Journal of
Bioengineering & Biomedical Science

Kamarapu, J Bioengineer & Biomedical Sci 2015, 5:2
DOI: 10.4172/2155-9538.1000152

Research Article Open Access

Pharmacognostic, Phytochemical and Pharmacological Studies of *Cassia roxburghii*

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Abstract

In the present study, the leaf of *Cassia roxburghii* and its powder were subjected to pharmacognostic evaluation in terms of macroscopic and microscopic evaluation. The powdered drug was subjected to extraction with various solvents such as petroleum ether, chloroform, ethyl acetate, methanol and aqueous extract by successive maceration. The methanolic and aqueous leaf extracts exhibited reducing power compared with ascorbic acid at similar concentrations. Based on the biochemical estimation of serum marker enzymes and histological study, all the extracts showed hepato protective activity and the maximum activity was seen in methanol extract at 200 mg/kg. The order of activity Me200>aq200>Me400>Aq400.

Keywords: Pharmacognostic evaluation; *Cassia roxburghii;* Enzymes

Introduction

The liver is the major organ in the body, contributing about 1/50 of the total weight of the body. It lies in the upper part of the abdominal cavity. More than 500 vital functions have been identified with the liver. The liver is important because a person's nutritional level is not only determined by what he or she eats, but by what the liver processes. It is difficult to detect symptoms of liver metabolic imbalances. Some of the common disorders of the liver include cirrhosis, viral hepatitis, alcoholic liver disease, hemochromatosis, liver cancer, jaundice and drug induced liver damage. Beyond the treatment of liver disorders, everyday care of the liver lays a cornerstone for total body health. Naturopaths and others, who look beneath the symptoms of an illness to its underlying cause, often discover that the liver had a role to play. People can suffer for a long time from a liver ailment without knowing of it. The incredible complexity of liver chemistry and its fundamental role in human physiology is so daunting to researchers that the thought that simple plant remedies might have something to offer is astonishing and incredible [1-5].

Cassia species have important role in phytochemical and pharmacological research due to their excellent medicinal values. Different classes of natural products, possessing potent physiological and pharmacological activities have been isolated from cassia species and they include anthracen derivatives, flavonoids and poly sacharides. Some of these compounds have been shown to possess Considerable antimicrobial activity. Cassia species are well known in folk medicine for their laxative and purgative uses. They are also used for treating skin diseases such as ring worm, scabies, eczema and wounds [6].

Materials and Methods

Collection of plant material

Cassia roxburghii is a uncommon in cultivation. The plant material collected from madikonda local areas of Warangal, India. Its parts were botanically authenticated by Taxonomist, Department of Botany, Kakatiya University, Warangal, India. A voucher specimen (CV-028) was maintained in the Department of Pharmacognosy and Phytochemistry, Vaagdevi College of pharmacy, India

Macroscopic, microscopic and physical evaluation of plant material

Macroscopic, Microscopic and physical evaluation of plant material was done by performing organoleptic, T.S, ash and extractive values.

Preparation of plant material

Cassia roxburghii leaves were washed under tap water and were efficiently dried under shade for about one week and protected from deterioration. The shade dried leaves were grinded made into powder with the help of blender.

Extraction

Maceration

The leaf material was weighed (250 g) and extracted by maceration using the solvents petroleum ether, chloroform, ethylacetate, methanol and aqueous at room temperature in a glass container for 3 days. The material was stirred from time to time to ensure proper extraction. After 3 days, the contents of the container were filtered through muslin cloth and the filtrate was concentrated under reduced pressure below 500C, until a soft mass obtained and then preserved in a desiccator. Finally phytochemical screening was done by performing the different identification tests [7-9].

Reducing power method

Different concentrations of the extracts from (100 µg/ml-1000 µg/ml) in 1 ml of distilled water were mixed with phosphate buffer (2.5 ml pH 6.8) and potassium ferricyanide (2.5 ml 1%). Then incubated at 500C for 20 min. To this, *trichloroacetic acid* was added and centrifuged at 3000 rpm for 10 min.

Then the upper layer was added with distilled water (2.5 ml), $FeCl_3$ (0.5 ml 0.1%) and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicates increased reducing power.

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Received: April 10, 2015; **Accepted:** April 23, 2015; **Published**: April 30, 2015

Citation: Kamarapu P (2015) Pharmacognostic, Phytochemical and Pharmacological Studies of *Cassia roxburghii*. J Bioengineer & Biomedical Sci 5: 152. doi:10.4172/2155- 9538.1000152

H_2O_2 scavenging assay method

H_2O_2 (43 mM) was prepared in phosphate buffer saline (pH 7.4). Positive control (Ascorbic acid) and extract solutions were prepared at concentrations of 50-250 µg/ml. Aliquots (different concentrations) of standard and extracts solutions (3.4 ml) were added to 0.6 ml of H_2O_2 solution. The reaction mixture was incubated at room temperature for 10 min and the absorbance was determined at 230 nm [10-13].

The % of scavenging was calculated as follows: % H_2O_2 scavenging = 100 X (absorbance of control – absorbance of sample)/absorbance of Control.

Acute toxicity testing

Acute toxicity and gross behavioral studies was carried out in mice after administration of various extracts of leaf of *Cassia roxburghii*. Albino mice weighing 20-25 gm were selected, weighed and marked. The mice were kept on overnight fasting before going to the test. The mice were divided into five groups with each group containing 6 mice. The test extracts were given orally in the form of suspension in arachis oil. The five groups of mice received the doses of extracts at 200, 400, 800 and 2000 mg/kg. Then the mice were continuously and carefully observed for 2 hrs followed by occasionally for further 4 hrs. The behavior and mortality of mice was observed up to 24 hrs. A one week washout period was allowed after studying each extract. Observation of behavioral changes will guide to go for further screening for the proposed activities [14].

Hepatoprotective Activity

In the present study, the animals were pretreated with test extracts and a standard drug silymarin (100 mg/kg) before inducing liver damage with CCl_4. The duration of the study was seven days. After acclimatization the rats were divided into thirteen groups (I-XIII). Each group consisting of six animals. All animals were kept on same diet for 7 days. The division of animals for Hepatoprotective activity of leaf extract of *Cassia roxburghii* is as follows.

Group I served as normal and received 1 ml/kg of arachis oil p.o. for seven days. Group II served as toxic control and was given 5 ml/kg of 50% v/v CCl_4 in olive oil i.p. on the seventh day. Group III (standard) animals were administered with 100 mg/kg of silymarin p.o. for seven days, followed by CCl_4 administration i.p. on the seventh day. Group IV–VII were treated in a similar way to that of group III (standard) using methanol extract and aqueous extract of leaf of *Cassia roxburghii* at doses of 200 mg/kg and 400 mg/kg in place of standard respectively, followed by CCl_4 administration i.p. on seventh day. All the rats were anaesthetized with thiopentone sodium (60 mg/kg i.p.) 36 h after administration of CCl_4. Then blood was collected from common carotid artery by carefully opening the neck region of the rat. After blood collection, the blood samples were allowed to coagulate at room temperature for at least one hour. Serum was separated by centrifugation at 3000 rpm for 30 minutes and then analysed for total bilirubin, Lactate dehydrogenase (LDH), Serum glutamate oxaloacetate transaminase (SGOT), Serum glutamate pyruvate transaminase (SGPT), ALP (Alkaline phosphatase), total protein and albumin levels. The animals were then dissected and the livers were carefully removed and washed with 0.9% saline solution and preserved in formalin solution (10% formaldehyde) for histopathological studies [15-18].

Processing of Liver Tissue

Liver tissues were taken out from fixing solution and dehydrated for 30 minutes each in 30, 50, 70, 90, and 100% alcohol successively. To remove the alcohol from the dehydrated tissues, they were kept for 30 minutes each in alcohol: xylene (1:1) followed by pure xylene. Then the tissue were then kept in xylene : paraffin wax mixture (1:1) for 1 hour and then in molten paraffin wax at 62°C, after which they were trimmed and mounted on wooden blocks for thin sectioning. Hand microtome (York precision rotary microtome, model no YS1114) was used to cut thin sections of liver tissues of 5 µm thickness [19,20].

Staining and mounting of liver tissues

Ribbons of thin sections of liver tissues were placed in rows on clean glass slides previously coated with albumin-glycerine mixture and few drops of water added to let the sections float. The slides were heated on hot plate to fix liver sections onto the slides. The slides were then placed for 5minutes each in xylene to remove wax, then in absolute alcohol to remove xylene from the liver sections. Hydration of liver sections was attained by keeping them in descending series of alcohol and water mixtures (90%, 70%, 50%, 30% alcohol and in pure water) for three minutes each. Hydrated sections were stained with *haemotoxylin* stain for one minute and washed in running tap water to remove excess stain. Liver sections were dehydrated again by keeping in ascending of alcohol water mixtures (30%, 50%, 70%, and 90% alcohol) for one minute. After that, the sections were kept for 5 minutes each in absolute alcohol and then in xylene. Finally, the stained liver sections were mounted in DPX (*Desterenedibutyls phthalate xylene*) and viewed under optical microscope for histological examination [21-24].

Results and Discussion

In this evaluation macroscopy and microscopy of leaf of *Cassia roxburghii* were studied. The observations of the investigations were, the leaf powder of the plant was studied for their organoleptic characters like colour, odour and taste. The results of this study were Color – Greenish, Taste –mucilaginous, Odour - Characteristic

The leaf powder of the plant has shown the presence of following plant tissue systems under microscopic evaluation:

Calcium oxalate crystals : Prismatic type

Starch grains : Simple, compound

Vascular tissue : Xylem and phloem

Trichomes : Unicellular covering trichomes

Quantitative microscopy of leaf/leaf powder *Cassia roxburghii:* The powder analysis of leaf powder of the plant was evaluated and the results obtained were shown below.The width range of phloem fibers in powdered leaf of *Cassia roxburghii*was found to be 15.46 µ. The diameter of starch grains in powdered leaf of *Cassia roxburghii* was found to be 2.45 µ (Simple and compound). The length of the calcium oxalate crystals in powdered leaf of *Cassia roxburghii* was found to be 3.2 µ. Vascular bundle: Xylem and Phloem (Figures 1-3).

Transverse section of leaf

Rubiaceous or Paracytic stomata.

Spongy tissue of parenchymatous cells.

Cluster crystals of calcium oxalate (prismatic type) in palisade and crystal sheath in mid rib region.

Palisade-a single layer below upper and lower epidermis.

Epidermal trichomes-Unicellular covering trichomes.

Figure 1: Powder analysis.

Figure 2: Trichomes.

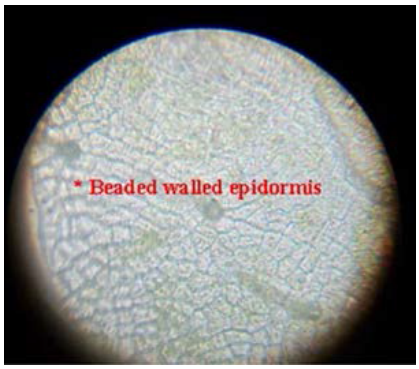

Figure 3: Epidermis.

Epidermis-beaded walled epidermis (Figure 4 and Tables 1-3).

Acute toxicity studies were performed for all the five extracts at doses of 200, 400, 800 and 2000 mg/kg body weight in mice. The behavioral changes were observed for 24 hours. The observation of the behavioral changes the animals did not show any toxic effects up to the dose of 2000 mg/kg.

Antioxidant activity

Reducing power method (Tables 4-6 and Figure 5): The reducing power of methanolic and aqueous extracts from *cassia roxburghii* compared with ascorbic acid as standard. The reducing power of both samples increased with the concentrations. The reducing power of methanolic, aqueous extracts were 1.01,0.55 at 1 mg/ml respectively however at 1 mg/ml ascorbic acid showed excellent reducing power of

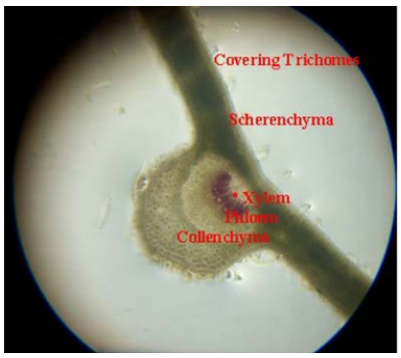

Figure 4: T.S of leaf.

Particulars	Leaf powder (%w/w)
Total ash	16
Acid insoluble ash	0.59
Water soluble ash	1

Table 1: Physicochemical evaluation.

S.No	Part of plant	Method of extraction	Solvent	Physical form	% Yield
1	Leaf	Maceration	Pet. ether	Resinous	1.42
2	Leaf	Maceration	Chloroform	Resinous	2.2
3	Leaf	Maceration	Ethyl acetate	Resinous	2.1
4	Leaf	Maceration	Methanol	Powder	2.1
5	Leaf	Maceration	Water	Powder	3.2

Table 2: Percentage yield of the *Cassia roxburghii* leaf extracts

Tests	Leaf extracts				
	P.E.E	C.E	E.A.E	M.E	A.E
Alkaloids - Dragendorff's test - Mayer's test - Wagner's test - Hager's test	- - - -	- - - -	- - - -	- - - -	- - - -
Carbohydrates - Molish's test - Fehling's test - Benedict's test - Barfoed's test	- - - -	+ + + +	+ + - +	+ + + +	+ + + -
Steroids - Liebermann-Burchard reaction - Salkowski test	+ +	- -	+ -	+ +	- -
Phenolics and Tannins - 5% ferric chloride test - Lead acetate solution test	- -	- -	+ -	+ +	+ +
Glycosides -Borntragers test -Keller killani test -Saponin test	- + -	- - -	- + +	+ + +	+ + +
Proteins -Millons test Xanthoprotein test	- -	- - -	- -	- -	- -

"+" Positive "-" Negative
P.E.E (Petroleum ether extract)
C.E (Chloroform extract)
E.A.E (Ethylacetate extract)
M.E (Methanol extract)
A.E (Aqueous extract)

Table 3: Qualitative phytochemical screening of different extracts of *Cassia roxburghii*.

Ascorbic acid Concentration (µg/ml)	Absorbance at 700 nm
100	0.26
200	0.42
400	0.60
600	0.72
800	0.84
1000	0.91

Table 4: Table showing ascorbic acid absorbance.

Leaf aqueous extract (µg/ml)	Absorbance at 700 nm
100	0.29
200	0.36
400	0.64
600	0.74
800	0.93
1000	1.01

Table 5: Table showing leaf methanol extract absorbance.

Leaf aqueous extract (µg/ml)	Absorbance at 700 nm
100	0.14
200	0.15
400	0.18
600	0.45
800	0.46
1000	0.55

Table 6: Table showing leaf aqueous extract absorbance.

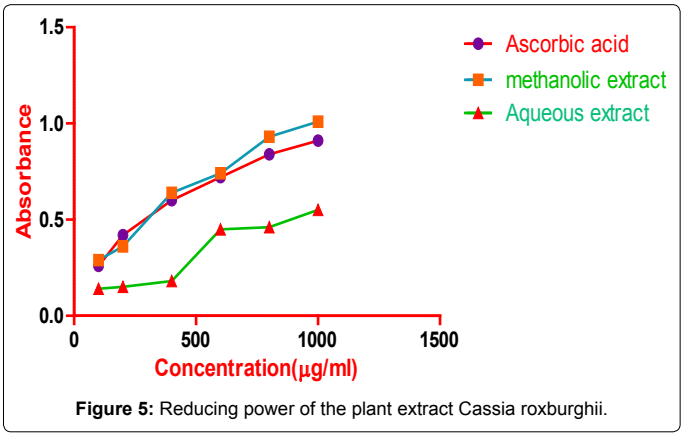

Figure 5: Reducing power of the plant extract Cassia roxburghii.

0.91, respectively which are significantly higher than that of methanol and aqueous extracts. In the present study methanolic extract showed higher reducing power than Aqueous extract.

H_2O_2 scavenging assay method (Tables 7-10 and Figure 6)

The methanolic and aqueous whole plant extracts exhibited higher H_2O_2 scavenging activity than ascorbic acid at similar concentrations. The IC50 values of the methanolic and aqueous extracts of leaves and ascorbic acid were 110.375, 136.599 and 136.633 µg/ml, respectively (Table 11 and Figures 7-10).

Histological study

Shown in Table 12 and Figures 11-14.

Conclusion

Whole plant and seeds are used to treat skin diseases like ringworm, antimicrobial, hepatoprotectivity. The methanolic and aqueous leaf extracts both exhibited Antioxidant activity compared with ascorbic acid. Methanolic extract found to be more potent Antioxidant activity compare with aqueous leaf extract. All the leaf extract of *Cassia roxburghii* showed hepatoprotective activity and methanolic extract (200 mg/kg) was found to be more potent hepatoprotective compare with the other extracts. The histological studies also indicated that methanol extract (200 mg/kg) showed significant activity when compared with other extracts. Therefore, it can be concluded that *Cassia roxburghii* may have "potential hepatoprotective activity thus further mechanism based studies are needed for its hepatoprotective activity".

Ascorbic acid concentrations (µg/ml)	Absorbance at 230 nm	% Inhibition
50	0.146	16.04
100	0.26	28.5
150	0.422	46.3
200	0.818	89.8
250	0.91	100

Table 7: Table showing ascorbic acid % inhibition.

Leaf aqueous extract concentrations (µg/ml)	Absorbance at 230 nm	% Inhibition
50	0.209	22.4
100	0.399	42.8
150	0.483	51.8
200	0.517	55.5
250	0.931	100

Table 8: Table showing leaf aqueous extract % inhibition.

Leaf methanolic extract concentrations (µg/ml)	Absorbance at 230 nm	% Inhibition
50	0.728	71.02
100	0.736	71.80
150	0.804	78.43
200	0.904	88.19
250	1.025	100

Table 9: Table showing leaf methanolic extract % inhibition.

S.No	Particulars	IC_{50} Values(µg/ml)
1	Ascorbic acid	136.633
2	Methanol extract of *Cassia roxburghii*	110.375
3	Aqueous extract of *Cassia roxburghii*	136.599

Table 10: Table showing IC50 values.

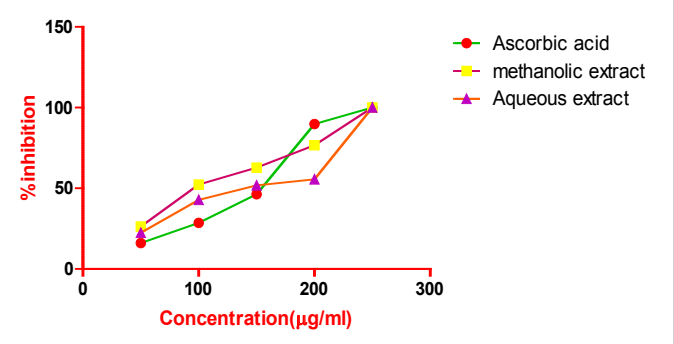

Figure 6: H_2O_2 scavenging activity of plant extracts of cassia roxburghii.

	SGPT	SGOT	ALP	Bilirubin	Protein	Cholesterol
Control	45.08 ± 1.22	38.52 ± 0.51	174.03 ± 1.672	0.48 ± 0.019	4.53 ± 0.001	67.5 ± 0.191
Toxic (ccl_4)	307 ± 8.38	389.3 ± 13.4	368 ± 2.8	0.83 ± 0.018	2.13 ± 0.024	34.45 ± 0.152
Standard (Silymarin)	43.64 ± 0.31	42.12 ± 0.20	182 ± 1.585	0.46 ± 0.006	5.26 ± 0.020	65.26 ± s0.138
Methanol 200 mg	54.26 ± 0.138	68.23 ± 0.157	136 ± 1.563	0.52 ± 0.012	6.81 ± 0.199	73.33 ± 1.202
Methanol 400 mg	64 ± 1.317	138.6 ± 0.112	266.6 ± 1.585	0.63 ± 0.010	4.7 ± 0.173	54.26 ± 0.138
Aqueous 200 mg	56.46 ± 0.140	75.06 ± 0.120	147 ± 1.555	0.53 ± 0.012	6.45 ± 0.125	64 ± 1.317
Aqueous 400 mg	73.33 ± 1.202	186.5 ± 0.002	336.48 ± 0.149	0.58 ± 0.014	5.56 ± 0.170	56.46 ± 0.140

N=six animals in each group; values are Mean ± SEM, when compare to control.

Table 11: Effect of different leaf extracts of *Cassia roxburghii* on serum biochemical parameters in CCl_4 induced liver toxicity.

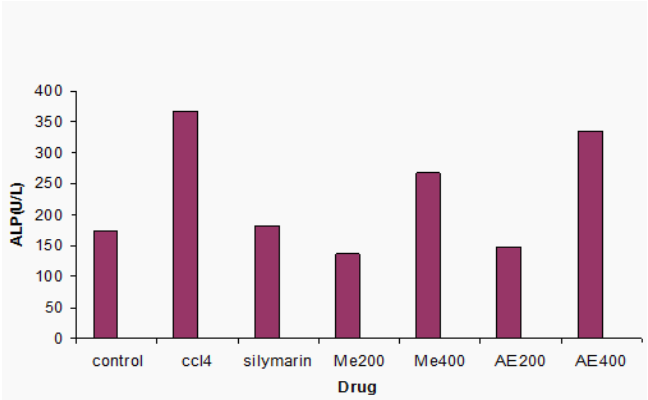

Figure 7: Effect of different leaf extracts of Cassia roxburghii on ALP (U/L) in serum.

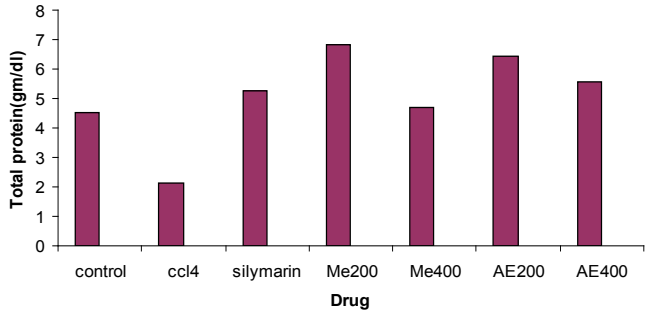

Figure 10: Effect of different leaf extracts of *Cassia roxburghii* on total protein in serum.

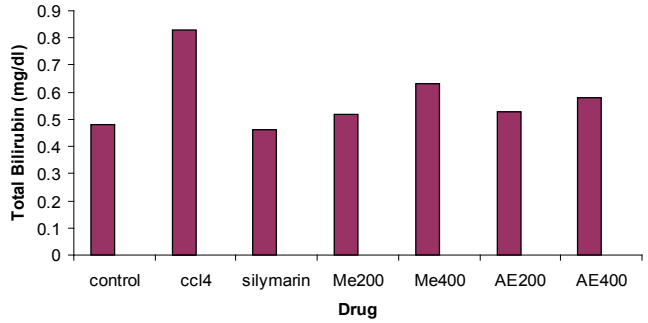

Figure 8: Effect of different leaf extracts of *Cassia roxburghii* on bilirubin in serum.

S.NO.	Group	Score	Observation
1.	Control	0	Showed normal hepatic cells w ith w ell preserved cytoplasm, nucleus, nucleolus and central vein.
2.	CCl_4	4	Showed necrosis, steatosis, fatty vaculaization and pseudolobule formation w ere seen.
3.	Reference standard	0	Showed significant restoration of liver architecture comparably w ith normal rats
4.	Methanolic (200 mg/kg)	1	Showed almost normal liver architecture w ith very mild degree of fatty changes w hen compared w ith normal and standard.
5.	Methanolic (400 mg/kg)	3	Moderated restoration of liver architecture w ith mild degree of necrosis.
6.	Aqueous (200 mg/kg)	2	Showed mild fatty change and mild sinusoidal Congestion.
7.	Aqueous (400 mg/kg)	3	Showed mild degree of necrosis w ith areas of inflammation.

Table 12: Grading of liver damage based on histological study.

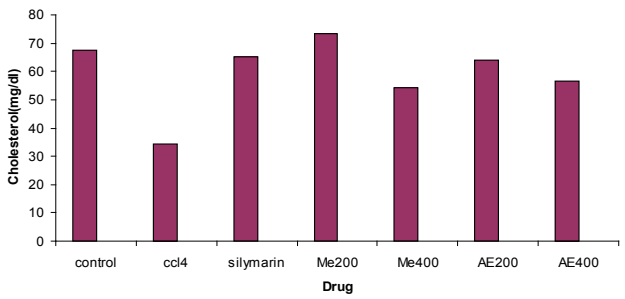

Figure 9: Effect of different leaf extracts of *Cassia roxburghii* on cholesterol in serum.

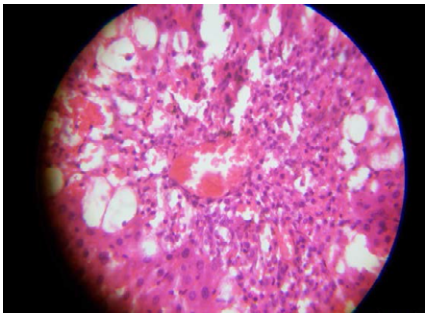

Figure 11: Toxic.

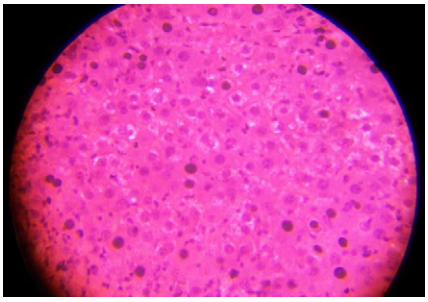

Figure 12: Control.

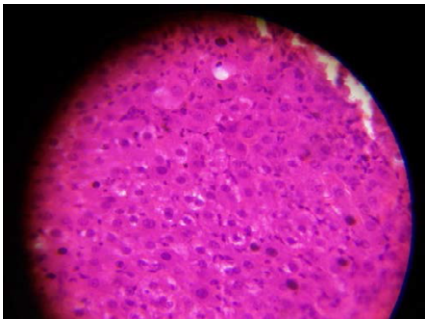

Figure 13: Reference standard.

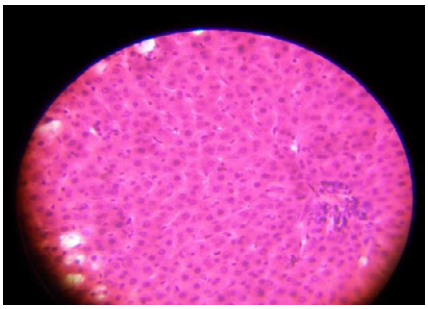

Figure 14: Methanol (200 mg/kg).

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