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PHYTOCHEMICAL INVESTIGATION AND PHARMACOLOGICAL EVALUATION OF *BOUGAINVILLEA SPECTABILIS* FOR HEPATOPROTECTIVE ACTIVITY

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ABSTRACT

The presence study aimed to evaluate the Hepatoprotective effect of Bougainvillea spectabilis stems extract against liver injury induced by carbon tetrachloride (CCl_4). Both male and female wistar albino rats were used, rats were injected i.p. with CCl_4 (1ml/kg) mixed in liquid paraffin in portion (1:1), three times in a seven days and treated orally with Bougainvillea spectabilis (100 mg/kg and 200 mg/kg) stems extract daily for seven days and compared with a group of rats injected i.p. with CCl_4 (1ml/kg) mixed in liquid paraffin in portion (1:1), three times in a seven days. Bougainvillea spectabilis plant belonging to family Nyctaginaceae used in various diseases like Hepatitis.(Leaves), Diarrhea, Inflammation, Stomach acidity, Cough, sore throat and other respiratory diseases, Leucorrhea, Ulcer, Microbial infection, Diabeties, Hyperlipidemia, Cancer, It have anti-fertility property. Carbon tetrachloride caused a significant elevation in enzyme levels such as AST, ALT, Total bilirubin and Total protein, this indicated the damaged structural integrity of liver, Due to Carbon tetrachloride inducing agent the levels of SGOT, SGPT, Total bilirubin were elevated and the total protein levels was decline than normal. The pre-treatment of B.S.Eth and B.S.Aqs stems extracts at dose levels of 100 and 200 mg/kg were shown a restored the ALT, AST, Total bilirubin and Total protein levels towards normalization and the effects were comparable with standard drug (Silymarin 100 mg/kg). Histopathological evaluation of livers revealed that the B.S. Eth and B.S.Aqs stems extracts reduced inflammation of hepatocytes, swelling, necrosis and no. liver lesions induced by CCl_4.

KEYWORDS: Bougainvillea spectabilis, CCl_4, AST, ALT, Hepatitis.

INTRODUCTION

Liver is vital organ which plays important role in metabolism, storage, detoxification, synthesis and regulation of various body processes.[7] Liver is largest and heaviest gland of the body weighing about 1.4 kg. In the average adult it is second largest organ of the body located in the diaphragm and occupies most of right hypochondrium and part of epigastrium of the abdomen.

The causes of liver disease are viruses, excessive drug therapy, environmental pollution, alcoholic intoxification etc. Liver receive blood supply from hepatic artery(20%) and portal circulation (80%) up to 20-25% of total cardiac output. Toxin, infectious agent medication and serum inflammatory mediator enter into the liver through the blood, may result in diverse range of disease processes, causing the loss of normal histological architecture reduced cell mass and loss of blood flow this may lead to decline liver function.

No effective hepatoprotective therapy is available. Conventional medicines used in liver treatments are often insufficient. Many chronic irreversible and acute hepatic disorders culminate in ultimately death due to lack of adequate remedies in modern medicines. It is therefore necessary to search for alternative drugs for treatment of liver diseases to replace currently used drugs of controversial efficacy and safety. In this condition there is greater demand of herbal formulation to treat liver diseases in developed as well as in developing countries for primary health care. Herbal medicines have minimum side effects, good biological activity, medicinal property, large safety margin and minimal cost. Modern drug are little to offer for curing of hepatic disorder, whereas most important representative of phytoconstituents used to treat liver disorder depend on the region are *Silyrium* (*Silybum marianum*) and catechin (*Anacardium occidentalis*) in Europe, glycyrrhizin (*Glycyrrhizaglabra*)in Japan and chizandrins(*Schizandra chinesis*) in China.

Bougainvillea spectabilis (Family-Nyctaginaceae) is referred as Paper flower, native to tropical south America, it grow around the world. It is known as Booganbel in hindi, Booganvel in Marathi and Kagithala puvvu in telugu.

It is an ornamental climber and shrub.[5] It's bracts are thin and papery. The purple or magenta colour is the most common Bougainvillea colour, The stem is a woody perennial vine, with multi-trunked and large clumping stems which spread up to 2-4 m. During the growth, the color of the stems turns from mid-green to dull green-brown. The bark is pale and

corky. The leaf is 5-10 cm long and 2-6 cm wide, with ovate to rounded shapes. Leaves are deep green, leathery in texture and hairy underneath. A cluster of three flowers arise in leaf axils. They are cream in color, small, slender, with hairy tubes and surrounded by showy. The fruit is an elongated five-lobed achene less than 1-2 cm long. It is rather inconspicuous, not showy, and has a dry, hard fruit cover.

Bougainvillea spectabilis contains alkaloids, flavonoids, furanoids, glycosides, phenols, phlobotannins, quinones, saponins, steroids, tannins and terpenoids which were extracted from stem, flowers and leaves of B. spectabilis. The other active constituents are bougainvinones peltogynoids, essential oils including methyl salicylate, terpinolene, α-(E)-ionone, pinitol, β-sitosterol, quercetin, and quercetin-3-O-rutinoside. In addition, the phytochemical constituents of B. spectabilis leaf extract revealed that tannins (27.64%), saponins (14.08%), glycosides (11.49%), flavonoids (10.05%), alkaloids (4.10%), phytate (49.27%) and oxalate (27.65%) contents are present.[4]

Bougainvillea spectabilis Willd is used in folk medicine for treatment of diarrhea, and to reduce stomach acidity, it is also used for cough, sore throat and other respiratory problems. For leucorrhea, flower is used, The plant leaves used as anti-diarrheal, Hepatoprotective, anti-ulcer and anti-microbial, as anti-diabetic due to the presence of pinitol which has insulin like activity and as anti-hyperlipidemic. Also it was proved to have anti-fertility activity. The stem bark of B. spectabilis shows cytotoxic effect upon five cancer cell lines which included KB, Hela S-3, HT-29, MCF-7 and HepG2.

MATERIALS AND METHODS

Collection, Identification & Authentication of plant material

Fresh stems of *Bougainvillea spectabilis* Willd. were collected from local market of Nanded and identified on the basis of its morphological features with the help of taxonomist. Herbarium of the plant specimen was authenticated by Dr. S. S. Bodke (Associate Professor & Head, Department of Botany & Horticulture, Yeshwant College, Nanded) which has been submitted to Nanded Pharmacy College, Nanded with specimen no: NPC/Herbarium/2018-2019.H-1 and authenticated as *Bougainvillea spectabilis* Willd. Stem (family-Nyctaginaceae).

Preparation of extract

The collected stems were dried under shade, segregated and further crushed to coarse powder by mechanical grinder and the powder was passed through sieve no. 10 and 40. the dried powdered stems of *Bougainvillea spectabilis* (300g) were first defatted with petroleum ether (60-80°c) and later extracted with ethanol by continuous hot extraction method in soxhlet apparatus and lastly extracted with water by using maceration with heat and agitation method. the extracts obtained were subjected to standardization and then utilized for evaluating in-vitro anti-oxidant and in-vivo hepatoprotective activity.

Phytochemical evaluation

Total Phenolic assay[9,19]

The total phenolic content of all the three extract of herbs was determined by using Folin-Ciocalteu method. A standard gallic acid curve was constructed by preparing the aliquot of (20, 40, 60, 80, and 100µg/ml) in methanol from standard 1 solution of gallic acid was added to 10 ml of volumetric flask, containing 3 ml of distilled water. A reagent blank using distilled water was prepared. 0.5 ml of Folin - Ciocalteu phenol reagent was added to the mixture and shaken. After 5 minutes 5 ml of 7% Na2CO3 solution was added to the mixture. The volume was then made up to the mark. After incubation for 90 minutes at room temperature, the absorbance against the reagent blank was determined at 765 nm with an UV-Visible spectrophotometer. Total phenolic content was expressed as mg Gallic acid Equivalents (mgGAE/g). All the experiments were performed in triplicate.

Flavonoid Assay[9,19]

Total flavonoid content was measured by the aluminium chloride colorimetric assay. Rutin was used as standard and flavonoid content was determined as Rutin equivalent. A calibration curve for rutin was drawn for this purpose. An aliquot (1ml) of extracts or standard solutions of rutin (20, 40, 60, 80 and 100 µg/ml) was added to 10 ml volumetric flask containing 4 ml of distilled water. To the flask was added 0.30 ml 5% NaNO2, after five minutes 0.3 ml 10% AlCl3 was added. After five minutes, 2 ml 1M NaOH was added and the volume was made up to 10 ml with distilled water. The solution was mixed and absorbance was measured against the blank at 510 nm. Appearance of pink colour showed the presence of flavonoids content. The total flavonoid content was expressed as mg rutin equivalents (RuE).

In-vitro Antioxidant activity DPPH free radical-scavenging assay[11,22]

The *Bougainvillea spectabilis* stems extracts capability to scavenge DPPH radicals was measured according to the method as previously described with some modifications. Antioxidants react with 1, 1- diphenyl-2-picryl-hydrazyl (DPPH) radical and convert it to 1, 1-diphenyl -2-picryl hydrazine. The degree of change in colour from purple to yellow can be used as a measure of the scavenging potential of antioxidant extracts. Initially, absorbance of DPPH solution (0.1 mM in methanol) was measured at wavelength 514 nm. Various concentrations of extracts (25,50,75,100 & 125µg/ml) were mixed with 1 ml of 0.1 mM DPPH radical solution in methanol and made up final volume of 10 ml with methanol. A plane sample was considered as a control. The mixture was shaken vigorously and incubated in the dark for 90 min at room temperature. For all the experiment each concentration of assay was run in triplicate. Gallic acid, ascorbic acid and rutin was used as a standard. The absorbance was measured at wavelength 514 nm by using uv visible spectrophotometer. The percent radical scavenging activity of tested samples was expressed by using following formula.

$$\% \text{ scavenging activity} = \frac{\text{Control absorbance} - \text{Test absorbance}}{\text{Control absorbance}} \times 100$$

Hepatoprotective activity

Hepatoprotective activity of *Bougainvillea spectabilis* Willd stems extracts was carried out using CCl_4 induced hepatotoxicity in albino wistar rats.

Selection and procurement of animals

The experiment was performed with the approval of Institutional Animal Ethics Committee (IAEC) following guidelines of CPCSEA. The male and female wistar rats with 150-200 g body weight was selected for study by using CCl_4induced hepatotoxicity model.

Housing facilities

The animal selected for experimental purpose maintained with standard procedure of laboratory condition in animal house of Nanded pharmacy college approved by the committee for purpose of control and supervision on experiments on animal (CPCSEA). All animal was placed in 12 hrs light /dark cycle with maintained temperature condition (22 ± 2^{o}c), feed with commercial pellet diet and water ad libitum. All animal under experiment were placed in the maintained animal house of college for at least 1 week before the start of

experiment. The experimental protocol for the study was followed according to the norms of Institutional Animal Ethics Committee.

Selection of the dose

CCl_4 : 1ml /kg

Silymarin : 100 mg/kg

Route : Oral, Intraperitoneal, Intravenous.

Sex : Male /female

Model : CCl_4 induced hepatotoxicity

Experimental design

Male or female wistar rats about 150 and 200 g body weight were selected for present study. The animals was divided into seven groups, six rats in each and subjected to the following treatments.

Grouping[18]

- Group I (Negative control) vehicle treated.
- Group II (Positive control) treated with CCl_4 dose (1ml/ kg)+liq.Paraffin (1:1).
- Group III treated with standard drug (Silymarin 100 mg/kg) for seven days.
- Group IV Test group 1(B.S.Eth 100 mg/kg) for seven days.
- Group V Test group 2 (B.S.Eth 200 mg/kg) for seven days.
- Group VI Test group 3 (B.S.Aqs 100mg/kg) for seven days.
- Group VII Test group 4 (B.S.Aqs 200mg/kg) for seven days.

Procedure[18]

- Male or female wistar rats weighing about 150 and 200 g were selected for the study.
- Animal were randomly divided into seven groups containing six animals in each group.
- Group I was Negative control group without treatment.
- Group II was treated with the CCl_4+ liq. Paraffin on 1^{st}, 4^{th}, 7^{th} day.
- Group III, IV, V, VI, VII were subjected treatment for 7 days.
- On 1^{st}, 4^{th}, 7^{th} day the test drug administered to the animal and after 1 hour CCl_4 (1ml/kg) in liquid paraffin in 1:1 proportion was given by intraperitoneal route.
- 24 hrs after CCl_4 treatment (8^{th} day) of the experiment, Blood (2-3ml) samples was collected in blood collecting tube from the retro orbital plexus of all the rat, under light anesthesia using ketamine+Xylazine (0.15ml of mixture per 100gm body weight) and

blood sample will collected and serum will separated after coagulating at 37º C for 30min. and centrifugation at 1200-1500 rpm for 15-20 min. and analysis for Serum Glutamate Pyruvate Transaminase (SGPT/ALT), Serum Glutamate Oxaloacetate Transaminase (SGOT/AST), Total Bilirubin, Total protein were estimated in blood serum by using Auto analyzer.

- Animal were sacrificed by using co_2 chamber and under the Euthanasia livers from animal were removed off, washed with saline solution, collected, preserved in 10% formalin and Histopathological study carried out**.**

Evaluation procedure for biochemical parameter

The blood was collected by puncturing retro orbital plexus under light anesthetia using ketamine+Xylazine. The blood was allowed to clot at room temperature for 30 min at 37^{o}c and then centrifuged at 1200-1500 rpm for 15-20 min. The hemolysed free serum sample were used for determination of biochemical parameters as per the standard procedure prescribed by the manufacturer's instruction manual provided in kit of Auto analyzer. Evaluation was carried out by estimating parameter such as SGOT, SGPT, Total protein, and Total bilirubin by using enzymatic kit.

Procedure for histopathology

The animal selected for protective study small portion of the median lobe of the liver was trimmed to a thickness of 3 mm. Then it was placed in plastic cassettes and fixed in 10% neutral buffer formalin solution for 24 hrs. The remaining livers were stored at -20^0 c for biochemical analysis. The washed tissue was dehydrated in descending grads of isopropanol and finally cleared in xylene. The tissue was embedded in molten paraffin wax. Sections were cut at 5 µm thickness deparaffinised and rehydrated using standard techniques, and the sections were stained with haematoxylin and eosin. The sections were then viewed under microscope for histopathological changes. The extent of carbon tetrachloride induced degeneration, fatty changes, necrotic changes was evaluated by assessing morphological changes in liver section using standard techniques.

Statistical analysis

The data were expressed as mean $\pm$ standard of mean (SEM).Statistical analysis were performed by one way analysis of variance (ANOVA).

RESULTS

Table 1: Phytochemical evaluation of *Bougainvillea spectabilis* stems extracts.

Chemical test	Petroleum ether	Methanol	Aqueous
Alkaloids	+	+	+
Flavonoids	+	+	+
Glycosides	-	+	-
Steroids	-	+	+
Protein	+	+	+
Carbohydrates	-	+	+
Tannin & Phenolic Compound	+	+	+

Table 2: TLC Analysis of *Bougainvillea spectabilis* stems Extracts.

Extracts	Petroleum Ether	Ethanol	Aqueous
Mobile Phase	Ethyl acetate : Methanol : Water (7 : 2 : 1)	Ethyl acetate : Methanol : Water (7 : 2 : 1)	Benzene: Chloroform : Methanol (4 : 4 : 2)
			
Rf Value	0.13 (Brown) 0.16 (Yellow) 0.22 (Green) 0.60 (Grey) 0.73 (Black)	0.08 (Brown) 0.10 (Grey) 0.16 (Green) 0.30 (Light green) 0.35 (Yellow) 0.47 (Purple) 0.66 (Black) 0.94 (Light orange)	0.11 (Brown) 0.15 (Black) 0.20 (Dark red) 0.25 (Grey) 0.35 (Purple) 0.56 (Light green) 0.58 (Orange) 0.70 (Violet) 0.81 (Yellow) 0.85 (Green) 0.95 (Pink)

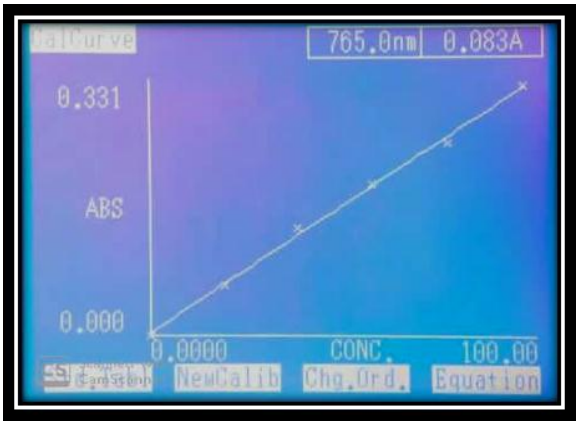
Estimation of phenols, flavonoids contents

Ethanolic extract contained more phenolic (59.39 mg GAE/g DW) and flavonoids (62.43 mg RU/g DW) as compared with aqueous (32.42 mg GAE/g DW, 52.19 mg RU/g DW) and petroleum ether (27.27 mg GAE/g DW, 40.00 mg RU/g DW) respectively.

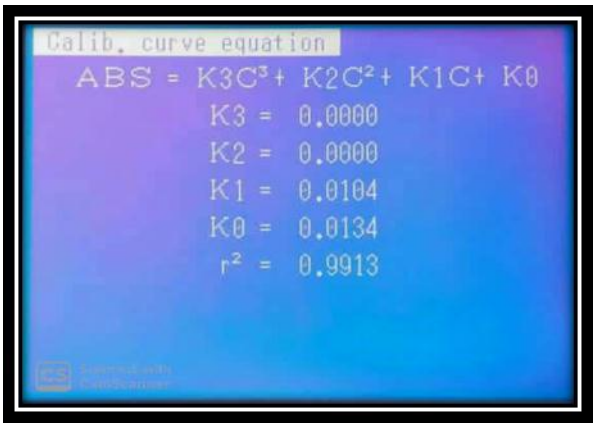
Table 3: Total phenolic and flavonoids content of *Bougainvillea spectabilis* stems extracts.

Sr. No.	Conc. µg/ml	Extracts	Phenolic content (mg GAE/g DW)	Flavonoid content (mg RU/g DW)
1	100	Petroleum ether	27.27 ± 0.16	40.00 ± 0.23
2	100	Ethanol	59.39 ± 0.19	62.43 ± 0.17
3	100	Aqueous	32.42 ± 0.27	52.19 ± 0.12

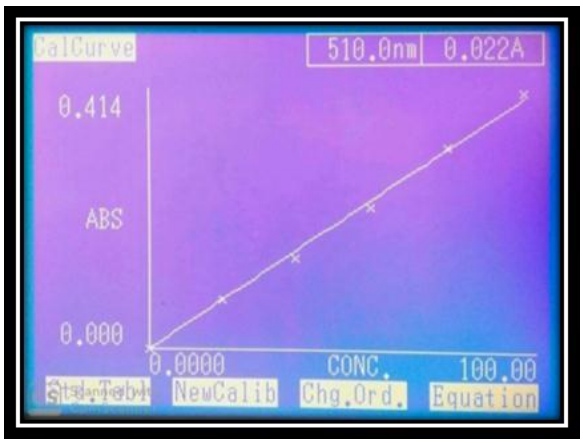
Note: GAE/g DW and RU/g DW denotes Gallic Acid Equivalent per gram dry weight, Rutin Equivalent per gram dry weight respectively.



Graph 1. Calibration curve of Gallic acid



Calibration curve equation for Total Phenolic Content



Graph 2. Calibration Curve of Rutin

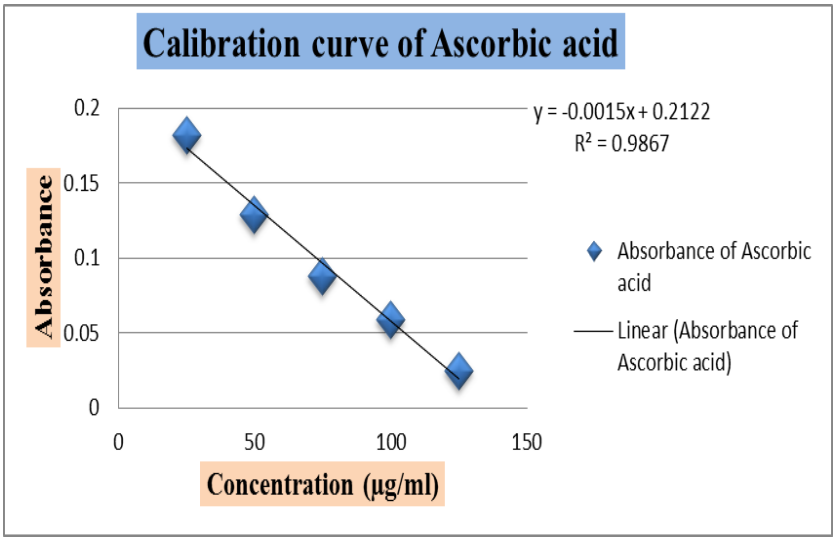
Calibration curve equation for Total Flavonoid

Estimation antioxidant activity

Antioxidant activity shows ethanolic extract inhibit more oxidative radicals at 25, 50, 75, 100 and 125 mg/kg (59.34%, 70.84%, 80.08%, 87.26%, 91.20%) than aqueous (57.08%, 61.19%, 77.41%, 83.20%, 89.80%) and petroleum ether extracts (54.62%, 58.93%, 70.84%, 75.00%, 77.41%) respectively, when compared with ascorbic acid are shown in Table 5.

Table 4: Total Antioxidant Content of Standard.

Sr. No.	Conc. µg/ml	Ascorbic acid % inhibition	Gallic acid % inhibition	Rutin % inhibition
1	25	62.62 ± 0.23	41.06 ± 0.33	41.47 ± 0.19
2	50	73.51 ± 0.22	54.00 ± 0.26	51.54 ± 0.17
3	75	81.93 ± 0.21	76.18 ± 0.27	74.53 ± 0.25
4	100	87.88 ± 0.04	80.90± 0.27	79.05 ± 0.31
5	125	95.07 ± 0.25	91.17 ± 0.28	93.42 ± 0.31



Graph 3: Calibration curve of Ascorbic acid.

Table 5: Total Antioxidant Content of *Bougainvillea spectabilis* stems Extracts.

Sr. No.	Conc. µg/ml	Petroleum ether % inhibition	Ethanol % inhibition	Aqueous % inhibition	Ascorbic acid % inhibition
1	25	54.62 ± 0.18	59.34 ± 0.35	57.08 ± 0.37	62.62 ± 0.23
2	50	58.93 ± 0.34	70.84 ± 0.06	61.19 ± 0.30	73.51 ± 0.22
3	75	70.84 ± 0.29	80.08 ± 0.25	77.41 ± 0.38	81.93 ± 0.21
4	100	75.00± 0.36	87.26 ± 0.31	83.20± 0.34	87.88 ± 0.04
5	125	77.41 ± 0.27	91.20 ± 0.31	89.80 ± 0.33	95.07 ± 0.25

Estimation of *Bougainvillea spectabilis* stems on Hepatoprotective activity

The effects of oral administration of Bouhainvillea spectabilis on liver structure and biochemical parameters such as SFOT, SGPT, Total bilirubin, Total protein in the Wistar rats DST are shown in images given below and Table 6. Administration of Carbon tetrachloride caused a significant elevation in enzyme levels such as AST, ALT, Total bilirubin and Total protein, this indicated the damaged structural integrity of liver, Due to Carbon tetrachloride inducing agent the levels of SGOT, SGPT, Total bilirubin were elevated and the total protein levels was decline than normal. The pre-treatment of B.S.Eth and B.S.Aqs stems extracts at dose levels of 100 and 200 mg/kg were shown a restored the ALT, AST, Total bilirubin and

Total protein levels towards normalization and the effects were comparable with standard drug (Silymarin 100 mg/kg).

Histopathological evaluation of livers revealed that the B.S.Aqs and B.S.Eth stems extracts reduced inflammation of hepatocytes, swelling, necrosis and no. liver lesions induced by CCl_4.

Post hoc analysis Tukey's multiple comparisons test found that B.S.Eth 200 mg/kg and B.S.Aqs 200mg/kg has significant difference when compared with positive control and has no significance difference when compared with Silymarin (standard) means it act like standard. The results indicated that Bougainvillea spectabilis showed significant Hepatoprotective -like effects.

Table 6: All Biochemical parameter.

Sr. no.	Groups	Total Bilirubin (mg/dl)	Total Protein (g/dl)	SGOT (U/L)	SGPT (U/L)
1	Group I (Negative control)	0.43±0.03	7.72±0.17	27.17±1.76	30.30±0.35
2	Group II (Positive control)	7.19±0.18	1.03±0.08	103.29±4.44	87.39±0.53
3	Group III (Std. Group)	0.55±0.01**	7.55±0.18**	32.52±0.61**	32.16±0.19**
4	Group IV (B.S.E.E. 100mg)	1.21±0.12**	6.53±0.14**	43.08±0.54**	36.16±0.43**
5	Group V (B.S.E.E 200mg)	0.56±0.02**#	7.03±0.20**#	35.61±0.34**#	33.84±0.19**#
6	Group VI (B.S.A.E 100mg)	2.36±0.22**	4.56±0.23**	63.41±0.96**	43.65±1.47**
7	Group VII (B.S.A.E 200mg)	0.77±0.01**#	6.78±0.15**	41.93±0.28**	34.33±0.33**#

Each value represents the mean ± S. E. M. (n=6), P < 0.05: when compared to control (One way ANOVA followed by Dennett's test),*-Significant difference (P<0.05), when test and standard compared with positive control; **-Highly significant difference (P<0.001), when test and standard compared with positive control;#- No significant difference, when test is compared with standard,$- Significant difference, when test is compared with standard but more activity than standard.

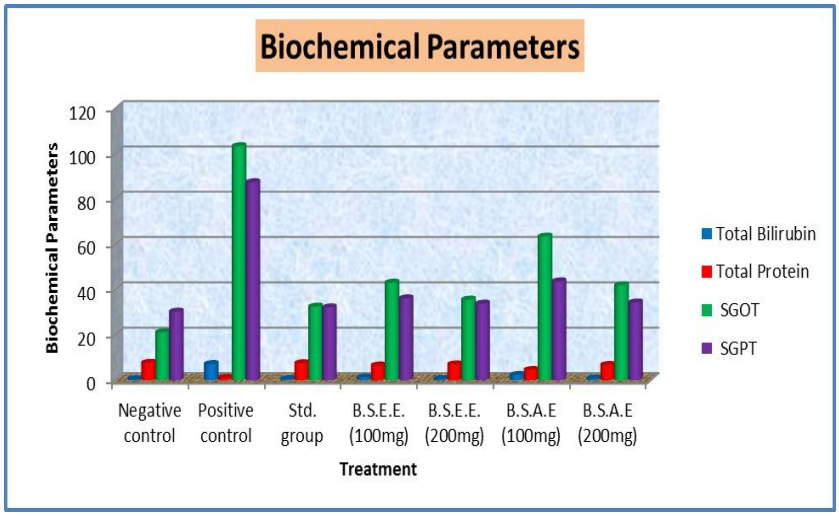


Chart 1: Biochemical Parameters of all groups.

Gross anatomy of liver

Group I: Negative control: Section shows normal architecture of liver (dark radish brown in colour) which was vehicle treated.

Group II: Positive control (CCl_4 treated): Section shows patches of liver cell necrosis with inflammatory collections.

Group III: Standard (Silymarin): liver shows almost near normal

Group IV: Test 1 (B.S.Eth 100 mg/kg): liver shows normal architecture with moderately damaged cell.

Group V: Test 2 (B.S.Eth 200 mg/kg): Test drug shows protective effect on liver**.**

Group VI: Test 3 (B.S.Aqs 100 mg/kg): liver shows normal architecture with some damage to cell.

Group VII: Test 4 (B.S.Aqs 200 mg/kg): Test drug shows protection effect on liver**.**

Image. Negative control

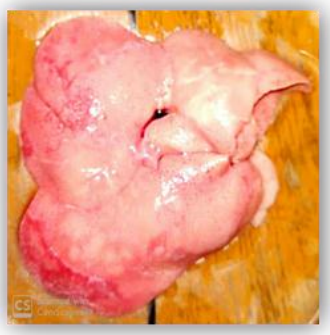


Image. Positive control

Image. Standard



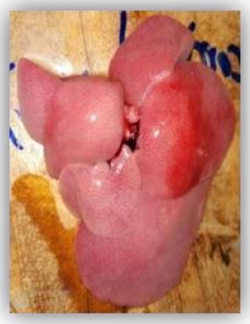



Image. B.S.Eth 100 mg/kg | **Image. B.S.Eth 200 mg/kg** | **Image. B.S.Aqs 100mg/kg** | **Image. B.S.Aqs 200mg/kg**

Histopathological evaluation of B.S.Eth and B.S.Aqs extract for hepatoprotective activity

Group I: Negative control: Section shows central vein surrounded by hepatic cord of cells (normal architecture).

Group II: Positive control (CCl_4 treated): Section shows destruction of normal structure, liver cell necrosis and (ballooning) fatty changes.

Group III: Standard (Silymarin): Section shows almost near normal

Group IV: Test 1 (B.S.Eth 100 mg/kg): liversection shows liver abscess is commonly solitary and irregular cell wall with (ballooning) necrosis

Group V: Test 2 (B.S.Eth 200mg/kg): liversection shows normal architecture of cell with irregular necrotic ballooning cell and protective effect against toxicant.

Group VI: Test 3 (B.S.Aqs 100 mg/kg): Section shows most of hepatocytes are distended with large lipid vacuoles with peripherally displaced nuclei, and necrosis.

Group VI: Test 4 (B.S.Aqs 200 mg/kg): Section shows normal architecture with mild necrosis**,** ballooning of cell and protective effect against toxicant.

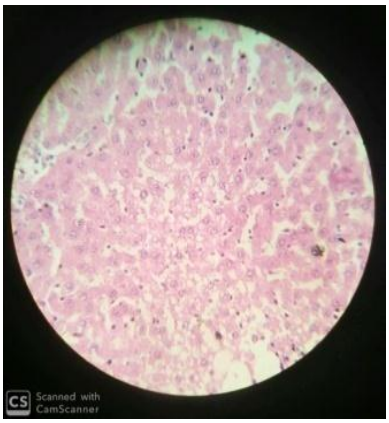
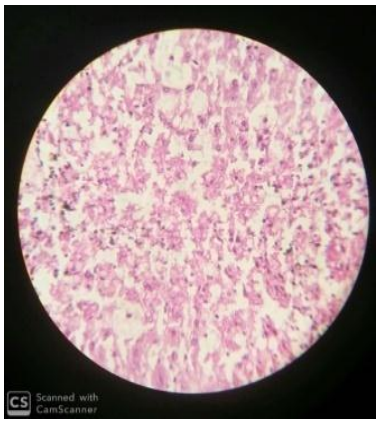
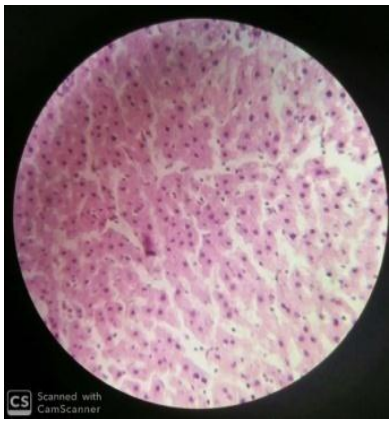




Image. Negative control | **Image. Positive control** | **Image. Standard**

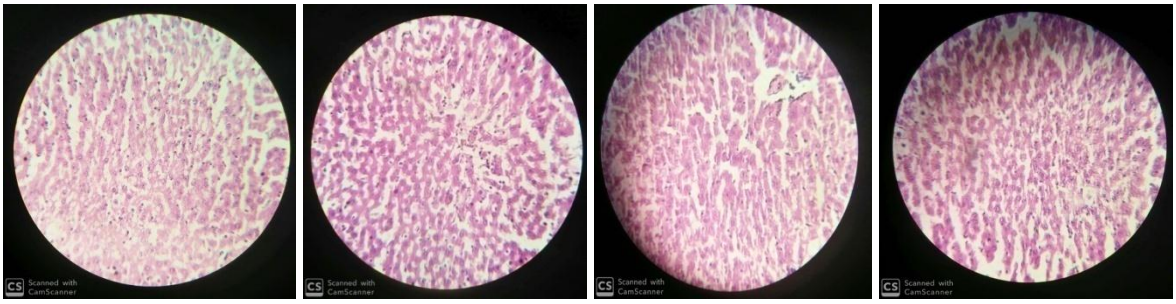


Image. B.S.Eth 100 mg/kg | **Image. B.S.Eth 200 mg/kg** | **Image. B.S.Aqs 100mg/kg** | **Image. B.S.Aqs 200mg/kg**

DISCUSSION

The liver is a major target organ for toxicity of xenobiotics and drugs, because most of the orally ingested chemicals and drugs firstly passes through the liver where they metabolized into inactive and toxic intermediates. At present, drug or chemical-induced liver injury has become a major clinical problem. Much attention should be paid to the mechanisms involving drug or chemical-induced liver injury. In addition, the search for effective therapeutical methods for the treatment of drug or chemical induced liver injury is also very important. Liver injury induced hepatotoxicity and is commonly used model for screening hepatoprotective drugs.

Liver injury due to carbon tetrachloride in the rats was first reported in 1936 and has been widely and successfully used by many investigators. Carbon tetrachloride is metabolized by cytochrome P-450 in endoplasmic reticulum and mitochondria with the formation of $CCl_3O_3^-$, reactive oxidative free radical, which initiates lipid peroxidation. Administration of single dose of CCl_4 to a rat within 24 hours produces a centrilobular necrosis and fatty changes. The toxicant reaches its maximum concentration in the liver within 3 hours of administration.

AST/SGOT predominantly found in mitochondria of hepatocytes. ALT/SGPT is more specific to the liver, is one of the most sensitive tests employed in the diagnosis of hepatic diseases and thus it is a better parameter for detecting liver injury. The AST/SGOT, ALT/SGPT, Total Bilirubin and Total protein levels are largely used as most common biochemical markers to evaluate liver injury.

Administration of Carbon tetrachloride caused a significant elevation in enzyme levels such as AST, ALT, Total bilirubin and Total protein, this indicated the damaged structural integrity of liver, because they are cytoplasmic in the location and released into circulation after cellular damages indicating development of hepatotoxicity. SGPT has comparatively

more activity in liver tissue. The increased activities in liver damage are due to necrotic or damaged hepatocytes and the enzyme is sensitive to hepatic dysfunction. Due to Carbon tetrachloride inducing agent the levels of SGOT, SGPT, Total bilirubin were elevated and the total protein levels was decline than normal. The pre-treatment of B.S.Eth and B.S.Aqs stems extracts at dose levels of 100 and 200 mg/kg were shown a restored the ALT, AST, Total bilirubin and Total protein levels towards normalization and the effects were comparable with standard drug (Silymarin 100 mg/kg).

The serum bilirubin increased due to large number of chemicals, drugs and diseases. Carbon tetrachloride, as inducing agent causes increase in the bilirubin, that is not hyperbilirubinanemia, as the raise is much less than double. But B.S.Eth and B.S.Aqs extracts at 100 mg/kg and 200 mg/kg were restored the approximate elevated level.

Histopathological evaluation of livers revealed that the B.S.Eth and B.S.Aqs stems extracts reduced inflammation of hepatocytes, swelling, necrosis and no. liver lesions induced by CCl_4.

The above results suggest that the B.S.Eth and B.S.Aqs stems extracts inhibits CCl_4 induced oxidative hepatic damage. It protects tissue from the effects of CCl_4 and reduce insidious progressive inflammation leading hepatic cell necrosis.

Phytochemical study of B.S.Eth and B.S.Aqs extracts shows the presence of carbohydrate, alkaloids, glycosides, protein, phenol, flavonoid, tannin, and steroid and thus both the extracts proved to be an antioxidant and herbal remedies.

CONCLUSION

Different extracts of *Bougainvillea spectabilis* stems shows significant Hepatoprotective activity. The ethanol extract shows more significant activity at respective doses compared to aqueous extract. This is a baseline work; further investigation is needed at molecular level.

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