e-ISSN: 2349-2678, p-ISSN:2961-6050

available online on www.ijpba.in

International Journal of Pharmaceutical and Biological Science Archive

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Index Copernicus Value 2019: 71.05

Volume 11 Issue 4; July-August; 2023; Page No. 35-51

ResearchArticle

Standardization & Hepatoprotective Activity of *Citrus sinensis* Peels

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Received: 20-05-2023 / Revised: 22-06-2023 / Accepted: 05-07-2023

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Conflict of interest: Nil

Abstract

Liver disease accounts for approximately 2 million deaths per year worldwide, one million due to complications of cirrhosis and one million due to viral hepatitis and hepatocellular carcinoma. Cirrhosis is currently the 11th most common cause of death globally and liver cancer is the 16th leading cause of death; combined, they account for 3.5% of all deaths worldwide. Cirrhosis is within the top 20 causes of disability-adjusted life years and years of life lost and account for 1.6% and 2.1% of worldwide burden. About 2 billion people consume alcohol worldwide and upwards of 75 million are diagnosed with alcohol use disorders and are at risk for alcohol associated liver disease. Drug induced liver injury continues to increase as a major cause of acute hepatitis. The liver is a major organ only found in vertebrates which performs many essential biological functions such as detoxification of the organism, and the synthesis of protein and biochemical necessary for digestion and growth.

Citrus sinensis is from Rutaceae family. Rutaceae are herbs, shrubs and trees with glandular punctate, commonly strongly smelling herbage comprising about 150 genera and 1,500 species. *Citrus sinensis* is one of the most important and widely grown fruit crops, with total global production reported to be around 120 million tons. Orange fruit is cultivated in more than 130 countries including India, UK, France, Germany, Holland, Brazil, China, USA and Spain. Oranges are generally available from winter through summer with seasonal variations dependingon the variety.

1. Introduction

Liver disease accounts for approximately 2 million deaths per year worldwide, one million due to complications of cirrhosis and one million due to viral hepatitis and hepatocellular carcinoma. Cirrhosis is currently the 11th most common cause of death globally and liver cancer is the 16th leading cause of death; combined, they account for 3.5% of all deaths worldwide. Cirrhosis is within the top 20 causes of disability-adjusted life years and years of life lost and account for 1.6% and 2.1% of worldwide burden. About 2 billion people consume alcohol worldwide and upwards of 75 million are diagnosed with alcohol use disorders and are at risk for alcohol associated liver disease. Approximately 2 billion adults are obese or overweight and over 400 million have diabetes; both serve as risk factors for the increase in non-alcoholic fatty liver disease as well as hepatocellular carcinoma. The global prevalence of hepatitis B (3.5%) and hepatitis C (1%) is high. Drug induced liver injury continues to increase as a major cause of acute hepatitis. Liver transplantation is the second most common solid organ transplantation after kidney transplantation worldwide. However, less than 10% of global needs of organ transplantation are met at current rates of

transplantation. Though these numbers are sobering, they offer an important opportunity to improve public health given that most causes of liver diseases are preventable

The liver is a major organ only found in vertebrates which performs many essential biological functions such as detoxification of the organism, and the synthesis of protein and biochemical necessary for digestion and growth.[1,2,3]

Citrus sinensis is from Rutaceae family. Rutaceae are herbs, shrubs and trees with glandular punctate, commonly strongly smelling herbage comprising about 150 genera and 1,500 species. These are further characterized by the common occurrence of winged petioles and spines.[4] *Citrus sinensis* is one of the most important and widely grown fruit crops, with total global production reported to be around 120 million tons. Orange trees are widely cultivated in tropical and subtropical climates for its tasty juice and medicinal value. In worldwide trades citrus fruits generate about 105 billion dollars per year all over the world. Orange fruit is cultivated in more than 130 countries including India, UK, France, Germany, Holland, Brazil, China, USA and Spain. Oranges are generally available from winter through summer with seasonal variations dependingon the variety.[5,6]

2. Materials & Methods

2.1 Plant Material

The plant part was (*Citrus sinensis* peels) collected from Kota (Rajasthan) in month of December 2022. The active constituents of plant were used to evaluate their hepatoprotective activity.

2.2 Standardization

The parameters for the standardization of *Citrus sinensis* peels was performed.[7] Parameters included are shown in table no.2.1.

Table 2.1: Standardization parameters of *Citrus sinensis* peels

S. No.	Parameter	Procedure
1.	Total ash value	Powdered peels of Citrus sinensis ↓ Incinerated at 450° C ↓ Percentage of ash was calculated with reference to dried drug.
2.	Swelling index	Powdered peels (1gm) + water (25ml) ↓ Shaken for every 10 mins for 1 hr. ↓ Allow to stand for 3 hrs. at room temperature ↓ Mean value of volume (ml) was calculated relating to 1gm of the plant material.
3.	Volatile matter	Fresh peels of citrus sinensis (25gm) ↓ Dipped in distilled water ↓ Flask was attached to the Clevenger apparatus ↓ Volatile oil was collected
4.	Foaming index	Plant material (1gm) + boiling water (100ml) ↓ Maintained at boiling for 30 mins. ↓

		Cooled and filtered in 100 ml volumetric flask ↓ Diluted to volume with water ↓ Decoction poured in 10 test tube in successive portion in 1ml up to 10 ml ↓ Volume adjusted up to 10 ml with water ↓ Tubes shaken for 15 sec and allowed to stand for 15 mins. ↓ Measure the height of foam ↓ Foaming index calculated ↓ 1000/A Where A- volume in ml of the decoction used for preparing the dilution in the tube where foaming to the height of 1cm observed
5.	Moisture content	Fresh citrus sinensis peels (2gm) ↓ Kept in an oven until moisture removed ↓ Weighed after drying
6.	Crude fibre	Coarse powder of Citrus sinensis peels (10gm) ↓ Extracted with ether ↓ Sulphuric acid (1.25%) (200ml) ↓ Mixture was refluxed for 30 mins. ↓ Liquid was filtered ↓ Residue was washed on filter paper with boiling water ↓ Dried and incinerated

2.3 Extraction of *Citrus sinensis* Peels

Citrus sinensis peels were dried and powdered. Coarsely powdered peels were placed in a "thimble" made up of muslin cloth, which was placed in the chamber of the soxhlet apparatus. The distilled water was heated in flask, and its vapours were condensed in condenser.

The condensed extractant dipped into the thimble containing the peels. When the level of liquid was raised to the tube of siphon tube, the liquid contents of the chamber were siphoned into the flask. This process was continuous and was carried out until a drop of solvent from the siphon tube does not leave residue when evaporated.

2.4 Phytochemical Screening of *Citrus sinensis* Peels

The chemical tests for the screening and identification of the phytoconstituents in medicinal plant under study (*Citrus sinensis*) was carried out for the aqueous extract as per the standard method.[8] Tests conducted for the phytochemical screening are described in table no. 2.2.

Table 2.2: Preliminary, phytochemical screening of the aqueous peel extract of *Citrus sinensis*

S. No.	Phytochemicals	Tests	Procedure
1	Carbohydrates	a) Molish' test	Aq. Extract (3ml) + α- naphthol (few drops); shaken; added conc. H_2SO_4 from the side
2	Reducing sugar	a) Fehling's test b) Benedict's test	Fehling's A + Fehling's B solution; Δ on water bath for 10 mins. Test solution + Benedict's reagent (2ml each); Δ for 5 mins.
3	Monosaccha-rides	a) Barfoed's test	Test solution + Barfoed's reagent (2ml each); Δ for 2 mins. on water bath & cooled
4	Proteins	a) Biuret's test b) Millon's test	Test solution (3ml) + NaOH (4%)(1ml) + $CuSO_4$ (1%)(1ml) Test solution (3ml) + Millon's reagent (5ml); white ppt. Warmed
5	Amino acids	a) Ninhydrin test	Test solution (3ml) + Ninhydrin solution (5%) (3drops); Δ on water bath for 10 mins.
6	Steroids	a) Salkowski reac-tion b) Liebermann – Burchard reaction c) Liebermann re-action	Extract + chloroform + conc. H_2SO_4 (2ml each other); shaken well Extract (2ml) + chloroform (1ml); acetic anhy-dride (1ml) + conc. H_2SO_4 (2 drops) from the sides of test tube Extract + acetic anhydride (2ml each); Δ & cooled; H_2SO_4 (few drops) added.
7	Glycosides (car-diac glycosides)	a) Baljet's test	Extract was treated with sodium picrate
8	Anthraquinone glycosides	a)Borntrager's test b) Modified Born-trager's test	Extract (3ml) + dil. H_2SO_4; boiled & filtered. To cold filtrate, benzene & chloroform (1ml each) was added; shaken; organic solvent sep-arated; ammonia added $FeCl_3$ (5%)(2ml) + dil. HCl (2ml); Δ on water bath for 5 min; cooled & benzene was added; shaken; organic layer separated; dil. ammonia (2ml) added
9	Saponin Glyco-sides	a) Foam test	Drug extract shaken with water
10	Flavonoids test	a) H_2SO_4 Test	Extract + Sulphuric acid (80%)
11	Alkaloids	a)Dragendroff's test b) Wagner's test c) Mayer's test d) Hager's test	Filtrate (3ml) + dragendroff's reagent (few drops) Filtrate (3ml) + Wagner's reagent (few drops) Filtrate (3ml) + Mayer's reagent (few drops) Filtrate (3ml) + Hager's reagent (few drops)
12	Tannins	a) $FeCl_3$ test b) Gelatin test	Extract (2ml) + $FeCl_3$ (few drops) Test solution (2ml) + gelatin (1%) (1ml) + NaCl (10%)

2.5 Isolation

Powdered peels of Citrus sinensis (100gm) were refluxed with 400 ml of petroleum ether (40 – 60^{o}C) on a water bath for 1 hour. The content were filtered hot through a Buckner funnel and the mark obtained was dried at room temp. The dried mark was further extracted with methanol (400 ml) for 3 hours. The content were filtered while hot and mark was washed with 50 ml of hot methanol. The washing was combined with filtrate and the resulting filtrate was kept under reduced pressure to form a syrupy mass and the crystallized hesperidine was obtained from the dilute acetic acid solution.[9]

2.6 Characterization

Characterization of isolated hesperidine was performed by IR, MASS ^{1}NMR and UV spectroscopic method.

52.6.1 IR – Spectroscopy

IR spectroscopy was performed at Guru Nanak Dev University, Amritsar. It gives information about functional group.

2.6.2 Mass-spectroscopy

Mass spectroscopy was performed from Guru Nanak Dev University, Amritsar. This is the method for determination of molecular weight and formula. In the spectra m+1 peak was found at 611.36

2.6.3 ^{1}HNMR-spectroscopy

^{1}HNMR was performed from Guru Nanak Dev University, Amritsar. It is used for determination of number of hydrogen and environment of hydrogen.

2.6.4 UV – Visible spectrophotometry

UV- Visible spectroscopy was performed from Guru Nanak Dev University Amritsar. Interpretation of spectra was performed on the basis of maximum absorbance (λmax) and specific absorptivity (A1%; 1cm) of standard and isolated hesperidine.

2.7 Experimental Animal

Female rats of wistar strain were used for the study. They were housed in different cages with not more than six animals per cage and maintained under standard conditions. The experimental protocol was approved by IAEC (Institutional Animal Ethical Committee) following the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) (Reg. No. 2005/PO/a/RcBT/S/18/CPCSEA).

2.8 Experimental Chemicals

Liver damage was induced in rats by administering allyl alcohol 1.25% (orally) at the dose of 0.4ml/kg body weight of each animal. Allyl alcohol (0.4ml/kg) orally was administered daily for a period of two days. Aqueous extract of *Citrus sinensis* peels.

2.9 Experimental Design

Thirty rats were divided into six groups of five each as follows:

Group 1: (Positive control) and received oral administration of vehicle (distilled water).

Group 2: (Negative control) animals were administered with allyl alcohol (1.25%) (0.4ml/kg, orally) for two days.

Group 3: (Silymarin treated group) animals were treated with Silymarin at dose of 200mg/kg, orally, before and after the administration of allyl alcohol (0.4ml/kg).

Group 4: (Treatment group) Animals were treated with aqueous extract of *Citrus sinensis* at dose of 100mg/kg, orally, before and after the administration of allyl alcohol (0.4ml/kg).

Group 5: (Treatment group) Animals were treated with aqueous extract of *Citrus sinensis* at dose of 200mg/kg, orally, before and after the administration of allyl alcohol (0.4ml/kg).

Group 6: (Treatment group) Animals were treated with aqueous extract of *Citrus sinensis* at dose of 400mg/kg, orally, before and after the administration of allyl alcohol (0.4ml/kg).

2.10 Biochemical Parameters Study

At the end of the experimental period the blood was obtained from all groups of rats after being lightly anaesthetized with ether by puncturing retro-orbital plexus.[10] The blood was allowed to flow into a clean dry centrifuge tube and left to stand 30 min before centrifugation to avoid hemolysis. The blood sample were centrifuged

for 15 min at 2500, rpm the clear supernatant serum was separated and collected by Pasteur pipette into a dry clean tube to use for determination of various biochemical parameters such as SGOT, SGPT, Total proteins, Albumin, Alkaline Phosphatase, Cholesterol, Bilirubin etc.[11]

2.11 Statistical Analysis

Data were expressed as mean ± S.E.M. Analysis will be performed with graphpad prism version 10 software using one – way analysis of variance (ANOVA) followed by ordinary nonparametric or mixed tests. $p<0.05$ was considered to be statistically significant.

2.12 Histopathological Study

Animals were sacrificed 24 h after the last treatment, the thoracic cavities opened, livers rapidly and carefully excised and all attached vessels and ligaments trimmed off. The removed livers were washed with cold saline, dried with filter papers and weighed, then dropped into a jar containing 10% formalin as a fixative and kept for histopathological examination. Liver slides were prepared and stained with hematoxylin and eosin staining.[12]

3 Results

The results of the proposed research work (Standardization & Hepatoprotective activity of *Citrus sinensis* peels) are explained in following way.

3.1 Standardization

The observation of parameters include in the standardization of *Citrus sinensis* peels are described in table no. 3.1.

Table 3.1: Observation of parameters include in the standardization parameters of *Citrus sinensis* peels

S. No.	Parameter	Observations
1.	Total ash value	Total ash value : 12.00%w/w
2.	Swelling index	Swelling index : 3
3.	Volatile matter	Volatile oil content: 4.0% v/w oil in the sample.
4.	Foaming index	Foaming index: 1000/A : 1000/9 : 111.11
5.	Moisture content	% Moisture content : 47%
6.	Crude fibre	% crude fibre : 33.33%

3.2 Extraction of *Citrus sinensis* PEELS

Weight & % yield calculated for aqueous extract of *Citrus sinensis* peels is shown in table no. 3.2.

Table 3.2: Weight of the aqueous extract of *Citrus sinensis* peels.

S. No.	Particulars	Weight (gm)
1	Dried powdered peels	80
2	Peels after extraction	58
3	Extract	22
4	% yield	27.5%

3.3 Phytochemical Screening of *Citrus sinensis* Peels

The observation of tests conducted for the phytochemical screening are described in table no 3.3.

Table 3.3: Observation of phytochemical screening of the aqueous peel extract of *Citrus sinensis*

S. No.	Phytochemicals	Tests	Inference	Remarks
1	Carbohydrates	a) Molish's test	Violet ring	+++
2	Reducing sugar	a) Fehling's test b) Benedict's test	First yellow then brick red ppt Appearance of green, yellow or red colour	+++ +++
3	Monosaccharides	a) Barfoed's test	Red ppt	+++
4	Proteins	a) Biuret's test b) Millon's test	Violet or pink colour Conversion of white ppt to brick red ppt. Dissolved to give red coloured solution	_ _
5	Amino acids	a) Ninhydrin test	Purple or bluish colour	_
6	Steroids	a) Salkowski reaction b) Liebermann Burchard reaction c) Liebermann reaction	Red layer of chloroform & greenish acid layer formed First red then blue & finally green ppt Blue colour	+++ +++ +++
7	Glycosides (cardiac glycosides)	a) Baljet's test	Yellow colour	+
8	Anthraquinone glycosides	a)Borntrager's test b) Modified Borntrager's test	Pink or red ammoniacal layer Ammoniacal shows pinkish red colour	+ +
9	Saponin Glycosides	a) Foam test	Persistent foam	+
10	Flavonoids test	a) H_2SO_4 Test	Deep yellow colour solution	+++
11	Alkaloids	a) Dragendroff's test b) Wagner's test c) Mayer's test d) Hager's test	Orange brown ppt Reddish brown ppt Ppt formation Yellow ppt	_ _ _
12	Tannins	a) $FeCl_3$ test b) Gelatin test	Deep blue black colour Ppt formation	_ _

Foot Note: +++ highly positive, ++ positive, + slightly positive, - negative

3.4 Isolation

Weight of hesperidine was calculated as shown in table no.3.4.

Table 3.4: Weight of isolated hesperidine from Citrus sinensis peels

S. No.	Particulars	Weight (gm)
1	Vial	6.74
2	Vial + hesperidine	7.12
3	Hesperidine	0.38

3.5 Characterization

3.5.1 IR – Spectroscopy

An IR spectra of isolated hesperidine is given in fig.3.1. Interpretation of spectra was performed and prominent group peaks are shown in table no.3.5.

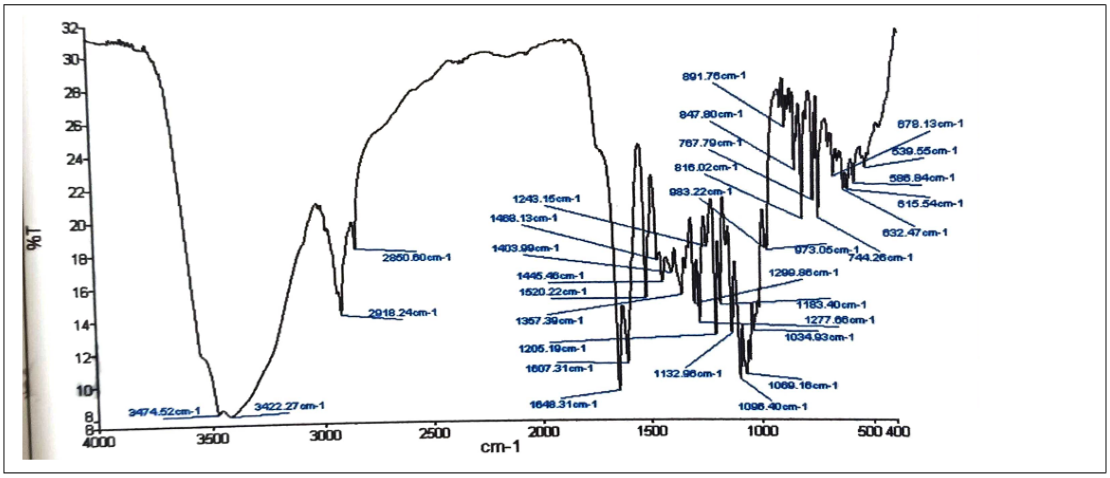


Figure3.1: IR spectra of isolated hesperidine

Table 3.5: Interpretation of IR spectra

S. No.	Peak maxima	Predicted groups
1	3500cm^{-1}	(-OH, str)
2	2900cm^{-1}	(-CH, str)
3	1733cm^{-1}	(-C=O)
4	1338cm^{-1}	(-CH, bending)

3.5.2 Mass-spectroscopy

A reported mass spectrum as well as of isolated hesperidine is shown in fig.3.2. This is the method for determination of molecular weight and formula. In the spectra m+1 peak was found at 611.36

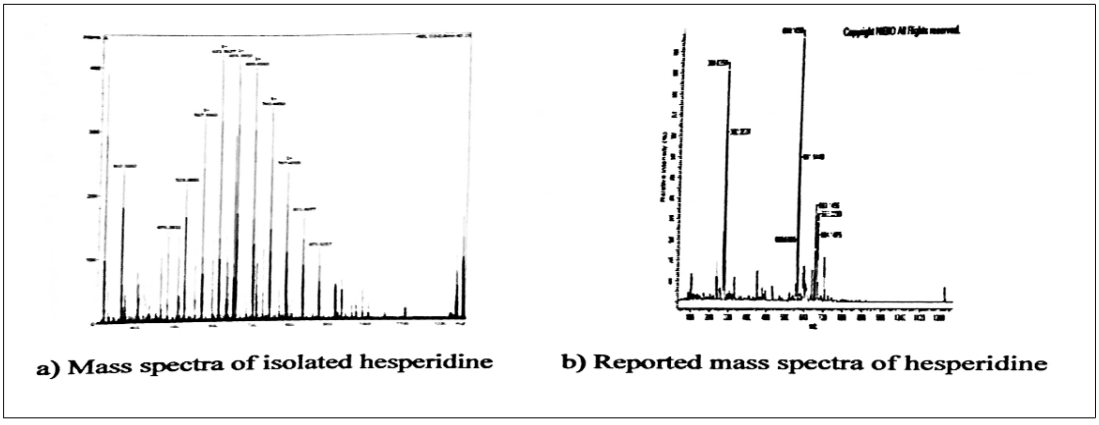
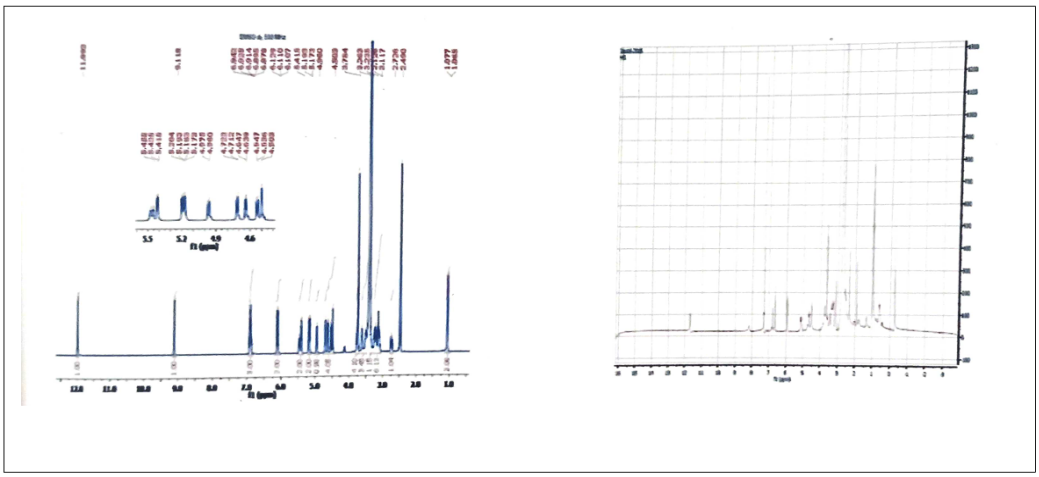


Figure 3.2: Mass spectra of hesperidine

3.5.3 ^{1}HNMR-spectroscopy

A reported ^{1}HNMR spectrum of hesperidine is given in fig, ^{1}HNMR spectra of isolated hesperidine is shown in fig.3.3.



A B

Figure 3.3(A): Reported NMR spectra
Figure 3.4(B): NMR spectra of hesperidine of hesperidine

3.5.4 UV – Visible spectrophotometry

Interpretation of spectra was performed on the basis of maximum absorbance (λmax) and specific absorptivity (A1%; 1cm) of standard and isolated hesperidine i.e. 283cm and 218.5 cm respectively. A spectrum of standard hesperidine is shown in fig.3.5.

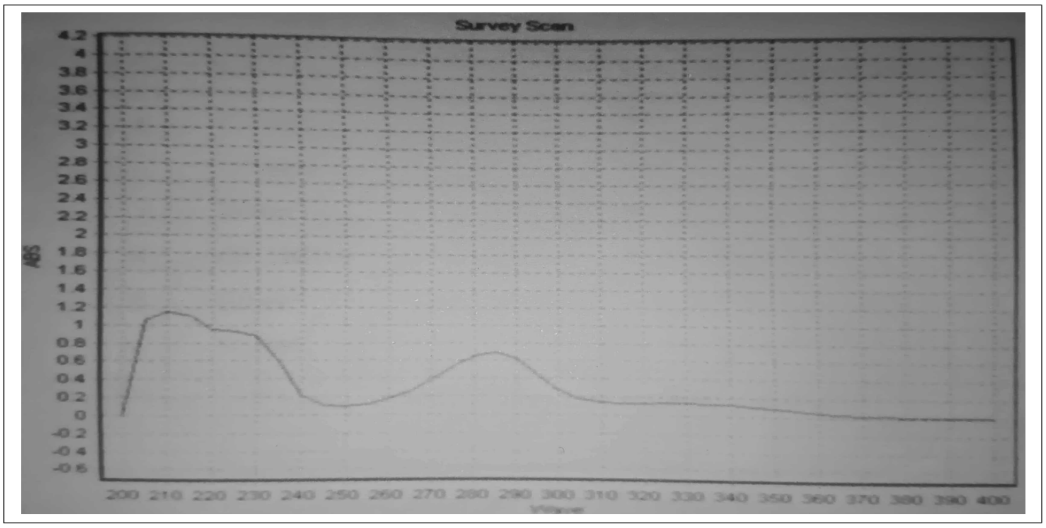


Figure 3.5: Survey scan of hesperidine (Standard)

3.6 Hepatoprotective Activity

The observation of hepatoprotective activity of *Citrus sinensis* peels extract described in the following way.

3.6.1 Biochemical parameters

3.6.1.1 Effect of Citrus sinensis peels extract on SGOT level

In control group treatment with allyl alcohol group in SGOT level as compare to sham group. Treatment with Citrus sinensis at doses 100mg/kg, 200mg/kg and 400mg/kg orally, attenuated the increase in SGOT level dose dependently.

Table 3.6: SGOT level

S. No.	Group Name	Treatment (Name of drug)	Dose	SGOT level (Unit/liter of serum)
1	Group 1	Distilled water	-	37.0±0.2887
2	Group 2	Allyl alcohol	0.4ml/kg	78.50±1.155
3	Group 3	Silymarin	200mg/kg	40.03±0.3180
4	Group 4	AECS	100mg/kg	67.50±1.041
5	Group 5	AECS	200mg/kg	60.13±1.065
6	Group 6	AECS	400mg/kg	48.63±1.090

**Normal range of SGOT level is between 5 to 40 units per liter of serum.

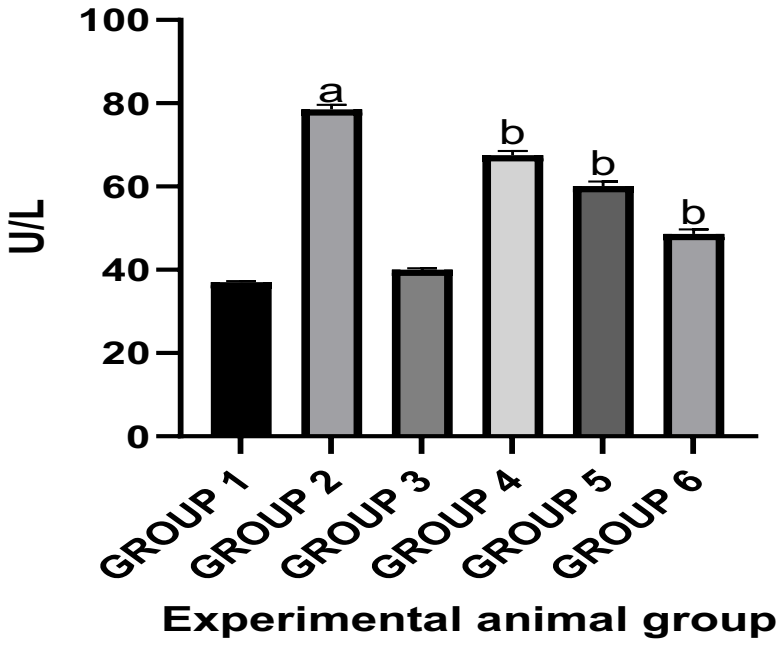


Figure 3.6: **Effect of Citrus sinensis peels extract on SGOT level**

Values are expressed as mean ± S.E.M.
a=*p≤ 0.005Vs sham, b=*p≤0.005 Vs control

3.6.1.2 Effect of Citrus sinensis on SGPT level

In control group treatment with allyl alcohol results in increase in SGPT level as compared to sham group. Treatment with Citrus sinensis at doses (100mg/kg, 200mg/kg and 400mg/kg) orally, attenuated the increase in SGPT level dose dependently.

Table 3.7: SGPT level

S. No.	Group Name	Treatment (Name of drug)	Dose	SGPT level (Unit/liter of serum)
1	Group 1	Distilled water	-	55.23±0.4631
2	Group 2	Allyl alcohol	0.4ml/kg	96.00±0.2887
3	Group 3	Silymarin	200mg/kg	65.97±0.7688
4	Group 4	AECS	100mg/kg	80.97±0.2603
5	Group 5	AECS	200mg/kg	78.57±0.3480
6	Group 6	AECS	400mg/kg	75.93±0.2333

**Normal range of SGPT level is between 7 to 56 units per liter of serum.

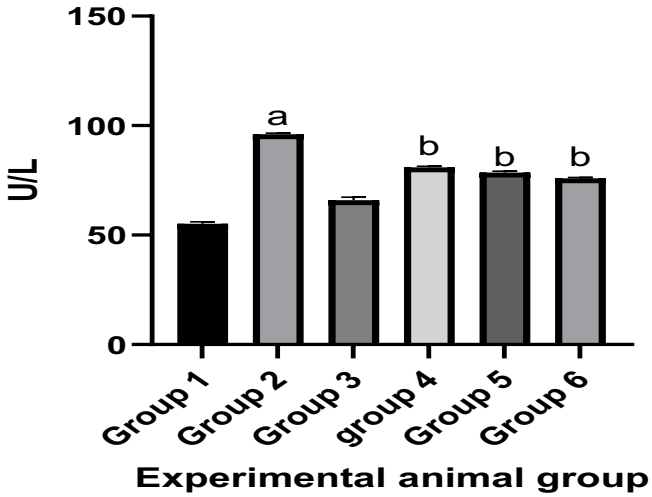


Figure 3.7: **Effect of Citrus sinensis peels extract on SGPT level**

Values are expressed as mean ± S.E.M.
a=*p≤ 0.005Vs sham, b=*p≤0.005 Vs control

3.6.1.3 Effect of Citrus sinensis peels extract on total protein level

In control group treatment with allyl alcohol results in decrease in total protein level as compared to sham group. Treatment with Citrus sinensis at doses (100mg/kg, 200mg/kg and 400mg/kg) orally, increase the decreased level of total proteins.

Table 3.8: Protein level

S. No.	Group Name	Treatment (Name of drug)	Dose	Protein level (grams/deciliter)
1	Group 1	Distilled water	-	7.500±0.2887
2	Group 2	Allyl alcohol	0.4ml/kg	3.200±0.1528
3	Group 3	Silymarin	200mg/kg	5.900±0.3786
4	Group 4	AECS	100mg/kg	3.900±0.3464
5	Group 5	AECS	200mg/kg	5.000±0.2646
6	Group 6	AECS	400mg/kg	5.765±0.4667

**Normal range of protein level is between 6.0 to 8.3 grams per deciliter.

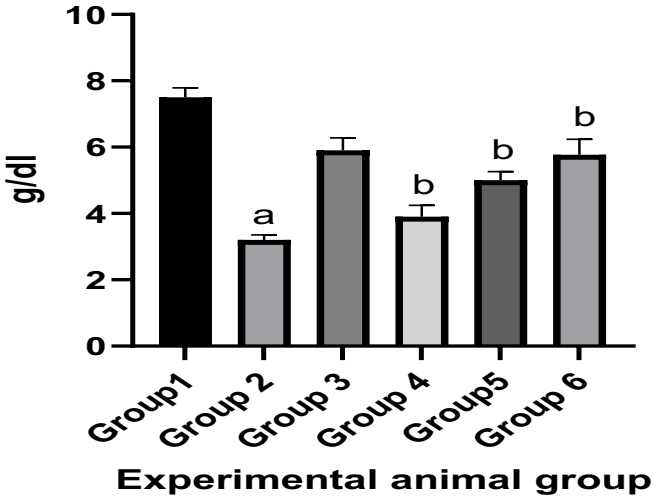


Figure 3.8: **Effect of Citrus sinensis peels extract on protein level**

Values are expressed as mean ± S.E.M.
a=*p≤ 0.005Vs sham, b=*p≤0.005 Vs control

3.6.1.4 Effect of Citrus sinensis peels extract on albumin level

In control group treatment with allyl alcohol results in decrease in albumin level as compared to sham group. Treatment with Citrus sinensis at doses (100mg/kg, 200mg/kg and 400mg/kg) orally, increase the decreased level of albumin dose dependently.

Table 3.9: Albumin level

S. No.	Group Name	Treatment (Name of drug)	Dose	Albumin level (grams/deciliter)
1	Group 1	Distilled water	-	4.400±0.2887
2	Group 2	Allyl alcohol	0.4ml/kg	1.233±0.1453
3	Group 3	Silymarin	200mg/kg	3.967±0.2906
4	Group 4	AECS	100mg/kg	1.933±0.3180
5	Group 5	AECS	200mg/kg	2.867±0.2603
6	Group 6	AECS	400mg/kg	3.567±0.3180

**Normal range of albumin level is between 3.4 to 5.4 grams per deciliter.

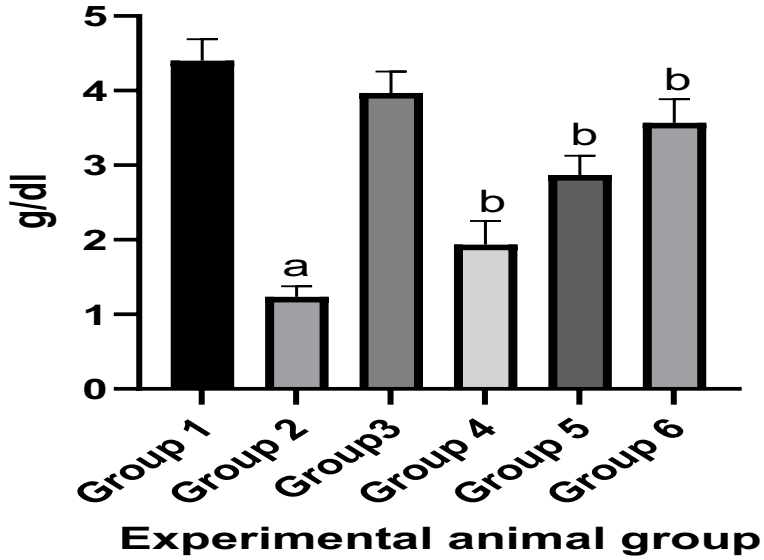


Figure 3.9: **Effect of Citrus sinensis peels extract on albumin level**

Values are expressed as mean ± S.E.M.
a=*p≤ 0.005Vs sham, b=*p≤0.005 Vs control

3.6.1.5 Effect of Citrus sinensis peels extract on phosphate level

In control group treatment with allyl alcohol results in increase in alkaline phosphate level as compared to sham group. Treatment with Citrus sinensis at doses (100mg/kg, 200mg/kg and 400mg/kg) orally, attenuated the increase in alkaline phosphate level dose dependently.

Table 3.10: Phosphate level

S. No.	Group Name	Treatment (Name of drug)	Dose	Phosphate level (Units/liter)
1	Group 1	Distilled water	-	89.50±0.2887
2	Group 2	Allyl alcohol	0.4ml/kg	189.6±0.3480
3	Group 3	Silymarin	200mg/kg	129.5±0.2603
4	Group 4	AECS	100mg/kg	179.6±0.2082
5	Group 5	AECS	200mg/kg	159.6±0.1856
6	Group 6	AECS	400mg/kg	135.5±0.4410

**Normal range of phosphate level is between 44 to 147 Units per liter.

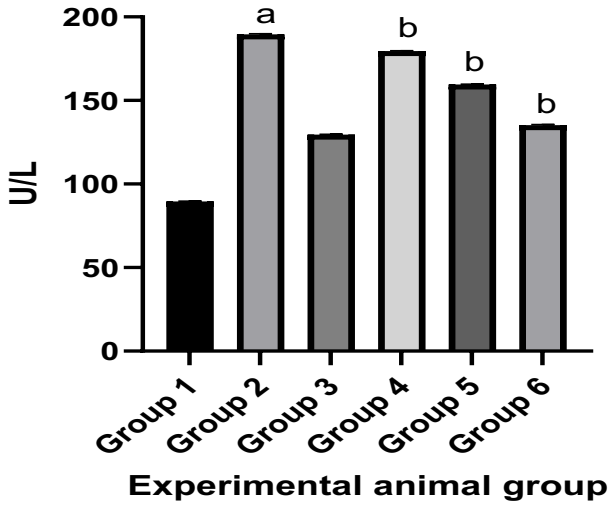


Figure 3.10: **Effect of Citrus sinensis peels extract on phosphate level**

Values are expressed as mean ± S.E.M.

a=*p≤ 0.005Vs sham, b=*p≤0.005 Vs control

3.6.1.6 Effect of Citrus sinensis peels extract on cholesterol level

In control group treatment with allyl alcohol results in increase in cholesterol level as compared to sham group. Treatment with Citrus sinensis at doses (100mg/kg, 200mg/kg and 400mg/kg) orally, attenuated the increase in cholesterol level dose dependently.

Table 3.11: Cholesterol level

S. No.	Group Name	Treatment (Name of drug)	Dose	Cholesterol level (mg/dl)
1	Group 1	Distilled water	-	178.8±0.6009
2	Group 2	Allyl alcohol	0.4ml/kg	299.2±0.4410
3	Group 3	Silymarin	200mg/kg	239.5±0.2887
4	Group 4	AECS	100mg/kg	288.8±0.7265
5	Group 5	AECS	200mg/kg	179.2±0.4163
6	Group 6	AECS	400mg/kg	159.3±0.3712

**Normal range of cholesterol level is less than 200mg/dl.

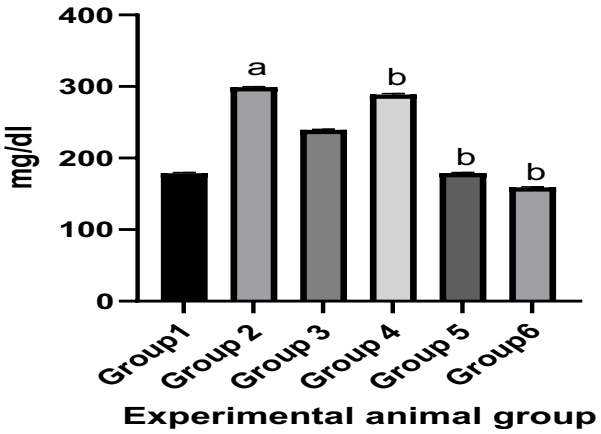


Figure 3.11: **Effect of Citrus sinensis peels extract on cholesterol level**

Values are expressed as mean ± S.E.M.

a=*p≤ 0.005Vs sham, b=*p≤0.005 Vs control

3.6.1.7 Effect of Citrus sinensis peels extract on bilirubin (direct) level

In control group treatment with allyl alcohol results in increase in bilirubin level as compared to sham group. Treatment with Citrus sinensis at doses (100mg/kg, 200mg/kg and 400mg/kg) orally, attenuated the increase in bilirubin (direct) level dose dependently.

Table 3.12 Bilirubin (direct) level

S. No.	Group Name	Treatment (Name of drug)	Dose	Bilirubin (direct) level (mg/dl)
1	Group 1	Distilled water	-	0.1667±0.03333
2	Group 2	Allyl alcohol	0.4ml/kg	3.233±0.1453
3	Group 3	Silymarin	200mg/kg	0.7667±0.O667
4	Group 4	AECS	100mg/kg	2.767±0.1453
5	Group 5	AECS	200mg/kg	2.133±0.2333
6	Group 6	AECS	400mg/kg	1.033±0.2906

**Normal range of bilirubin (direct) level is less than 0.3mg/dl.

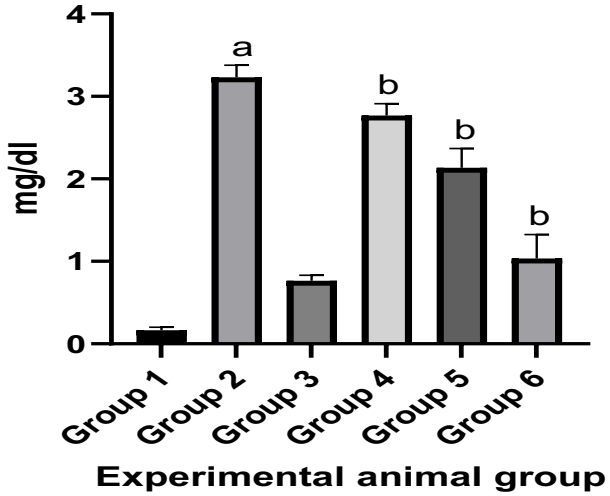


Figure 3.13: **Effect of Citrus sinensis peels extract on bilirubin (direct) level**

Values are expressed as mean ± S.E.M.
a=*p≤ 0.005Vs sham, b=*p≤0.005 Vs control

3.6.1.8 Effect of Citrus sinensis peels extract on bilirubin (total) level

In control group treatment with allyl alcohol results in increase in bilirubin (total) level as compared to sham group. Treatment with Citrus sinensis at doses (100mg/kg, 200mg/kg and 400mg/kg) orally, attenuated the increase in bilirubin (total) level dose dependently.

Table 3.13 Bilirubin (total) level

S. No.	Group Name	Treatment (Name of drug)	Dose	Bilirubin (total)level (µmol/L)
1	Group 1	Distilled water	-	7.300±0.1155
2	Group 2	Allyl alcohol	0.4ml/kg	2.933±0.4702
3	Group 3	Silymarin	200mg/kg	6.133±0.2333
4	Group 4	AECS	100mg/kg	4.300±0.1528
5	Group 5	AECS	200mg/kg	4.700±0.2082
6	Group 6	AECS	400mg/kg	5.433±0.1453

**Normal range of bilirubin (total) level is 1.71 to 20.5µmol/L

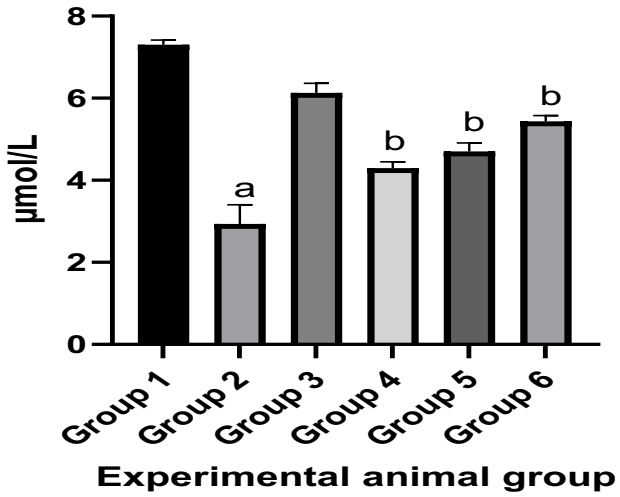
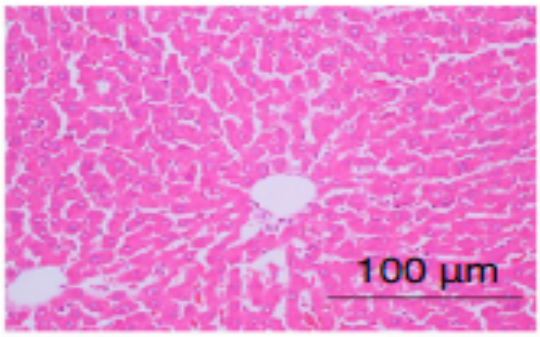


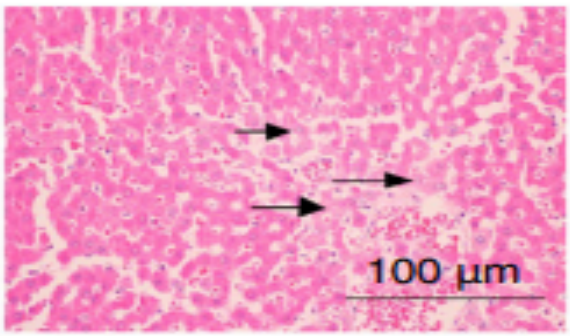
Figure 3.13: **Effect of Citrus sinensis peels extract on bilirubin (TOTAL) level**
Values are expressed as mean ± S.E.M.
a=*p≤ 0.005Vs sham, b=*p≤0.005 Vs control

3.6.2 Histopathological changes in liver

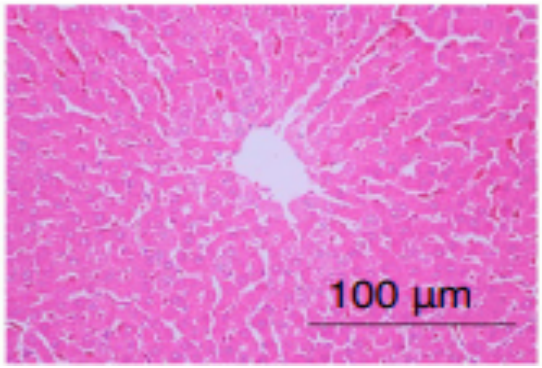
Histopathological examination of liver tissue of rats received distilled water (positive control) showed apparently normal hepatic tissue; the hepatic cells are radially placed and each cell has a large spherical nucleus and granules cytoplasm without any injury (figure a). Liver tissue of rats treated with allyl alcohol revealed congestion of the portal vein with mild to moderate periportal area infiltration with some inflammatory cells mainly macrophages and lymphocytes (figure b). Treatment with Citrus sinensis peels extract at dose of 200mg/kg & 400mg/kg improved liver condition and showed very mild portal vein congestion and leucocytic infiltration with no vacuolation of the periportal hepatocytes (figure d & e). This situation was comparable to that of animal liver treated with Silymarin (200mg/kg) (figure c).



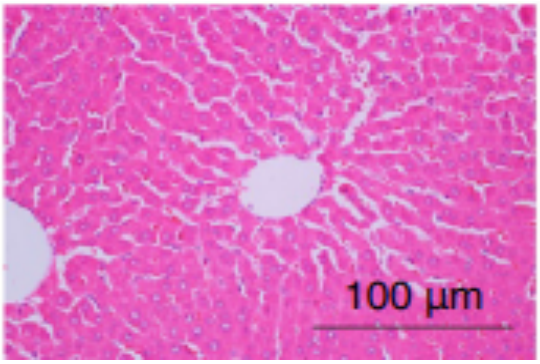
a) Positive control



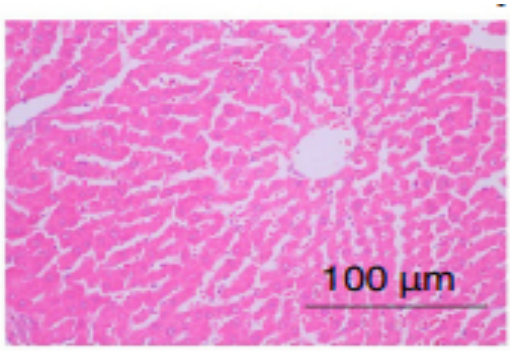
b) Negative control



c) Silymarin 200mg/kg



d) AECS 200mg/kg



e) AECS 400mg/kg

Figure 3.14: Effect Citrus sinensis peels extract on the liver histopathological photomicrographs of the experimental group of rats.

Histopathological photomicrographs of liver of various groups stained with haematoxylin and eosin. (a) Normal architecture of rat liver, (b) Necrosis and hepatocellular fatty degeneration (eccentric nuclei) in allyl alcohol intoxicated liver and congestion of portal vein and periportal infiltration of inflammatory cells, (c) Very lesser damage of hepatocytes and low index of necrosis in Silymarin (200mg/kg) group, narrow arrows refers to inflammatory cells infiltration, wide arrows refers to congestion of portal vein, (d) Improvement in hepatic cells (e) Minimal damage of hepatocytes and very low index of necrosis in *Citrus sinensis* peels extract (400mg/kg) group.

4 Discussion

In the proposed research work the peels of the plant Citrus sinensis were subjected to continuous extraction for the preparation of aqueous extract. The percentage yield of the extract obtained was 27.5%. The evaluation of preliminary phytochemical screening of aqueous extract of Citrus sinensis revealed the presence of phytoconstituents like flavonoids, carbohydrates, reducing sugars, monosaccharide sand steroids.

Important parameters were evaluated like total ash value (12.00% v/w), swelling index (3), foaming index (111.11), volatile matter (4.0% v/w oil in the sample), moisture content (47%), and crude fibre (33.33%).

Isolated hesperidine was characterized by IR-spectroscopy, Mass-spectroscopy, ^{1}HNMR-spectroscopy and UV-Vis. Spectrophotometric methods.

The hepatoprotective activity was screened by allyl alcohol induced hepatotoxicity. Various biochemical parameters like serum glutamic oxaloacetic transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), bilirubin (total), bilirubin (direct), albumin, total proteins, alkaline phosphate, and cholesterol were estimated to determine the functional state of the liver. The hepatoprotective activity was further strengthened by the histological studies of the liver.

The result of screening of hepatoprotective activity indicated the aqueous extract possess significant hepatoprotective activity at the dose of 400mg/kg body weight.

References

1. Elias H.; Bengelsdorf H.; "The Structure of the Liver in Vertebrates"; Cells Tissue Organs; 1 July 1952; 14 (4): 297-33.
2. Abdel Misih; Sherif R.Z.; Bloomston Mark; "Liver Anatomy"; Surgical Clinics of North America; 2010; 90(4): 643-653.
3. "Anatomy and Physiology of the Liver – Canadian Cancer Society"; Cancer. ca. Archived from the original on 2015-06-26.
4. Perea, V., (2016-17). Citrus sinensis monograph. Agricultural science 2016-17.
5. Sissayb.Mecbib, ThierryJ, RegnierC, and LiseKorsten, Citrus sinensis disease survey: knowledge, attitude and management practices in Ethiopia, Tropical research journal 2006; 4: 1-9.
6. Parle Milind and Chaturvedi Dev "Orange: Range of Benefits"; International Research Journal of Pharmacy; 2012; 3(7): 59-62.
7. Khandelwal CK.; "Practical Pharmacognosy"; 19th Edition; Pune: Nirali Prakashan; 2009; P. 25.1.
8. Mukherjee P.K.; "Quality control of herbal drugs: an approach to evaluation of

botanicals"; New Delhi; Business Horizons; 2007; P. 183-221.
9. Kokate CK.; "Practical Pharmacognosy"; 4th Edition; New Delhi; Vallabh Prakashan; 2008; P.147.
10. Sorg, D. A., Buckner, B., 1964. A Simple method for obtaining venous blood from small laboratory animals, Proceed. Soc. Exp. Biol. Med. 115(4), 1131-1132.
11. Retiman, S., Frankel, S., 1957 July 1. A colorimetric method for determination for serum glutamic oxaloacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathol. 28(1), 56-63.
12. Bancroft, John D., Gamble, Marilyn (Eds.), 2008. Theory and practice of Histological Techniques, Elsevier health sciences.