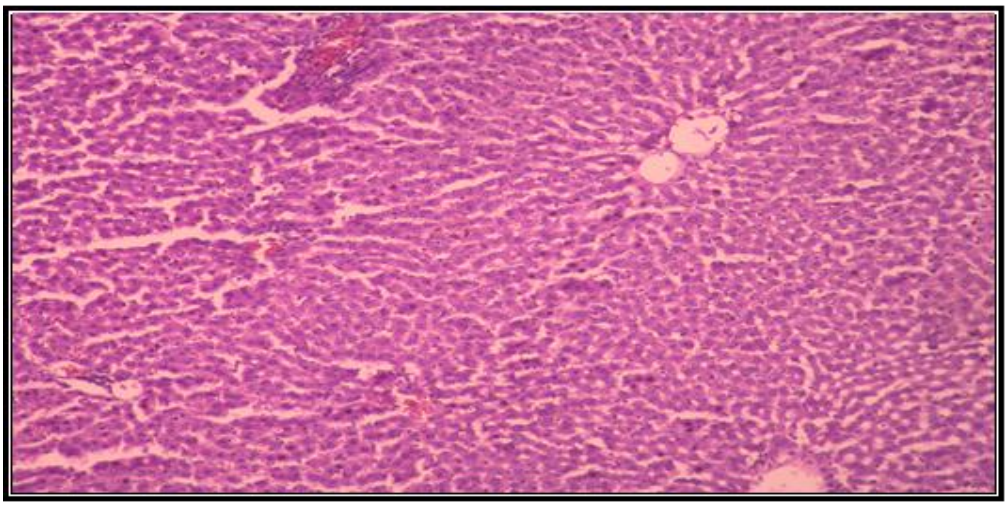


Plate No: 01: Photograph of normal liver biopsy (Normal group).

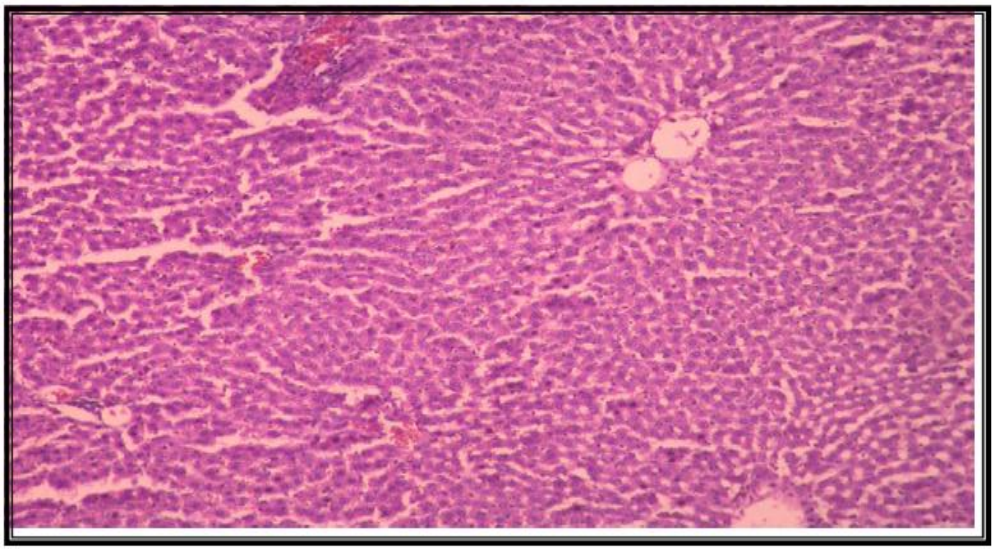


Plate No: 02: Photograph showing necrosis of hepatocytes (CCl_4 group).

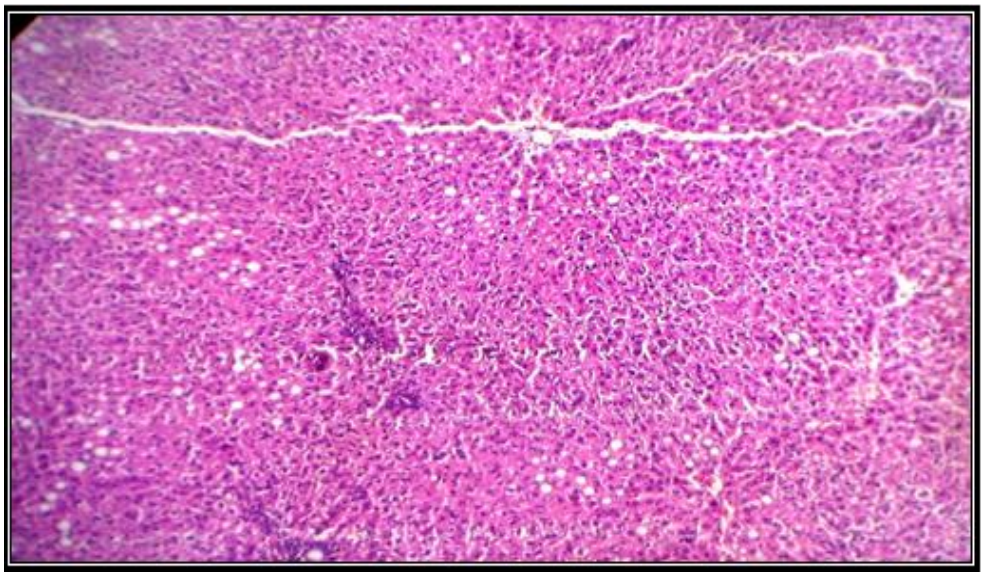


Plate No: 03: Photograph showing effect of liv-52 on regeneration of Hepatocytes.

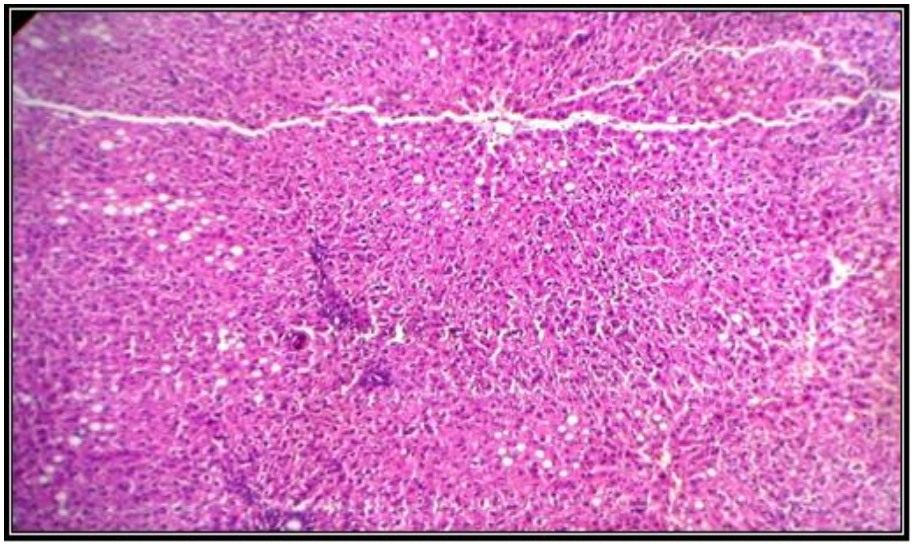


Plate No: 04: Photograph showing effect of Successive Methanol extract on regeneration of Hepatocytes.

CONCLUSION

The result of pharmacognostic, fitokimyəvi studies and for the study of poor circulation on the bark of *Lawsonia inemis* L. the following results were achieved.

Morphological evidence for the identification and authentication of drugs are.

Then the bark of *Lawsonia inemis* L. itself outstanding histological features, cork, cortex, medullary gives phloem fibers, cellulose, sclereids, cork cells, fiber crystals, medullary rays of the sun with fiber attachment and cortical cells that contain starch, grains.

Initial phytochemicals studies of chloroform, methanol and water, rich and resolved in the ethyl acetate fraction of methanol affected the presence of alkaloides, steroids, triterpenoids, tannins, flavonoids and phenolic compounds.

The successive methanol and aqueous extracts exhibited more promising hepatoprotective activity at the dose of 200mg/kg body weight which is supported by Histopathology. Collectively these natural flavonoids and tannins of *Lawsonia inemis* barks are promising in this research. However further investigations are needed to give some more evidences to support this research.

The isolated COMP-A was characterized by UV, FT-IR, H^1-NMR, ^{13}C-NMR and Mass spectral data co-relates with member of natural flavonoids.

The product of acid butanol reaction yields cyanidin. This confirms the proanthocyanidin present in *Lawsonia inemis* L. bark contains catechin or epicatechin units.

The results of the investigations justify the folklore use of bark of *Lawsonia inemis* in the treatment of liver diseases and the plant is worth for further isolation of more bioactive molecules.

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