ANNEXURE – A

FINAL PROJECT REPORT / EXECUTIVE SUMMARY

Phytochemical Characterization and Bioactivity Evaluation of Some Ethnomedicinal Plants of Southern Assam

UGC Sanction Order No.: 39/228/2010(SR) dated 28/12/ 2010

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Project Title	**Phytochemical Characterization and Bioactivity Evaluation of Some Ethnomedicinal Plants of Southern Assam**
Project Sanction No.:	**F. 39/228/2010(SR) dated 28 / 12 / 2010**
Date of Implementation:	**01 / 02 / 2011**
Duration of the Project:	**Three (03) Years**
Brief Objective of the project	Following the objectives of the project: (a). Documentation of ethnomedicinal plants of Southern Assam of North East India. (b). Phytochemical screening of some selected plants. (c). Bioactivity evaluation of some selected plants in relation to antimicrobial and Hepatoprotective activities. (d). Isolation and characterization of compounds.

Statement of the problem

The use of plants as a source of medicine dates back to the middle Paleolithic age around 60,000 years ago[1]. Approximately, 80% of world population depends upon plants as their primary source of health care[2]. It is now known that 87% of all categorized human diseases including hepatic disorders can be cured by drugs of natural product origin[3,4]. In spite of remarkable advances in contemporary medicine, no effective compounds to treat complex ailments and infections without imparting any side – effects. For example, free radicals or reactive oxygen species (ROS) like superoxide radical ($O_2^{\bullet -}$), hydroxyl radical ($OH^{\bullet}$) and hydrogen peroxide (H_2O_2) are considered detrimental to cells[6]. Redox regulations, which involves ROS is important for the modulation of essential cellular functions[7]. Increase in the level of ROS in cells results in disruption of cellular functions, activities of important enzymes and triggers specific signaling pathways leading to oxidative stress, which is related to various human diseases[6,7]. One of the important cellular injuries that are triggered by ROS is the initiation of lipid peroxidation in biological membranes[8]. Natural medicines derived from plants are always considered effective and can be used as a safe alternative treatment for several diseases and as such several plants have been evaluated for their medicinal efficacy against infections and other aliments[10-19].

To achieve the objectives of the project, the work has been distributed into various phases, which primarily comprises of documentation of ethnomedicinal plants from Southern Assam of North East India followed by preliminary phytochemical screening, bioactivity evaluation and finally aimed for isolation and characterization of compounds.

Collection and documentation of ethnomedicinal plants

Intensive field work has been undertaken for a period of two years covering different seasons so as to gather information on each plant species found to be used in traditional healing practices. Information were gathered by taking interview of local medicine men using structured questionaries', while in some cases verbal information and personal observations were noted. Medicine men were selected on the basis of information received from the local informant(s). Prior to the interview, the respondents were made aware about the objective of the study, followed by their verbal consent. Each medicine men / healer were selected on the basis of their previous experience using medicinal plants in treatment of diseases and in certain cases, the data obtained from one healer is cross verified and examined with the other healer(s). The vernacular name, mode of preparation, etc. were recorded. In certain, where the healer was not aware of the actual name of the disease or ailment, name of the disease was given on the basis of symptoms described by them.

Phytochemical Screening of ethnomedicinal plants

Preparation of plant extract

The qualitative phytochemical analysis were carried out to evaluate the presence of secondary metabolites in the plant extract. For the preparation of the plant extract, the fresh plant part(s) were cleaned, cut into small pieces and shade dried after which the samples were milled to get a fine powder. Approximately 500g of dried and powdered plant samples were extracted initially with petroleum ether followed by subsequent extraction with eythyl acetate, methanol and 70% (v/v) ethanol by dipping the plant sample in 1.5 to 2 liters of the extraction solvent. The extracts obtained were filtered and concentrated or made solvent free using a Rotary Evaporator (IKA, Fischer Scientific,

Germany). These extracts were used for phytochemical analysis as per the accepted protocol mentioned in Table 1.

Table 1. Qualitative analysis of secondary metabolites in plant extracts

TEST SAMPLE	TEST SOLUTION	OBSERVATION	INFERENCE
2ml Plant Extract + 2 ml Dil. HCl	1 ml of Mayer's Reagent	Yellowish Buff Colored Precipitate	Alkaloid present
2ml Plant Extract + 0.2 ml Dil. HCl	0.1ml of iodine solution	Reddish brown precipitate	Alkaloid present
3.2ml Plant Extract + 0.2ml Dil HCl	0.1ml of Phosphomolybdic acid	Buff Colored Precipitate	Alkaloid Present
4.5 ml of the Plant Extract	5ml Fehlin A & B Solution	Brick Red Colored Precipitate	Reducing Sugar Present
5.5ml of plant extract hydrolyzed with H_2SO_4 and neutralized with 10% Sodium Carbonate	5ml Fehlin A & B Solution	Brick Red Colored Precipitate	Reducing Sugar Present
6.5 ml of plant extract hydrolyzed with H_2SO_4	a. 1ml Dil. Ammonia b. 1ml Dil. Sodium Carbonate c. 1ml Dil. Sodium Hydroxide Solution	Greenish Yellow Precipitate Pale Yellow Colouring Yellow Colouring	Flavanoids Present
10mg plant extract dissolved in 5ml glacial acetic acid	1 drop of concentrated H_2SO_4	Reddish ring at the bottom	Steroid Present
5ml of plant extract + water, shaken vigorously	-	Stable forth	Saponins Present
5ml of plant extract	1ml of 10% $K_2Cr_2O_7$	Yellowish brown Precipitate	Tannin Present
5ml of plant extract	1ml of 10% $FeCl_3$	Yellowish brown Precipitate	Tannin Present

Bioactivity Evaluation of selected medicinal plants

The bioactivity evaluation of the selected ethnomedicinal plants were carried out in terms of Hepatoprotective of acetone extracts. The extraction was carried out as described earlier. The final extract obtained was stored at -20^{0}C for further analysis.

Chemicals

All the chemicals were of analytical grade and procured either from Merck (India & Germany) and HiMedia Labs, India. The biochemical assay kits were procured from Ozone Pvt. Ltd., India.

Animal experiments for assessment of Hepatoprotective activity

Pathogen free Swiss albino mice (~28±2g b.w) of both the sexes were procured from Pasteur Institute, Shillong, India. The animals were grouped and housed in polyacrylic cages (38 × 28 × 10 cm) with not more than six animals per cage and maintained under standard conditions (25±2^{0}C with 14/10h dark / light cycles and RH 55±5%). They were allowed free access to dry pellet diet and water *ad labitum*. The mice were acclimatized to the laboratory conditions for 7d prior to the onset of experimental conditions. The experimental protocol was strictly designed following standard guide for care and use of laboratory animals.

Biochemical analysis

Toxicity Study

Toxicity study was performed as per OECD guideline 423. Swiss Albino mice weighting 24-26g were used for toxicity study. Animals were treated with graded dose (200, 400, 600, 800, 1000, 1200, 1600 and 2000 mg/kg b.w.p.o.) of the extracts. After dose administration the individual animals were under observation for any symptoms of toxicity and abnormality in the behavior up to 21 days. After 21 days, blood was collected and liver was excised out for histological observations

CCl_4 induced hepatic damage:

The hepatic cell injury was induced by using carbon tetrachloride (CCl_4). Mice were divided into nine groups (n=6), Group I received a single dose of water (1mL/kg b.w.) and liquid paraffin (1mL/kg) (1:1) daily for 5 days. Group II received CCl_4 (0.5 mL/kg body weight): liquid paraffin (1:1) once daily for 5 days. Group III received standard drug silymarin (50 mg/kg b.w.) once daily for 5 days.Test groups animals (Groups IV–IX) were administered at a dose of 100, 200 and 300 mg/kg body weight per orally of bark extract and methanol bark extracts respectively, in the form of suspension with water. Groups III–IX animals were administered simultaneously CCl_4: liquid paraffin (1:1, 2 mL/kg body weight) once daily for 5 days after 30 min of administration of the doses. Animals were sacrificed 24 h after the last treatment.

Analysis of hepatoprotective activity by using serum biochemical parameters:

Blood serum was used for biochemical analysis, such as the serum glutamic pyruvates transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), serum alkaline phosphatase (SALP) and total bilirubin.The SGOT and SGPT level were measured as per the method suggested by Reitman and Frankle (1957). The total blirubin content was measured as per the method of Grof (1938) and the SALP level was measured as per the method of Sood (1999).

Method for the measurement of SGPT (Serum glutamic pyruvic transaminase) in blood serum samples

Assay Procedure:

1.0 mL of working reagent (2mL of R_2 with 8mL of R_1) was added to 100 µl of serum / plasma, mixed well, incubated for 1 min and the change in optical density was observed every 60 seconds for 180 seconds against distilled water at 340nm.

Calculations:

Average change was observed in absorbance per minute (ΔAbs/min) and release of GPT was expressed in U/L.

At 340 nm in U/L = ΔAbs^{-1} x 1746

At 334 nm in U/L = ΔAbs^{-1} x 1780

At 365 nm in U/L = ΔAbs^{-1} x 3235

where, ΔAbs= change in absorbance; 1746, 1780, 3235= dilution factor.

Method for the measurement of SGOT (Serum glutamic oxaloacetic transaminase) in blood serum samples:

Assay Procedure:

1.0mL of working reagent (2mL of R_2 with 8mL of R_1) was added to 100 µl of serum / plasma, mixed well, incubated for 1 min and the change in optical density was observed every 60 seconds for 180 seconds against distilled water at 340nm.

Calculations:

Average change was observed in absorbance per minute (ΔAbs/min) and release of GOT in U/L.

At 340 nm in U/L = ΔAbs^{-1} x 1746

At 334 nm in U/L = ΔAbs^{-1} x 1780

At 365 nm in U/L = ΔAbs^{-1} x 3235

where, ΔAbs= change in absorbance; 1746, 1780, 3235= dilution factor.

Method for the measurement of SALP (Serum alkaline phosphatase) in blood serum samples:

Assay procedure:

Four clean dry test tubes were taken and labelled as Blank (B), Standard (S), Control (C) and Test (T). 1.05 mL of distilled water in (B) and 1.0 mL of distilled water each in (S), (C) and (T) was pipetted out. Then 1 mL of Buffer Reagent (L1) and 0.1 mL of Substrate Reagent (L2) was added in all the four test tubes, mixed well and was allowed to stand for 3 minat 37ºC. 0.05 mL of sample was added in (T), 0.05 mL of Phenol Standard (S) was added on (S), mixed well and was allowed to stand for 15 min at 37ºC. Then 1 mL of Colour Reagent (L3) was added in all the four test tubes, 0.05 mL of sample was added in (C), mixed well and absorbance was measured against distilled water at 510 nm.

Calculation:

$$\text{Total ALP activity in K.A. units} = \frac{\text{Abs.T-Abs.C}}{\text{Abs.S-Abs.B}} \text{ X } 10$$

Method for the measurement of Total bilirubin (TB) in blood serum samples:

Assay procedure:

Two clean dry test tubes were taken and labelled as T_1 and T_2. 0.5 mL of Reagent Diazo A in T_1 and T_2 and 0.05 mL of Diazo B in T_1 was added and mixed thoroughly. Then 0.5 mL of Activator, 1.0 mL of distilled water, 0.1 mL of serum sample was added in T1 and T2,

mixed well, kept at room temperature in the dark for 5 min and absorbance was read at 540 nm.

Calculation

Total bilirubin mg/dl A= of T_1 -T_2 x 26.31

where, 26.31= dilution factor.

Preparation of liver homogenate:

Liver samples from the sacrificed mice was quickly removed and pursued with ice-cold saline. A portion of the liver was homogenized in chilled sodium phosphate buffer (0.1M, pH 7.4) using a Potter Eleven homogenizer. The homogenate obtained was centrifuged in a cooling centrifuge at 12,000xg for 30 min at 4ºC to separate the nuclear debris. The supernatant was collected and used for the subsequent assays (Mohandas *et al.*, 1984).

Analysis of antioxidant activity by measuring antioxidant enzymatic and non enzymatic levels:

Lipid Peroxidation (LPO) assay:

Determination of Hepatic TBARS which signifies the rate of lipid peroxidation was carried out by following the standard method (Uchiyama and Mihara, 1978; Lee and Lim, 2008). Briefly the tissue was homogenized in chilled 0.1M potassium chloride solution. The assay mixture contained 0.5 mL of liver homogenate, 3mL of 1% H_3PO_4 and 1 mL of 0.6% TBA. The mixture was heated at 100ºC for 45 min, the reaction mixture was then allowed to cool at room temperature and 3 mL of n-butanol was added to it and shaken vigorously so as to separate the butanolic phase, it was then subjected to centrifugation at 4000xg for 10 min and absorbance was determined at 535 nm.

Reduced Glutathione (GSH) content:

Reduced Glutathione content was determined by the method of Ellman et al., (1958). 0.2 mL of tissue homogenate was mixed with 1.8 mL of EDTA solution. To this 3.0 mL of precipitating reagent (1.67 g of meta phosphoric acid, 0.2g of EDTA disodium salt, 30g sodium chloride in 1 L of distilled water) was added mixed thoroughly and kept for 5 min before centrifugation To 2.0 mL of the filtrate, 4.0 mL of 0.3 M disodium hydrogen phosphate solution and 1.0 mL of DTNB (5.5-dithiobis-2nitrobenzoic acid) reagent were added and absorbance was read at 412 mm.

Catalase (CAT) assay:

CAT assay was performed following the method based on the disappearance of $H_2 0_2$ at 25^0C (Pedraza-chaverri et al., 2005).5µl of dilute homogenate (1:40) was mixed with 720 µl of 30mM H_20_2 in 10 mM potassium phosphate solution and the reaction was followed at 240 mm. Decomposition of H_20_2 by CAT contained follows a first order kinetics as K=2. 3/t log Ao/ A where K is the first order rate constant, it is the time for which the decrease of H202 due to CAT activity was measured for 15 s and A o/ A is the optical density at time 0 and at 15.5 respectively.

Superoxide dismutase (SOD) activity:

Superoxide dismutase activity was measured by the method of Dhindsa et al., (1981) The reaction mixture consisted of 1.5 mL phosphate buffer (0.1 M, pH 7.4) 0.1 mL NBT (2.25 mM, 0.1mL tissue homogenate, 0.1mL sodium carbonate (1.5 M), 0.2 mL methionine (200 mm), 0.1 mL EDTA (3 mM) 1mL distilled water and 0.1 riboflavin (60mM) in the total volume of 3 mL. It was incubated in light for 60 min at room temperature. The rate of reaction was measured by recording changes in absorbance at 560 mm due to formation of formazone, a reaction product of NBT.

Glutathione Peroxidase (GPx) assay:

100 µl of the tissue homogenate diluted in 1:100 was added to 800 µl of reaction mixture containing 50 mM potassium phosphate solution pH 7.0, 1mM EDTA, 1mM sodium azide, 0.2mM NADPH, 1Unit/mL of GR and 1mM GSH. The reaction mixture was incubated at room temperature for 5 min before addition of 32 µl of 2.5 mM H_20_2solution for the initiation of the reaction and finally absorbance at 340 nm was recorded for 3 min and the activity was calculated focussing on millimolar absorption co –efficient for NADPH being 6.22. In case of blank reactions homogenates were replaced by distilled water which was subtracted from each assay (Pedraza-Chaverri et al., 2005).

Histopathological observation:

The mice liver tissues were fixed in 10% formalin for 24 hours and dehydrated with a solution of ethanol and embedded in paraffin. The serial sections were cut 5µm thick and stained with Haematoxylin-Eosin dye and observed under microscope (BX41, OLYMPUS) to note the changes in the liver tissue (Jothy et al., 2011).

Statistical analysis:

The data presented here were expressed as mean ± SE. The results were analysed by one way ANOVA with Tukey- Kramer multiple comparisons Test. The level of significance was accepted at $P<0.001$, $P<0.01$ and $P<0.05$.

Isolation and characterization of compounds

Thin Layer Chromatography (TLC)

The chromatographic analysis of the four extracts was performed by thin layer chromatography (TLC) technique on analytical plates over silica gel (TLC-grade; Merck India). For each extract, seven different solvent systems were used on the basis of their polarity. They were as follows: Hexane **,** Ethyl acetate (9:1, 9.5:0.5, 8.5:1.5, 4:1) and Hexane **,** Ethyl acetate **,** Methanol (9:0.5: 0.5, 8:1:1 and 7.5:2:0.5). In each case the spots were visualized under UV light at 370 nm.

Column Chromatography

The sample was dissolved in minimum amount of solvent and absorbed on silica gel (60-120 mesh) and allowed to dry. The column was eluted with n- hexane, and then the polarity was gradually increased using ethyl acetate. Eluents were collected in 500 mL reagent bottle (Qualigens), and the progress of the separation was monitored by thin layer chromatography, using the solvent system Hexane **,** Ethyl acetate (9:1, 9.5:0.5, 8.5:1.5, 4:1) and Hexane **,** Ethyl acetate **,** Methanol (9:0.5: 0.5, 8:1:1 and 7.5:2:0.5) a total of 30 fractions were collected. Fractions (4-6) after Preparative thin layer chromatography gave a single spot on TLC using different solvent system with different ratios.The other fractions gave two- three spots in Preparative thin layer chromatography from the same eluting solvent.

Isolation of compounds using column chromatography

Column chromatography was carried out with acetone bark extract in a usual manner using solvents of increasing polarities. After collecting the fractions, all fractions were subjected to preparative TLC.

HPLC analysis of the isolated compound

The analytical HPLC chromatogram of the isolated compound indicated that the material to be farely pured (impurity present in minor quantity). The chromatogram was obtained by eluting the test material with acetonitrile and water eluted for twenty minutes.

Structure elucidation of the isolated compound

The structure of the isolated compound is achieved using FT-IT, ^{1}H and ^{13}C NMR.

The isolated compound was subjected to bioactivity evaluation.

PHASE – I

Results

1. Documentation of ethnomedicinal plants

The various ethnomedicinal plants that were observed and recorded during the field studies are detailed in **Appendix – I** containing botanical name, family, vernacular name, parts used, mode of use, disease, etc.

2. Bioactivity evaluation

The results of the Experiments done in Phase – I are enclosed as Appendix – II

Experiments Conducted in Phase – II

Swiss albino mice of either sexes were divided into six groups containing six (n=06) animals. The animals were treated with different dosage of acetone extract of *A. carambola* followed by administration of carbon tetrachloride (CCl_4) (0.5ml / kg b.w. i.p.) once daily for 5 days. On 6d, the blood was collected through retro-orbital venous sinus to collect serum for assessment of biochemical parameters. Liver were excised out and used for further analysis.

2.1. Hepatoprotective activity of Pajanelia longifolia

Measurement of toxic effect of crude bark extract on serum biochemical parameters:

The acute toxicity study of the acetone bark extract was performed by using a graded dose of 200, 400, 600, 800, 1000, 1200, 1600 and 2000 mg/kg b.w.p.o. None of the doses showed any mortality or any visible behavioural changes after 21 days of treatment. Treatment with higher dose levels (1600 mg/kg b.w. and 2000 mg/kg b.w.) exhibited hepatic damage by the formation of cyst like structure [fig. 1.(E) and (F)].In lower dose levels (200, 400, 600 mg/kg b.w.) there was no any cyst like structure could be observed. From histopathological observation, it was found that treatment with lower dose concentrations (200, 400, 600 mg/kg b.w.p.o.) exhibited lesser zone of necrotic lesions in tissues but treatment with higher dose concentrations(1200, 1600 and 2000 mg/kg b.w) showed higher level of hepatic necrosis with tissue degradation (Fig. 1.). The findings of toxicity results were further cleared by measuring the serum biochemical parameters where it was observed that the levels of SGOT, SGPT and bilirubin were significantly ($P<0.001$) increased from lower dose to higher dose levels which was the indication of increasing the rate of toxicity from lower dose level to higher dose levels. But the toxic effect which was observed in lower dose levels were negligible (Table 2).

Table 2. Measurement of toxic effect of acetone crude bark extract of *Pajanelia longifolia* (Willd.) K. Schuman on serum biochemical parameters.

Plant Extract	SGOT (IU/L)	SGPT (IU/L)	Bilirubin (IU/L)
Control	21.62 ± 1.31	14.68 ± 0.72	0.29±0.01
200 mg/kg body weight	28.63 ± 1.75	20.38 ± 1.30	0.34 ± 0.01
400 mg/kg body weight	44.80 ± 1.45***	37.82 ± 1.78***	0.73 ± 0.01***
600 mg/kg body weight	66.75 ± 1.63***	56.34 ± 1.83***	1.04 ± 0.01***
800 mg/kg body weight	94.46 ± 1.03***	85.19 ± 1.14***	1.85 ± 0.01***
1000 mg/kg body weight	137.64 ± 1.42***	123.84 ± 1.02***	2.49 ± 0.01***
1200 mg/kg body weight	205.96 ± 1.11***	187.12 ± 2.67***	3.34 ± 0.01***
1600 mg/kg body weight	336.86 ± 2.48***	276.98 ± 1.02***	5.03 ± 0.01***
2000 mg/kg body weight	414.87 ± 1.42***	382.36 ± 1.45***	6.84 ± 0.00***

n=6 animal, values are given as mean± SE, values are statistically significant at ***P<0.001 control vs. treated group.

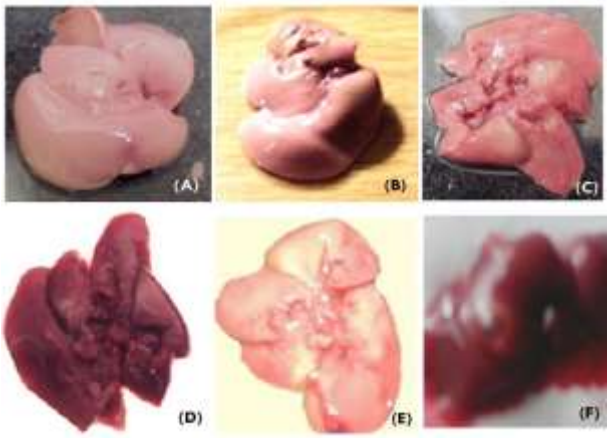


Fig. 1. Formation of Cyst like structure in higher dose level [1600mg/kg (E, arrow) and 2000 mg/kg (F, arrow)] but no any Cyst like structure formed in lower dose level [200 mg/kg (B), 800 mg/kg (C), 1200 mg/kg (D)] compared to control (A).

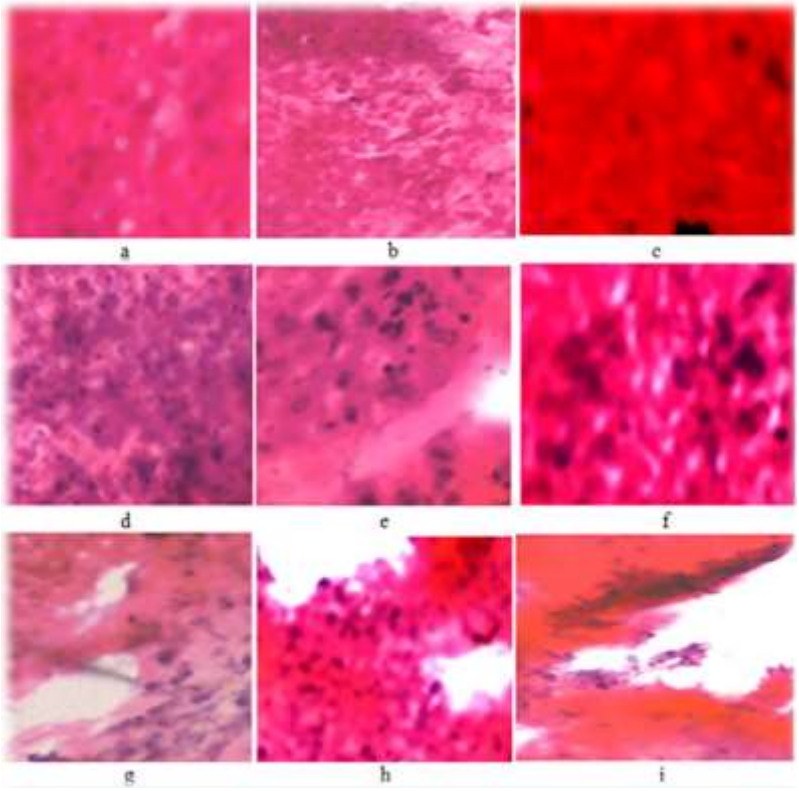


Fig. 2. Histopathological studies of sections of mice liver for the determination of toxicity level after 21 days of treatment with acetone bark extract.[(a) control group, (b) 200 mg/kg dose, (c) 400 mg/kg dose, (d) 600 mg/kg dose, (e) 800 mg/kg dose, (f) 1000mg/kg dose, (g) 1200 mg/kg dose, (h) 1600 mg/kg dose,(i)2000 mg/kg dose]. (arrow mark indicates tissue degradation)

Measurement of toxic effect of acetone crude bark extract on serum biochemical parameters

The acute toxicity study of the acetone bark extract was performed by using a graded dose of 200, 400, 600, 800, 1000, 1200, 1600 and 2000 mg/kg b.w.p.o. None of the doses showed any mortality or any visible behavioural changes after 21 days of treatment. Treatment with higher dose levels (1600 mg/kg b.w. and 2000 mg/kg b.w.) exhibited hepatic damage by the formation of cyst like structure [fig. 3 (E) and (F)].In lower dose levels (200, 400, 600 mg/kg b.w.) there was no any cyst like structure could be observed. From histopathological observation, it was found that treatment with lower dose concentrations (200, 400, 600 mg/kg b.w.p.o.) exhibited lesser zone of necrotic lesions in tissues but treatment with higher dose concentrations (1000, 1200, 1600 and 2000 mg/kg b.w) showed higher level of hepatic necrosis with tissue degradation (fig. 4). The findings of toxicity results were further cleared by measuring the serum biochemical parameters where it was observed that the levels of SGOT, SGPT and bilirubin were significantly ($P<0.001$) increased from lower dose to higher dose levels which was the indication of increasing the rate of toxicity from lower dose level to higher dose levels. But the toxic effect which was observed in lower dose levels were negligible (Table 3).

Table: 3. Measurement of toxic effect of methanol crude bark extract of *Pajanelia longifolia* (Willd.) K. Schuman on serum biochemical parameters

Plant Extract	**SGOT (IU/L)**	**SGPT (IU/L)**	**Bilirubin (IU/L)**
Control	21.62±1.31	14.68±0.72	0.29±0.01
200 mg/kg body weight	36.15±1.44**	29.14±1.59*	0.44±0.01***
400 mg/kg body weight	63.97±1.04***	58.37±2.45***	0.98±0.01***
600 mg/kg body weight	102.90±0.08***	94.83±3.39***	1.41±0.00***
800 mg/kg body weight	153.15±2.46***	128.65±1.42***	2.11±0.01***

1000 mg/kg body weight	231.53±1.56***	167.22±0.99***	2.94±0.01***
1200 mg/kg body weight	298.71±2.52***	213.83±2.40***	3.68±0.01***
1600 mg/kg body weight	423.71±0.72***	306.28±1.27***	5.14±0.01***
2000 mg/kg body weight	519.033±1.36***	435.50±1.42***	7.23±0.01***

n=6 animal, values are given as mean ± SE, values are statistically significant at ***P<0.001, P<0.01, *P<0.05, control vs. treated group

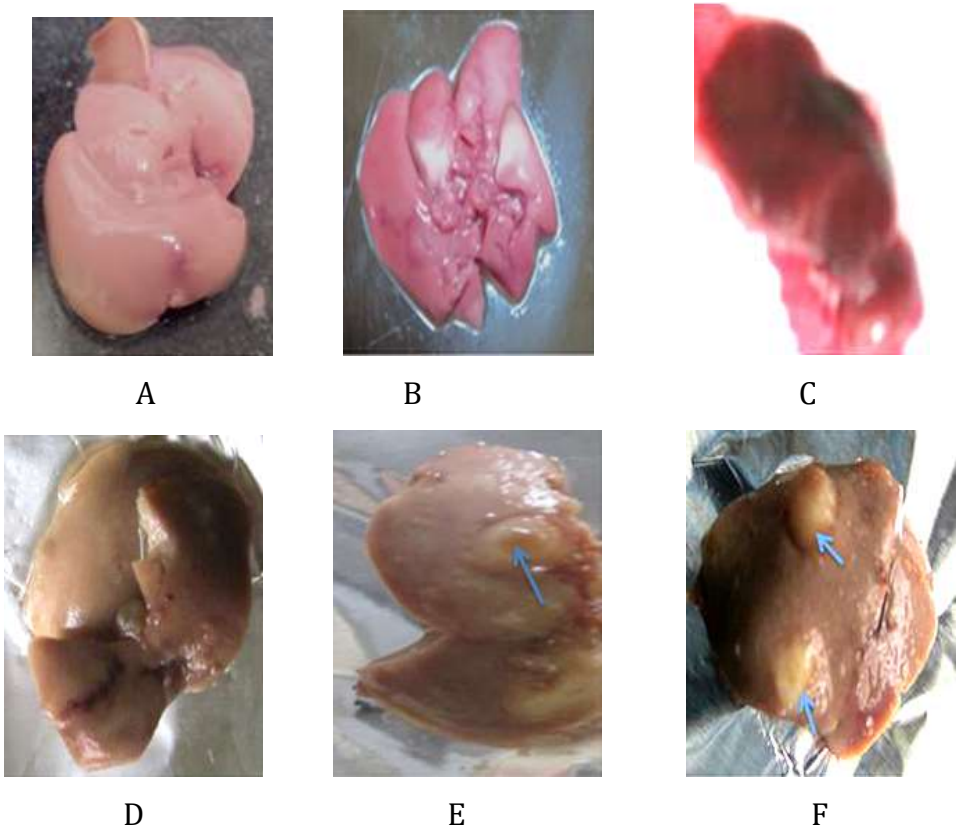


Fig 3. Formation of Cyst like structure in higher dose level [1600mg/kg (F) and 2000 mg/kg (E)] but no any Cyst like structure formed in lower dose level [200 mg/kg (B), 800 mg/kg (C), 1200 mg/kg (D)] compared to control (A)

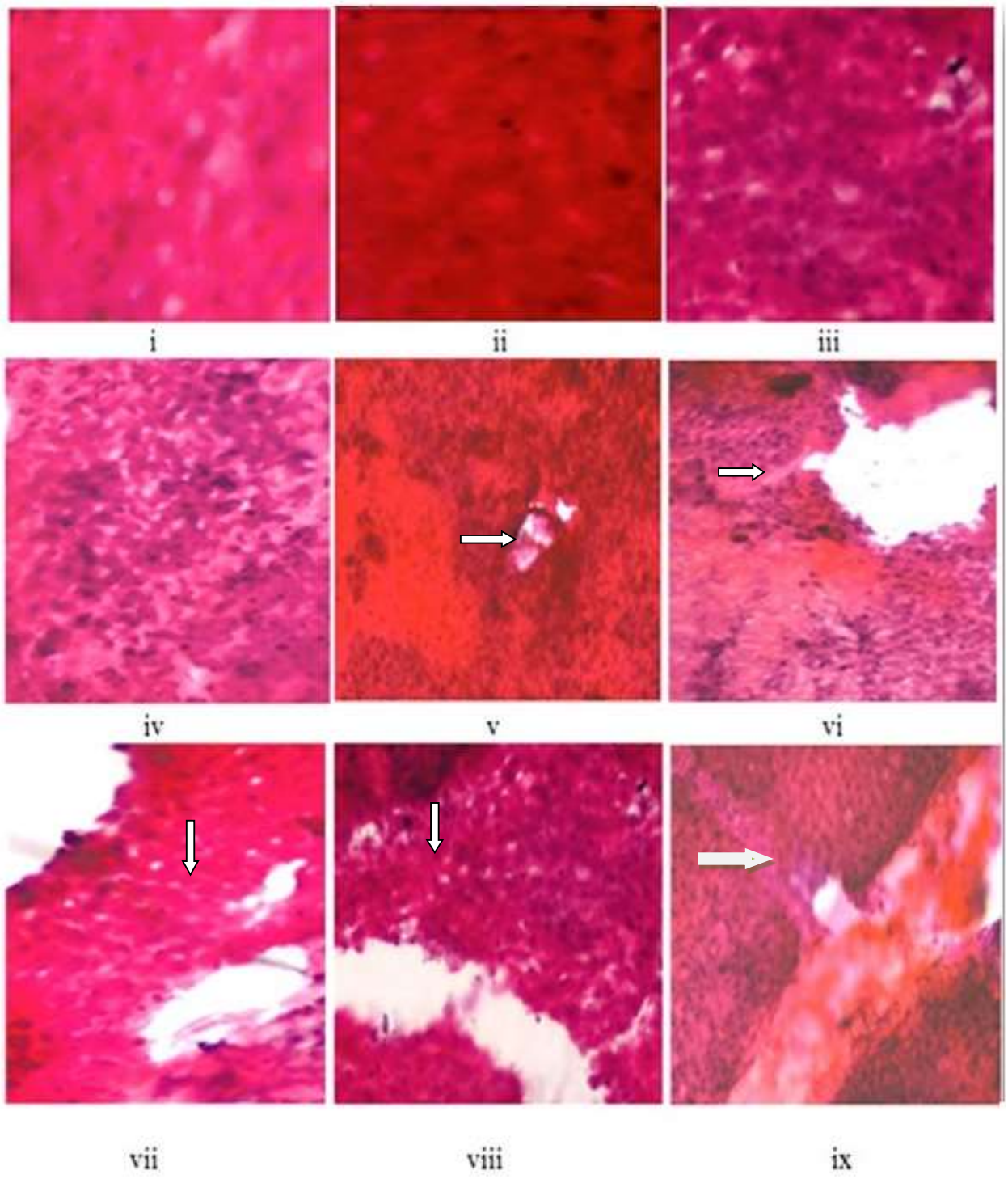


Fig: 4. Histopathological studies of sections of mice liver for the determination of toxicity level after 21 days of treatment with methanol bark extract.[(i) control group, (ii) 200 mg/kg dose, (iii) 400 mg/kg dose, (iv) 600 mg/kg dose, (v) 800 mg/kg dose, (vi) 1000mg/kg dose, (vii) 1200 mg/kg dose, (viii) 1600 mg/kg dose,(ix)2000 mg/kg dose] (arrow mark indicates: tissue degradation).

Protective effect of *Pajanelia longifolia* (Willd.) K. Schuman on serum biochemical parameters against CCl_4 induced hepatotoxicity in Swiss albino mice:

On the basis of acute toxicity study the dose concentration of 100, 200 and 300 mg/kg b.w.p.o. of acetone bark extracts and methanol bark extracts were selected. Mice treated with a single dose of CCl_4 (0.5 ml/kg b.w.i.p.) developed significant hepatic damage as observed from elevated serum levels of different liver function parameters (Table: 4). Levels of SGOT, SGPT, SALP and serum bilirubin were significantly increased in CCl_4 intoxicated animals which were revealed by the increased levels of SGOT (78.29 ± 2.19 IU/L, $P<0.001$ compared to control 26.61±1.55 IU/L), SGPT (94.54 ±1.51 IU/L, $P<0.001$ compared to control 18.85±2.05IU/L), SALP (127.25±2.19 IU/L, $P<0.001$ compared to control 41.67±2.11IU/L) and bilirubin (IU/L, $P<0.001$ compared to control 0.31±0.01IU/L). Significant ($P<0.001$, compared to toxic group, standard group and control group respectively) declines of SGOT, SGPT, SALP and bilirubin levels were also noticed after the treatment with different dose levels of *Pajanelia longifolia* (Willd.) K. Schuman acetone and methanol bark extracts. The data indicated that the dose concentration of 100, 200, 300 mg/kg body weight of acetone crude extracts and methanol crude extracts conferred hepatoprotective activity (Table 4). The most protective effect (significant at $P<0.001$, compared to toxic group and control group) was observed by the pretreatment with 200 mg/kg b.w.p.o. the dose of acetone crude bark extracts and least protective efficacy (significant at $P<0.001$, compared to toxic group, standard group and control group respectively) was offered by 300 mg/kg dose of methanol crude bark extracts in serum enzymes and bilirubin levels (Fig. 5). The standard drug Silymarin (50 mg/kg) significantly ($P<0.001$) decreased the SGOT (35.11 ± 1.62 IU/L), SGPT (23.08 ±1.11), SALP (58.79±0.85) and bilirubin (0.74±0.01) levels as compared to toxic (CCl_4) group.The results were also supported by histopathological observations (fig.5).

Table: 4. Protective effect of *Pajanelia longifolia* (Willd.) K. Schuman on serum biochemical parameters against CCl_4 induced hepatotoxicity in Swiss albino mice

Doses	**SGOT (IU/L)**	**SGPT (IU/L)**	**SALP (IU/L)**	**Bilirubin (IU/L)**
Group I	26.61 ± 1.55	18.85 ± 2.05	41.67 ± 2.11	0.31 ± 0.01
Group II	78.29 ± 2.19[a]	94.54 ± 1.51[a]	127.25 ± 2.19[a]	3.75 ± 0.02[a]
Group III	35.11 ± 1.62[f]	23.08 ± 1.11[f]	58.79 ± 0.85[b,f]	0.74 ± 0.01[a,f]
Group IV	51.99 ± 1.43[a,e,f]	39.63 ± 1.51[a,d.f]	86.51 ± 2.23[a,c,f]	1.04 ± 0.00[a,c.f]
Group V	38.95 ± 1.11[f]	29.37 ± 1.23[f]	72.24 ± 1.56[a,f]	0.85 ± 0.01[a,f]
Group VI	47.97 ± 1.12[a,e,f]	36.59 ± 1.82[b,e,f]	81.55± 2.07[a,c,f]	0.93 ± 0.02[a,d,f]
Group VII	49.50 ± 1.52[a,e,f]	42.51 ± 1.38[a,c,f]	83.25± 2.97[a,c,f]	1.08 ± 0.01[a,c,f]
Group VIII	56.09 ± 1.30[a,c,f]	51.50 ± 1.82[a,c,f]	91.92± 1.49[a,c,f]	1.22 ± 0.02 [a,c,f]
Group IX	64.71± 1.55[a,c,g]	60.85 ± 1.55[a,c,f]	98.88± 2.12[a,c,f]	1.37 ± 0.01[a,c,f]

n=6 animal, values are given as mean±SE, values are statistically significant at P<0.001, P<0.01 and P<0.05 level of significance.

a- compared with control P<0.001, b- compared with control P<0.01,

c- compared with standard P<0.001, d- compared with standard P<0.01, e- compared with standard P<0.05,

f- compared with toxic P<0.001, g- compared with toxic P<0.05.

Group I-control, GroupII- toxic (CCl_4), Group III- standard (Silymarin), Group IV- 100 mg/kg b.w.p.o. dose of acetone crude extract, Group V-200 mg/kg b.w.p.o. dose of acetone crude extract, GroupVI- 300 mg/kg b.w.p.o. dose of acetone crude extract, GroupVII- 100 mg/kg b.w.p.o. dose of methanol crude extract, Group VIII- 200 mg/kg b.w.p.o. dose of methanol crude extract, Group IX- 300 mg/kg b.w.p.o. dose of methanole crude extract.

Table: 5. % of Hepatoprotection offered by different crude extracts of *Pajanelia longifolia* (Willd.) K. Schuman at different dose concentration manner on serum biochemical parameters against CCl_4 induced hepatotoxicity in Swiss albino mice

Doses	**SGOT (%)**	**SGPT (%)**	**SALP (%)**	**Bilirubin (%)**
Group III	83approx	94 approx	79 approx	87 approx
Group IV	50 approx	72 approx	47 approx	78 approx
Group V	76 approx	86 approx	64 approx	84 approx
Group VI	58 approx	76 approx	53 approx	81 approx
Group VII	55 approx	68 approx	51 approx	77 approx
Group VIII	42 approx	56 approx	41 approx	73 approx
Group IX	26 approx	44 approx	33 approx	69 approx

Group III- standard (Silymarin), Group IV- 100 mg/kg b.w.p.o. dose of acetone crude extract, Group V-200 mg/kg b.w.p.o. dose of acetone crude extract, GroupVI- 300 mg/kg b.w.p.o. dose of acetone crude extract, GroupVII- 100 mg/kg b.w.p.o. dose of methanol crude extract, Group VIII- 200 mg/kg b.w.p.o. dose of methanol crude extract, Group IX- 300 mg/kg b.w.p.o. dose of methanole crude extract.

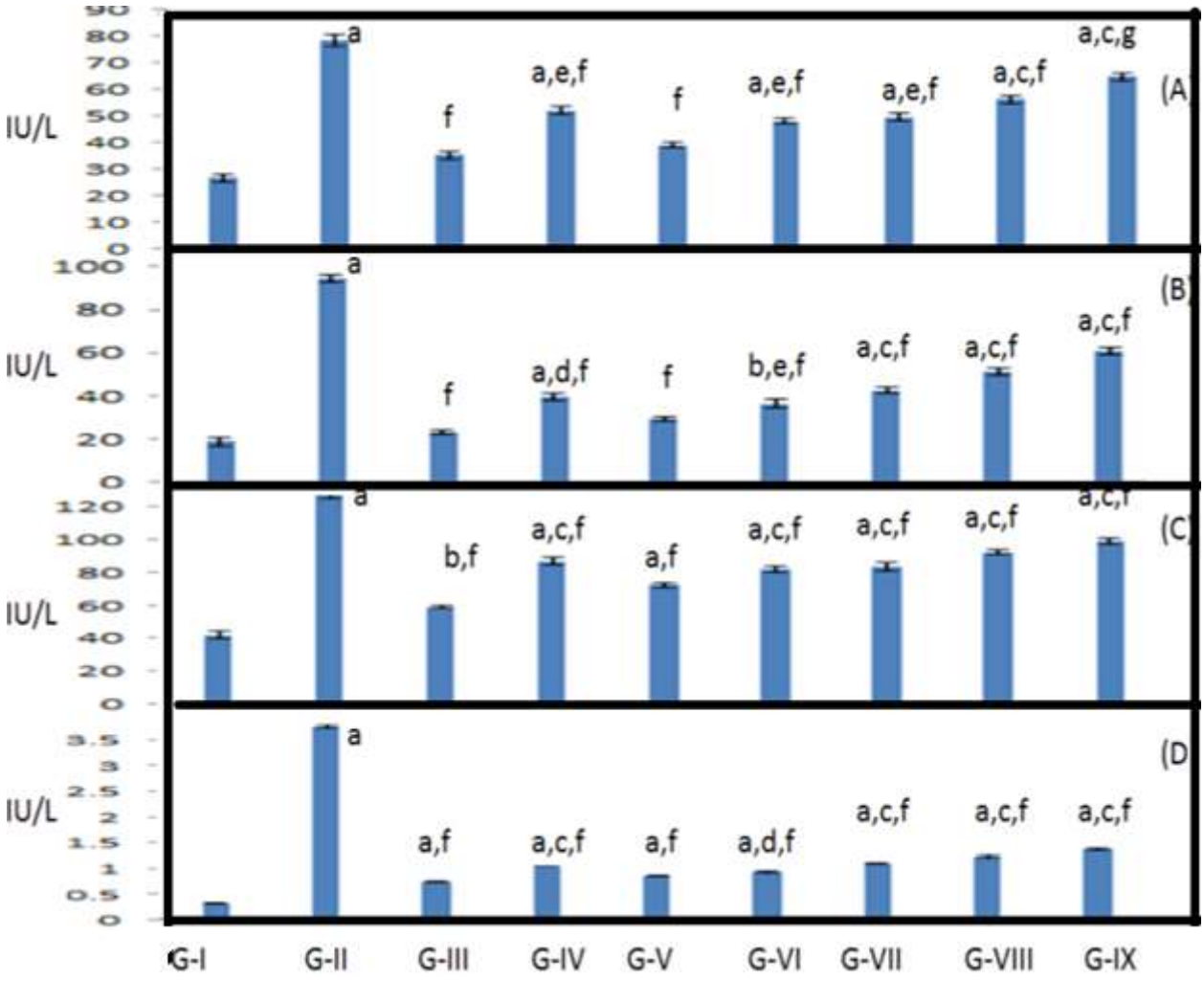


Fig. 5. Protective efficacy offered by crude bark extracts of *Pajanelia longifolia* (Willd.) K. Schuman at different dose concentration manner on serum biochemical parameters against CCl_4 induced hepatotoxicity in Swiss albino mice. (A) changes in SGOT level, (B) changes in SGPT level, (C) changes in SALP level, (D) changes in Bilirubin level

a- compared with control P<0.001, b- compared with control P<0.01, c- compared with standard P<0.001, d- compared with standard P<0.01, e- compared with standard P<0.05, f- compared with toxic P<0.001, g- compared with toxic P<0.05.

Group I-control, GroupII- toxic (CCl_4), Group III- standard (Silymarin), Group IV- 100 mg/kg b.w.p.o. dose of acetone crude extract, Group V-200 mg/kg b.w.p.o. dose of acetone crude extract, GroupVI- 300 mg/kg b.w.p.o. dose of acetone crude extract, GroupVII- 100 mg/kg b.w.p.o. dose of methanol crude extract, Group VIII- 200 mg/kg b.w.p.o. dose of methanol crude extract, Group IX- 300 mg/kg b.w.p.o. dose of methanole crude extract.

Antioxidant efficacy of *Pajanelia longifolia* (Willd.) K. Schuman on tissue enzymatic and non-enzymatic levels of Swiss albino mice against CCl_4 induced hepatic damage:

CCl_4 intoxication decreased the enzymatic and non- enzymatic antioxidant levels in mice liver as compared to the normal group. In CCl_4 treated group the level of glutathione peroxidase was significantly ($P<0.001$ compared to control group) decreased by approximately 77%, catalase, superoxide dismutase and reduced glutathione level were decreased by approximately 75%, 71 % and 78% respectively, whereas the lipid peroxidation level of CCl_4 intoxicated mice liver was significantly ($P<0.001$ compared to control group) increased by approximately 60% compared to control group. But treatment with *Pajanelia longifolia* (Willd.) K. Schuman acetone and methanol crude bark extracts (100, 200, 300 mg/kg b.w.p.o. respectively) significantly ($P<0.001$, $P<0.01$ and $P<0.05$ compared to toxic, standard and control group) restored the levels of SOD, CAT, GPx, GSH and LPO. The data indicated that the dose concentration of 100, 200, 300 mg/kg b.w.p.o. dose of acetone and methanol crude bark extracts conferred antioxidant activity. The most antioxidant effect ($P<0.001$ compared to toxic and control group respectively and $P<0.05$ compared to standard group) was observed by the treatment with 200 mg/kg b.w. p.o. dose of the acetone crude bark extract and least antioxidant activity was offered by 100 mg/kg dose of methanol crude extract. The standard drug Silymarin significantly ($P<0.001$, $P<0.01$ control vs standard, $P<0.001$ toxic vs, standard) altered the changes occurred in antioxidant enzymatic and non-enzymatic levels by CCl_4 intoxication. The results were also supported by histopathological observations (fig.6).

Table: 6. Antioxidant efficacy of *Pajanelia longifolia* (Willd.) K. Schuman on tissue enzymatic and non enzymatic levels of Swiss albino mice against CCl_4 induced hepatic damage:

Doses	LPO (nmolesTBARS/ mg protein)	GSH (unit/mg protein)	CAT (unit/mg protein)	SOD (unit/mg protein)	GPx (unit/mg protein)
Group I	0.56 ± 0.01	54.67 ± 1.31	0.58 ± 0.02	1.12 ± 0.00	67.36 ± 1.13
Group II	3.97 ± 0.04[a]	11.84± 0.51[a]	0.14 ± 0.01[a]	0.32 ± 0.01[a]	14.98± 1.01[a]
Group III	0.83 ± 0.03[a,f]	43.03±1.41[b,f]	0.46± 0.01[a,f]	0.86±0.01[a,f]	48.30±1.71[a,f]
Group IV	1.21 ± 0.01[a,c,f]	30.55±0.98[a,d,f]	0.33±0.00[a,d,f]	0.70±0.01[a,d,f]	32.50±1.38[a,c,f]
Group V	0.97 ± 0.01[a,e,f]	38.75 ± 0.40[a,f]	0.45 ± 0.01[a,f]	0.81 ± 0.02[a,f]	43.62 ± 1.29[a,f]
Group VI	1.05 ± 0.02[a,c,f]	31.76±0.76[a,d,f]	0.38 ± 0.01[a,f]	0.76 ± 0.03[a,f]	37.94 ± 1.24[a,f]
Group VII	1.34 ± 0.03[a,c,f]	28.31±2.02[a,c,f]	0.31±0.01[a,c,f]	0.67±0.01[a,c,f]	30.87±0.77[a,c,f]
Group VIII	1.52 ± 0.02[a,c,f]	25.81±0.94[a,c,f]	0.29 ± 0.00[a,c,f]	0.63±0.01[a,c,f]	26.44±0.82[a,c,g]
Group IX	2.02 ± 0.06[a,c,f]	20.90±0.85[a,c,g]	0.22±0.0[a,c,f]	0.52±0.0[a,c,f]	19.04 ± 1.39[a,c]

n=6 animal, values are given as mean±SE, values are statistically significant at P<0.001, P<0.01 and P<0.05 level of significance.

a- compared with control P<0.001, b- compared with control P<0.01,

c- compared with standard P<0.001,d- compared with standard P<0.01,e- compared with standard P<0.05,

f- compared with toxic P<0.001,g- compared with toxic P<0.05.

Group I-control, GroupII- toxic (CCl_4), Group III- standard (Silymarin), Group IV- 100 mg/kg b.w.p.o. dose of acetone crude extract, Group V-200 mg/kg b.w.p.o. dose of acetone crude extract, GroupVI- 300 mg/kg b.w.p.o. dose of acetone crude extract, GroupVII- 100 mg/kg b.w.p.o. dose of methanol crude extract, Group VIII- 200 mg/kg b.w.p.o. dose of methanol crude extract, Group IX- 300 mg/kg b.w.p.o. dose of methanole crude extract.

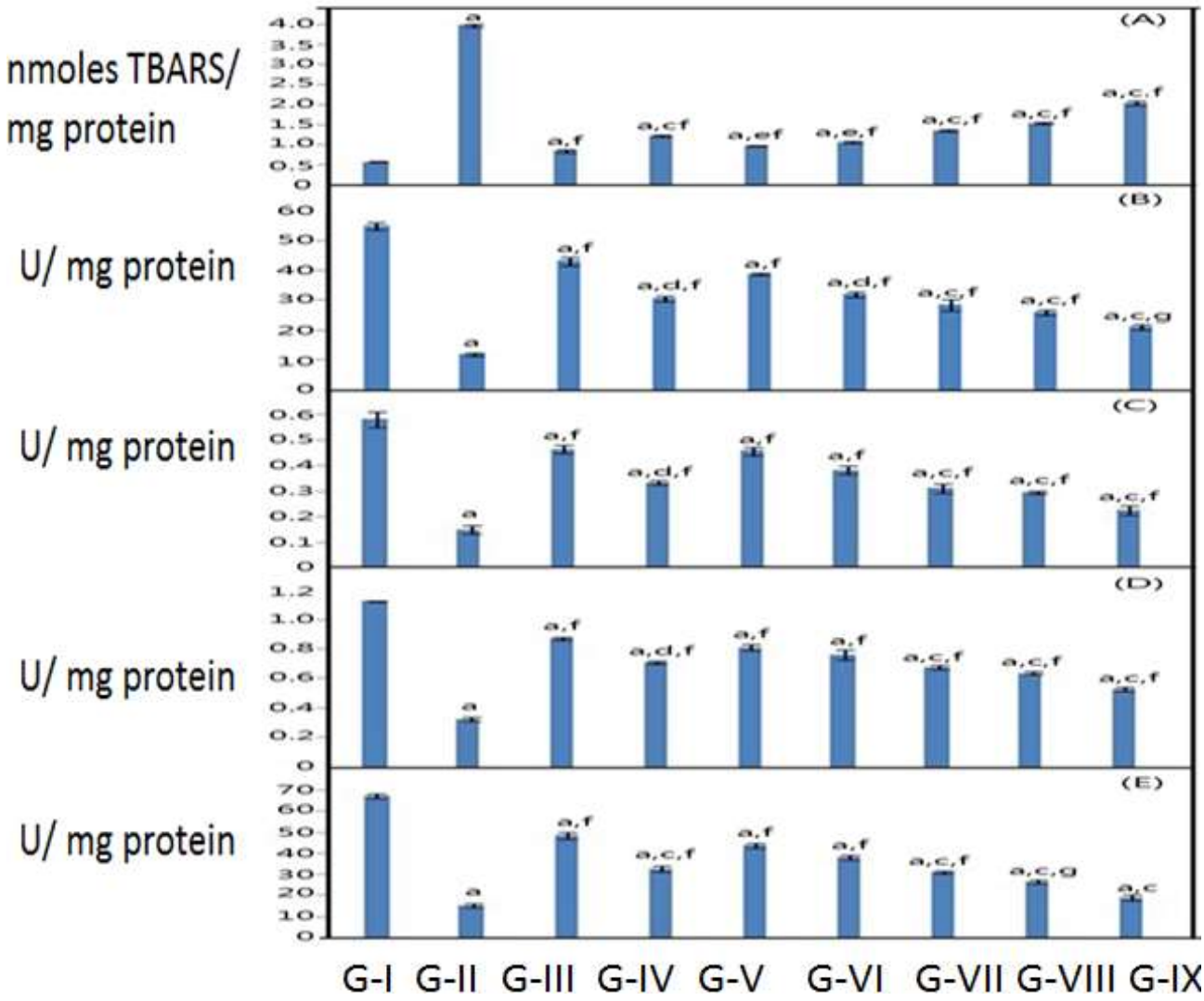


Fig. 6. Antioxidant efficacy offered by crude bark extracts of *Pajanelia longifolia* (Willd.) K. Schuman at different dose concentration manner on tissue enzymatic and non enzymatic levels of Swiss albino mice against CCl_4 induced hepatic damage. (A) changes in LPO level, (B) changes in GSH level, (C) changes in CAT level, (D) changes in SOD level, (E) changes in GPx.

a- compared with control P<0.001, b- compared with control P<0.01, c- compared with standard P<0.001, d- compared with standard P<0.01,e- compared with standard P<0.05, f- compared with toxic P<0.001,g- compared with toxic P<0.05.
Group I-control, Group II- toxic (CCl_4), Group III- standard (Silymarin), Group IV- 100 mg/kg b.w.p.o. dose of acetone crude extract, Group V-200 mg/kg b.w.p.o. dose of acetone crude extract, Group VI- 300 mg/kg b.w.p.o. dose of acetone crude extract, Group VII- 100 mg/kg b.w.p.o. dose of methanol crude extract, Group VIII- 200 mg/kg b.w.p.o. dose of methanol crude extract, Group IX- 300 mg/kg b.w.p.o. dose of methanol crude extract.

Histopathology of treated mice livers after treatment with different dose concentration manner of acetone and methanol crude bark extracts of *Pajanelia longifolia* (Willd.) K. Schuman:

The protective efficacy of acetone and methanol crude extracts of *Pajanelia longifolia* (Willd.) K. Schuman bark at dose level of 100, 200, 300 mg/kg b.w.p.o. respectively were further analysed by histopathological study (fig.7). The microscopy examination of the transverse section of control mice liver clearly depicted well formed hepatocytes (fig.7A). The liver sections of CCl_4 intoxicated mice showed cellular degradation and necrosis (Fig 7B).Treatment with acetone crude bark extract at a dose of 200 mg/kg b.w.p.o and methanol crude bark extract at a dose of 300 mg/kg b.w.p.o.exhibited maximum and minimum level of healing of necrosis respectively (Fig 7E and 7I) as compared to other doses of crude bark extracts.Silymarin treated group illustrates normal hepatocytes (Fig. 7C).

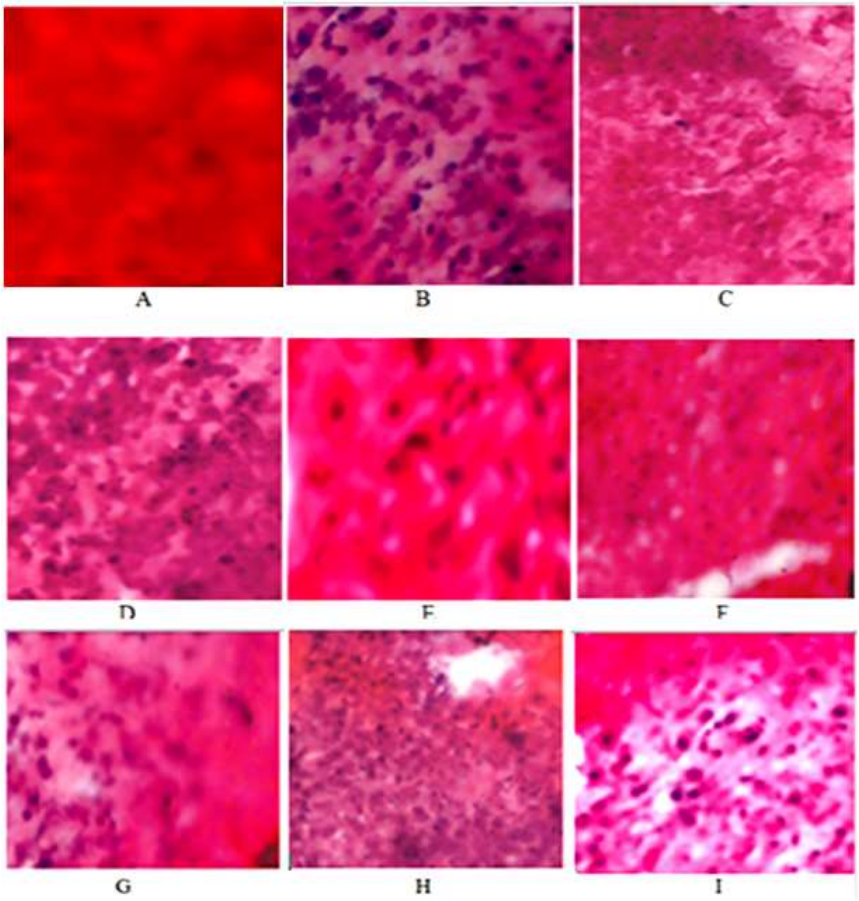


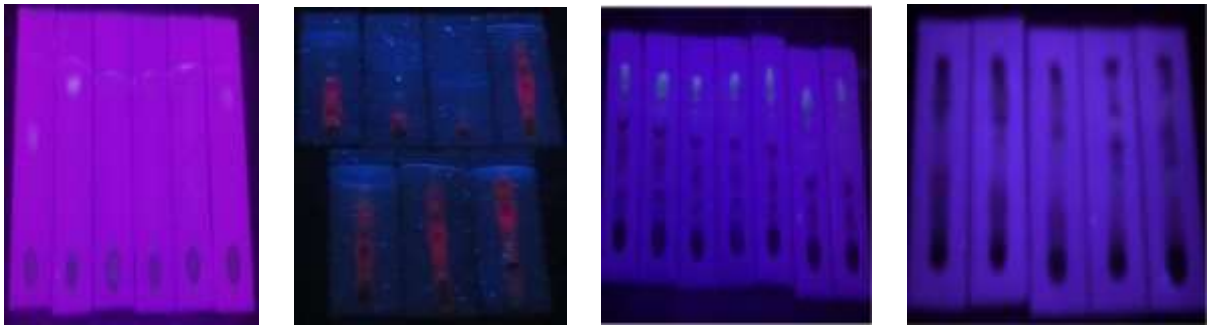
Fig. 7. Histopathological studies of sections of mice liver on 6th day after treatment. (A) Control, (B) CCl_4 (o.5 ml/kg b.w.i.p.),(C) Silymarin (50 mg/kg b.w.p.o.),(D) 100 mg/kg dose of acetone extract,(E) 200 mg/kg dose of acetone extract,(F) 300 mg/kg dose of

acetone extract, (G) 100 mg/kg dose of methanol crude extract,(H) 200 mg/kg dose of methanol crude extract,(I) 300 mg/kg dose of methanol crude extract.(arrow mark in (B) indicates : necrotic lesions) and (C, D, E, F,G, H, I respectively indicates: healing of necrosis)

ISOLATATION AND CHARACTERIZATION OF COMPOUNDS

TLC fingerprint results (under 370 nm. UV light) of different crude extracts:

Each of the four extracts was checked by thin layer chromatography (TLC) on analytical plates over silica gel G. For each extracts seven different solvent systems were used on the basis of polarity. These were as follows: PE:EE (9.5:0.5), PE:EE (9:1), PE:EE (8.5:1.5), PE:EE (4:2), PE:EE:ME (9:0.5:0.5), PE:EE:ME (8:1:1), PE:EE:ME (7.5:2:0.5).In each of the seven solvent systems, the spots on the plates were visualized under UV light at 370 nm. The TLC monitoring of acetone extract of bark of *Pajanelia longifolia* (Willd.) K. Schuman exhibited maximum spots which were well separated in all seven different solvent systems compared to other extracts. Based on the results of TLC profiling and phytochemical screening, the acetone bark extract was taken for column chromatography.



(a) **(b)** **(c)** **(d)**

Fig: 8. TLC fingerprint of different crude extracts of *Pajanelia longifolia* (Willd.) K. Schuman. eluted with different concentration of PE:EE (9.5:0.5), PE:EE (9:1), PE:EE (8.5:1.5), PE:EE (4:2), PE:EE:ME (9:0.5:0.5), PE:EE:ME (8:1:1), PE:EE:ME (7.5:2:0.5) respectively.The plates were visualized under UV light at 370 nm (a) n-hexane crude extract, (b) ethyl acetate crude extract, (c) acetone crude extract, (d) methanol crude extract.

Isolation of single organic molecule by Column Chromatography technique:

Column chromatography was carried out with acetone bark extract in a usual manner using solvents of increasing polarities. After collecting the fractions, all fractions were subjected to preparative TLC which showed that Fractions 4-6 exhibited two spots in all the solvent systems, whereas the solvent system n-hexane, ethyl acetate, dichloromethane, 7.5:2:0.5 yielded three spots (named- F5A, F5B and F5C). These three spots were taken for analytical HPLC analysis to check their purity level and it was found that the spot F5A existed in pure form (a small amount of impurity was present). The retention factor (Rf-value) of the spot F5A was 0.45. The isolated component was subjected to HPLC and the chromatogram was obtained by eluting the test material with acetonitrile and water for twenty minutes (Fig. 9).

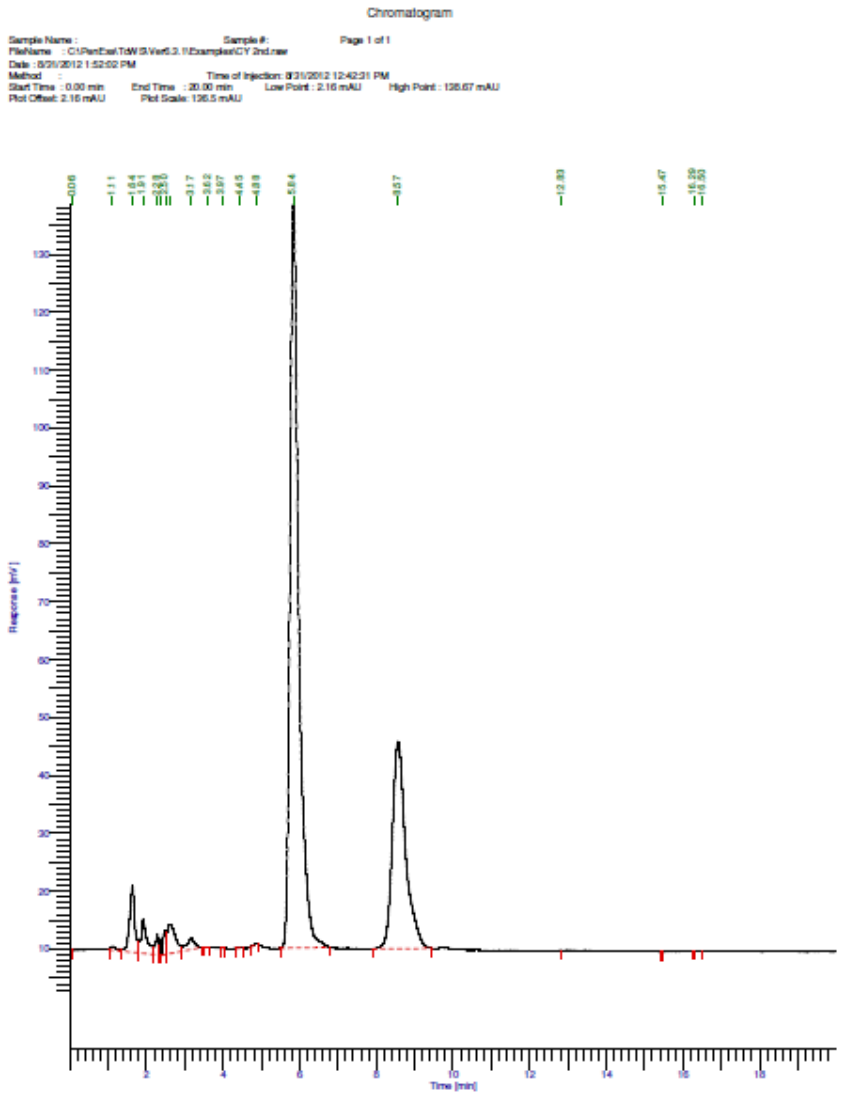


Fig. 9. HPLC result of compound F5A isolated from bark of *Pajanelia longifolia* (Willd.) K. Schuman

Structure elucidation of isolated compound:

The spectroscopic analysis of the compound F5A was carried out by IR, and NMR spectrum. The IR spectrum was obtained using an IR affinity spectrometer. The ^{1}H and ^{13}C NMR spectrum was obtained using Bruker Avena 400 MHz NMR spectrometer.

IR Spectroscopic data:

The **IR spectrum** (Fig. 9) of the isolated compound exhibited a characteristic strong band at about 1728cm^{-1} attributable to the stretching vibration of the C=O bond of the carbonyl group. The presence of a moderate bend at 2719cm-1 indicates carbonyl moiety to be an aldehyde (=C-H stretching). The peak at 1382 cm^{-1} is attributable to C=C stretching vibration. The peaks at 2927 and 2858 cm^{-1} are attributable to C-H stretching of alkyl moiety.

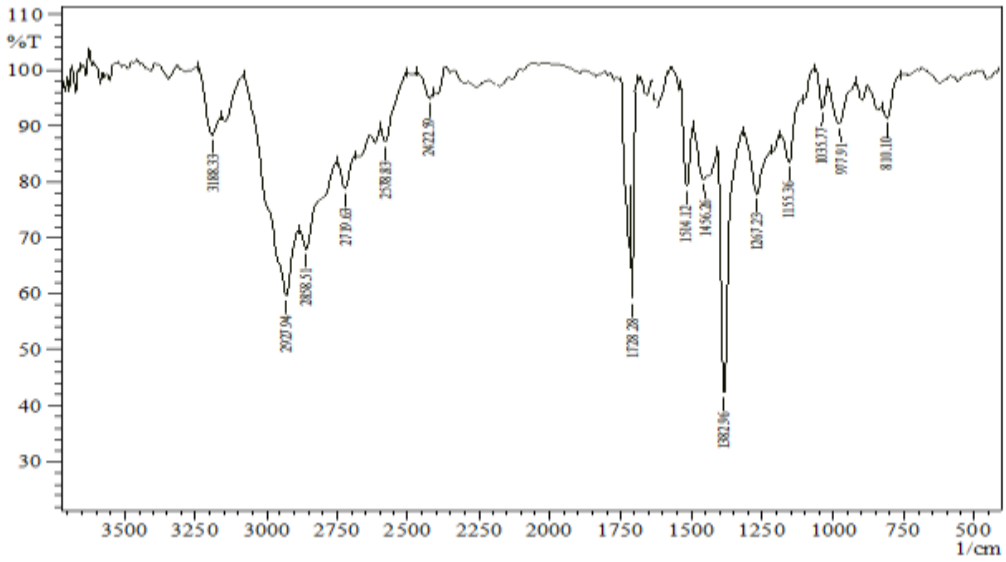


Fig. 10. IR Spectra of isolated compound F5A from bark of *Pajanelia longifolia* (Willd.) K. Schuman

^{1}H NMR Spectroscopic data:

The **^{1}H NMR spectrum** (Fig. 10) of the compound exhibited peak for –CHO proton as singlet at δ 9.942ppm [H1]. The alkenyl protons H7 resonated at δ4. 175ppm as quartet due to the the interaction with the three neighbouring methyl protons [H8], which in turn resonated at δ1. 592 ppm. Other alkyl protons resonated at their characteristic region of δ0. 8-2.0ppm. ^{1}HNMR assignment of the protons is tabulated below.

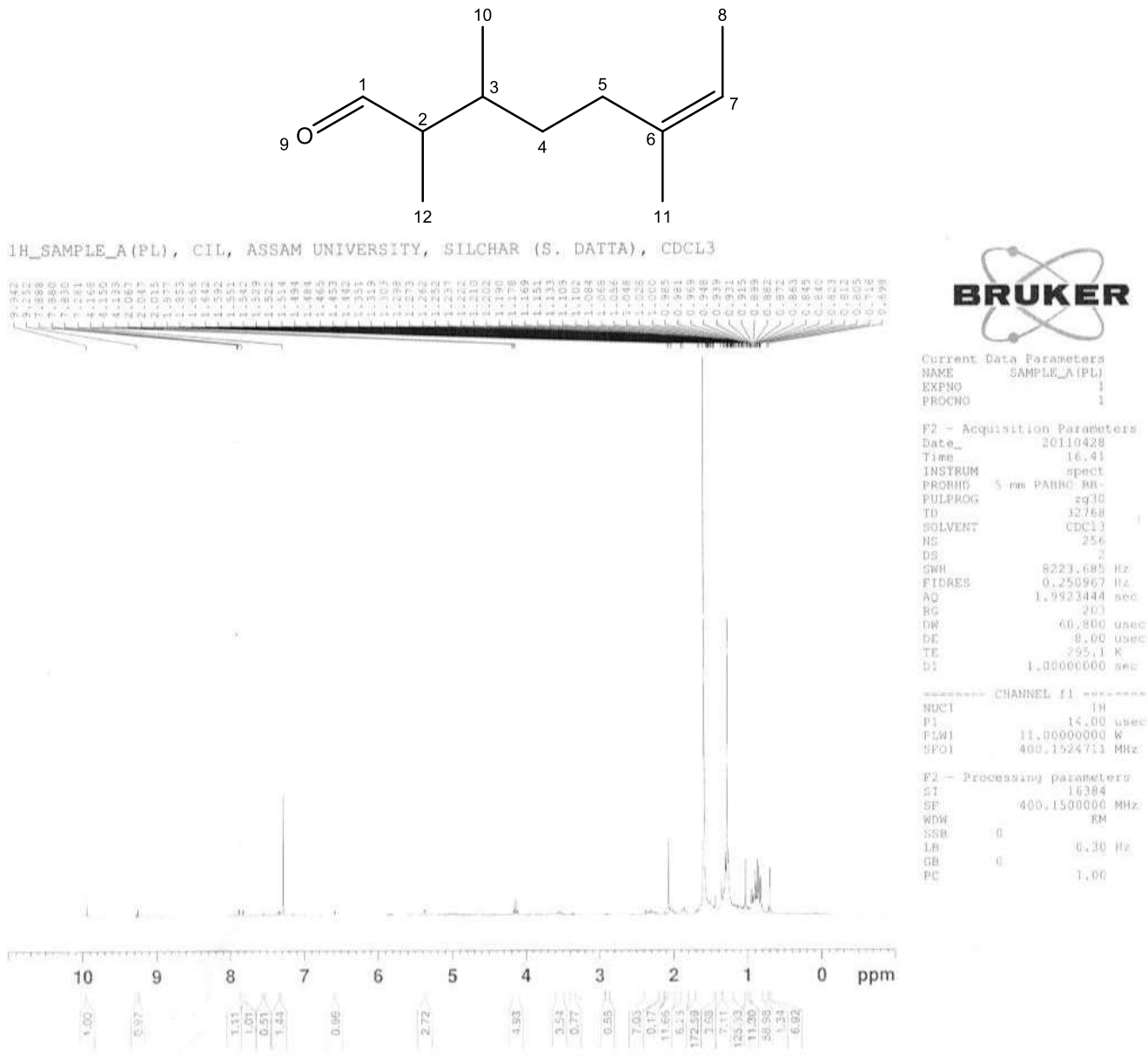
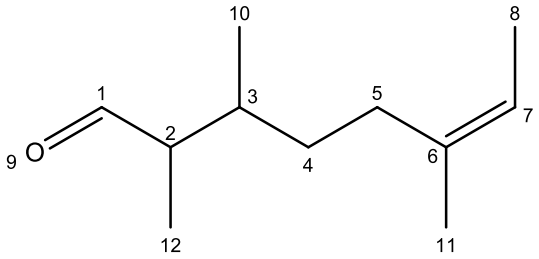


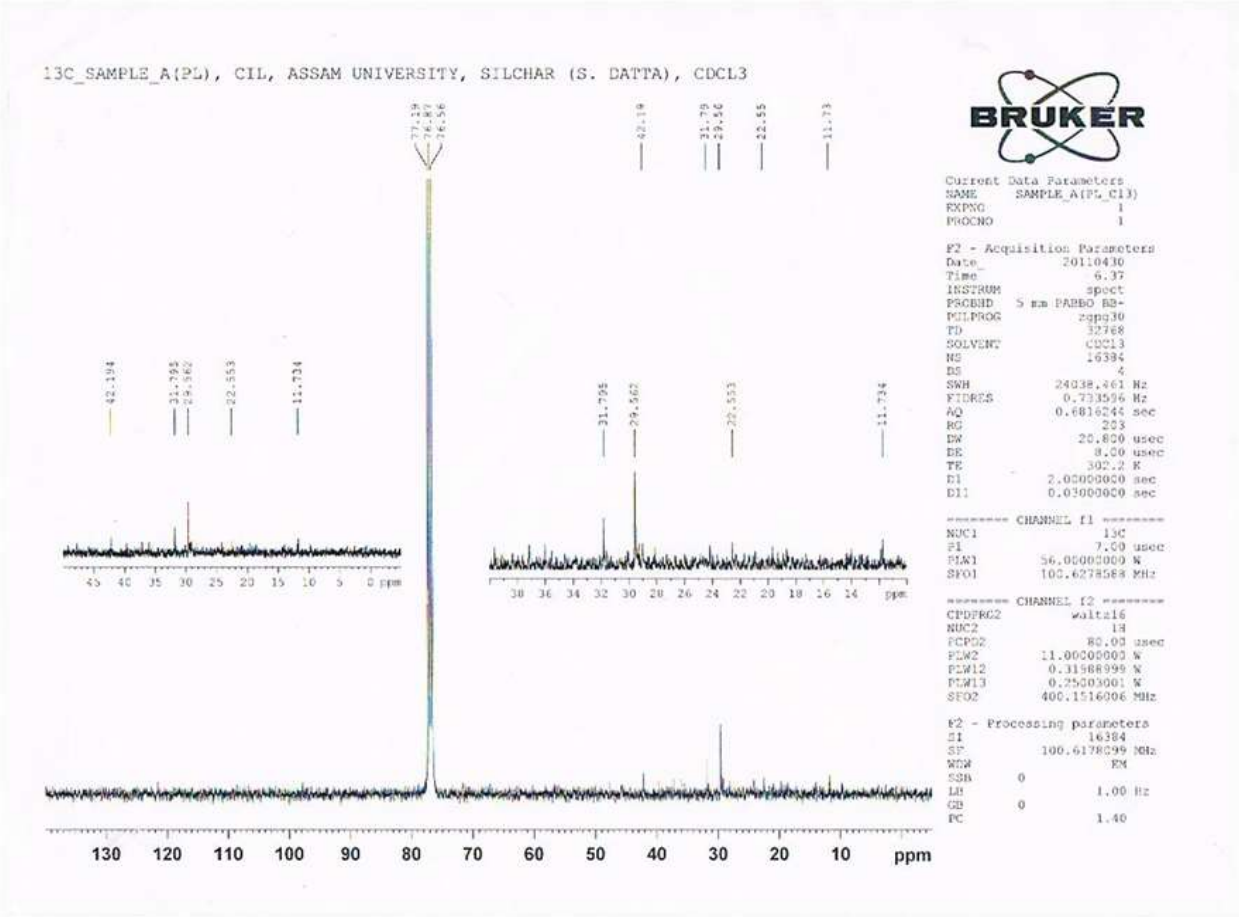
Fig. 11. [1]H NMR Spectra of isolated compound F5A from bark of *Pajanelia longifolia* (Willd.) K. Schuman

[13] C NMR Spectroscopic data:

The **[13]C NMR spectrum** of the compound is presented in Figure 4.5.3. The spectrum documented the peak corresponding to the alkenyl carbon [C7] in the down field region δ120ppm. The peak due to the carbonyl carbon could not be detected in 13C NMR spectrum (confirmed through 1HNMR and IR). The assignments of the peaks are tabulated below:

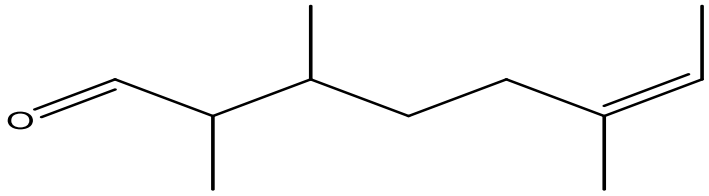


Peak δ (ppm)	Assignment to Carbon
50.00	2
36.02	3
32.56	4
33.80	5
120.02	7
14.01	8
19.28	10
22.28	11
11.86	12



^{13}C NMR Spectra of isolated compound F5A isolated from bark of *Pajanelia longifolia* (Willd.) K. Schuman

Considering these spectral data the probable structure of the compound is elucidated as



2,3,6-trimethyloct-6-enal

The compound possesses skeletal similarity with citronellal.

BIOACTIVITY EVALUATION OF 2,3,6 trimethyloct-6-enal

For the determination of the biological (Hepatoprotective) activity of the isolated compound 2,3,6-trimethyloct-6-enal, the biochemical and histological as described above were considered.

Protective effect of 2,3,6 trimethyloct-6-enal on serum biochemical parameters against CCl_4 induced hepatotoxicity in Swiss albino mice:

Mice treated with a single dose of CCl_4 developed significant ($P<0.001$, control vs. toxic group) hepatic damage as observed from elevated levels of SGOT (78.29 ± 2.19), SGPT (94.54± 1.51), SALP (127.25±2.19) and serum bilirubin (3.75±0.02). Treatment with 100, 200, 300 mg/kg b.w. p.o. dose of 2,3,6 trimethyloct-6- enal exhibited significant ($P<0.001$ compared to toxic group and $P<0.001$,$P<0.01$ and $P<0.05$ compared to control group) hepatoprotective activity in serum enzyme and bilirubin levels of swiss albino mice (Table 7). 100 mg/kg b.w. p.o. dose exhibited maximum level of (significant at $P<0.001$ compared to toxic group) hepatoprotective effect in SGOT (32.20 ± 0.74), SGPT (23.44± 1.13), SALP (60.24±0.93) and serum bilirubin (0.72±0.01) compared to other group.The protective efficacy of 100 mg/kg dose of 2,3,6 trimethyloct-6- enal was similar to that of protective efficacy exhibited by standard drug Silymarin (Fig.11).The results were also supported by histopathological observations (Fig. 11).

Table 7. Protective effect of 2,3,6 trimethyloci-6-enal on serum biochemical parameters against CCl_4 induced hepatotoxicity in Swiss albino mice:

Doses	**SGOT (IU/L)**	**SGPT (IU/L)**	**SALP (IU/L)**	**Bilirubin (IU/L)**
Group I	26.61 ± 1.55	18.85 ± 2.05	41.67 ± 2.11	0.31 ± 0.01
Group II	78.29 ± 2.19[a]	94.54 ± 1.51[a]	127.25 ± 2.19[a]	3.75 ± 0.02[a]
Group III	35.11 ± 1.62[e]	23.08 ± 1.11[e]	58.79 ± 0.85[b,e]	0.74 ± 0.01[a,e]
Group IV	32.20 ± 0.74[e]	23.44 ± 1.13[e]	60.24 ± 0.93[b,e]	0.72 ± 0.01[a,e]
Group V	39.76 ± 3.04[c,e]	27.16 ± 1.65[e]	69.15 ± 2.11[a,e]	0.81 ± 0.03[a,e]
Group VI	47.25 ± 2.19[a,e]	34.89 ± 2.95[b,e]	89.83 ± 1.88[a,e]	0.98 ± 0.03[a,d,e]

n=6 animal, values are given as mean±SE, values are statistically significant at $P<0.001$, $P<0.01$ and $P<0.05$ level of significance.

a- compared with control $P<0.001$,

b- compared with control $P<0.01$,

c- compared with control $P<0.05$,

d- compared with standard $P<0.001$,

e- compared with toxic $P<0.001$.

Group I-control, Group II- toxic (CCl_4), Group III- standard (Silymarin), Group IV- 100 mg/kg b.w.p.o. dose of 2,3,6,trimethyloct-6- enal, Group V-200 mg/kg b.w.p.o. dose of 2,3,6,trimethyloct-6- enal, GroupVI- 300 mg/kg b.w.p.o. dose of 2,3,6,trimethyloct-6-enal.

Table 8. % of Hepatoprotection offered by 2,3,6 trimethyloci-6-enal at different dose concentration manner on serum biochemical parameters against CCl_4 induced hepatotoxicity in Swiss albino mice:

Doses	**SGOT (%)**	**SGPT (%)**	**SALP (%)**	**Bilirubin (%)**
Group III	83approx	94 approx	79 approx	87 approx
Group IV	89 approx	93 approx	78 approx	88 approx
Group V	74 approx	89 approx	67 approx	85 approx
Group VI	60 approx	78 approx	43 approx	80 approx

Group III- standard (Silymarin), Group IV- 100 mg/kg b.w.p.o. dose of 2,3,6 trimethyloct-6-enal, Group V-200 mg/kg b.w.p.o. dose of 2,3,6 trimethyloct-6-enal, GroupVI- 300 mg/kg b.w.p.o. dose of 2,3,6 trimethyloct-6-ena.

Antioxidant efficacy of 2,3,6 trimethyloct-6-enal on tissue enzymatic and non enzymatic levels of Swiss albino mice against CCl_4 induced hepatic damage:

Mice treated with a single dose of CCl_4 developed significant hepatic damage as observed from elevated levels of LPO and decreased levels of SOD, CAT, GPX, and GSH in hepatic cells. Pretreatment with 2,3,6 trimethyloct-6-enal at dose concentration of 100, 200, 300 mg/kg b.w. p.o. conferred significant ($P<0.001$ and $P<0.01$ compared to control, $P<0.001$ compared to toxic and $P<0.01$ compared to standard) antioxidant activity in enzymatic and non-enzymatic levels of swiss albino mice by lowering the elevated levels of LPO and by increasing the decreased levels of SOD, CAT, GPX, and GSH (Table 5.3). 100 mg/kg b.w. p.o. dose of 2,3,6 trimethyloct-6-enal exhibited a maximum level ($P<0.001$ compared to toxic) of antioxidant activity and 300 mg/kg b.w.p.o. dose exhibited least level ($P<0.001$ compared to toxic, standard and control respectively) of antioxidant activity.The protective efficacy of 100 mg/kg dose of

2,3,6 trimethyloct-6-enal was similar to that of protective efficacy exhibited by standard drug Silymarin (50 mg/kg b.w.p.o.). The result was also supported by histopathological observations (Fig. 12)

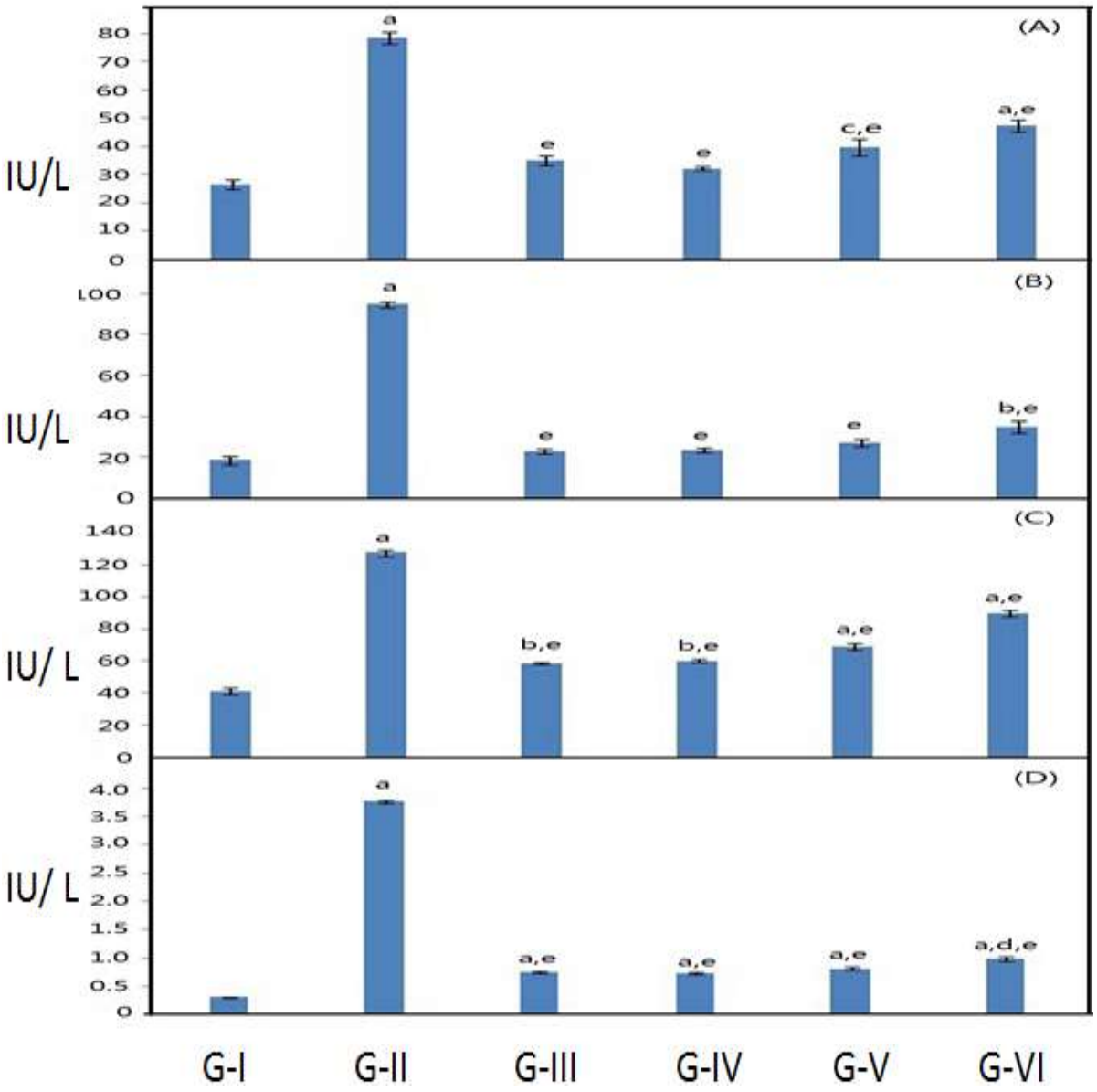


Fig. 11. Protective efficacy offered by 2,3,6 trimethyloct-6-enal at different dose concentration manner on serum biochemical parameters against CCl_4 induced hepatotoxicity in Swiss albino mice. (A) changes in SGOT level, (B) changes in SGPT level, (C) changes in SALP level, (D) changes in Bilirubin level

a- compared with control P<0.001,b- compared with control P<0.01,c- compared with control P<0.05, d- compared with standard P<0.001, e- compared with toxic P<0.001.

Group I-control, Group II- toxic (CCl_4), Group III- standard (Silymarin), Group IV- 100 mg/kg b.w.p.o. dose of 2,3,6,trimethyloct-6- enal, Group V-200 mg/kg b.w.p.o. dose of 2,3,6,trimethyloct-6- enal, GroupVI- 300 mg/kg b.w.p.o. dose of 2,3,6,trimethyloct-6- enal.

Table 9. Antioxidant efficacy of 2,3,6 trimethyloci-6-enal at different dose concentration manner on tissue enzymatic and non enzymatic levels of Swiss albino mice against CCl_4 induced hepatic damage**:**

Doses	**LPO (nmoles TBARS/mg protein)**	**GSH (unit/mg protein)**	**CAT (unit/mg protein)**	**SOD (unit/mg protein)**	**GPx (unit/mg protein)**
Group I	0.56 ± 0.01	54.67 ± 1.31	0.58 ± 0.02	1.12 ± 0.00	67.36 ± 1.13
Group II	3.97 ± 0.04[a]	11.84± 0.51[a]	0.14 ± 0.01[a]	0.32 ± 0.01[a]	14.98 ± 1.01[a]
Group III	0.83 ± 0.03[a,e]	43.03±1.41[b,e]	0.46 ± 0.01[e]	0.86 ± 0.01[a,e]	48.30±1.71[a,e]
Group IV	0.81 ± 0.02[a,e]	42.72±1.46[b,e]	0.49 ± 0.02[e]	0.82 ± 0.01[a,e]	52.95±2.70[b,e]
Group V	0.92 ± 0.01[a,e]	37.60±1.32[a,e]	0.42±0.01[b,e]	0.79 ± 0.01[a,e]	46.38±0.90[a,e]
Group VI	1.12±0.02[a,d,e]	31.76±0.90[a,f,e]	0.35±0.01[a,e]	0.72±0.01[a,e,f]	35.67±1.98[a,e,g]

n=6 animal, values are given as mean±SE, values are statistically significant at $P<0.001$, $P<0.01$ and $P<0.05$ level of significance.

a- compared with control $P<0.001$, b- compared with control $P<0.01$,c- compared with control $P<0.05$, d- compared with standard $P<0.001$, e- compared with toxic $P<0.001$.f- compared with standard $P<0.01$, g - compared with standard $P<0.001$

Group I-control, Group II- toxic (CCl_4), Group III- standard (Silymarin), Group IV- 100 mg/kg b.w.p.o. dose of 2,3,6,trimethyloct-6- enal, Group V-200 mg/kg b.w.p.o. dose of 2,3,6,trimethyloct-6- enal, GroupVI- 300 mg/kg b.w.p.o. dose of 2,3,6,trimethyloct-6- enal.

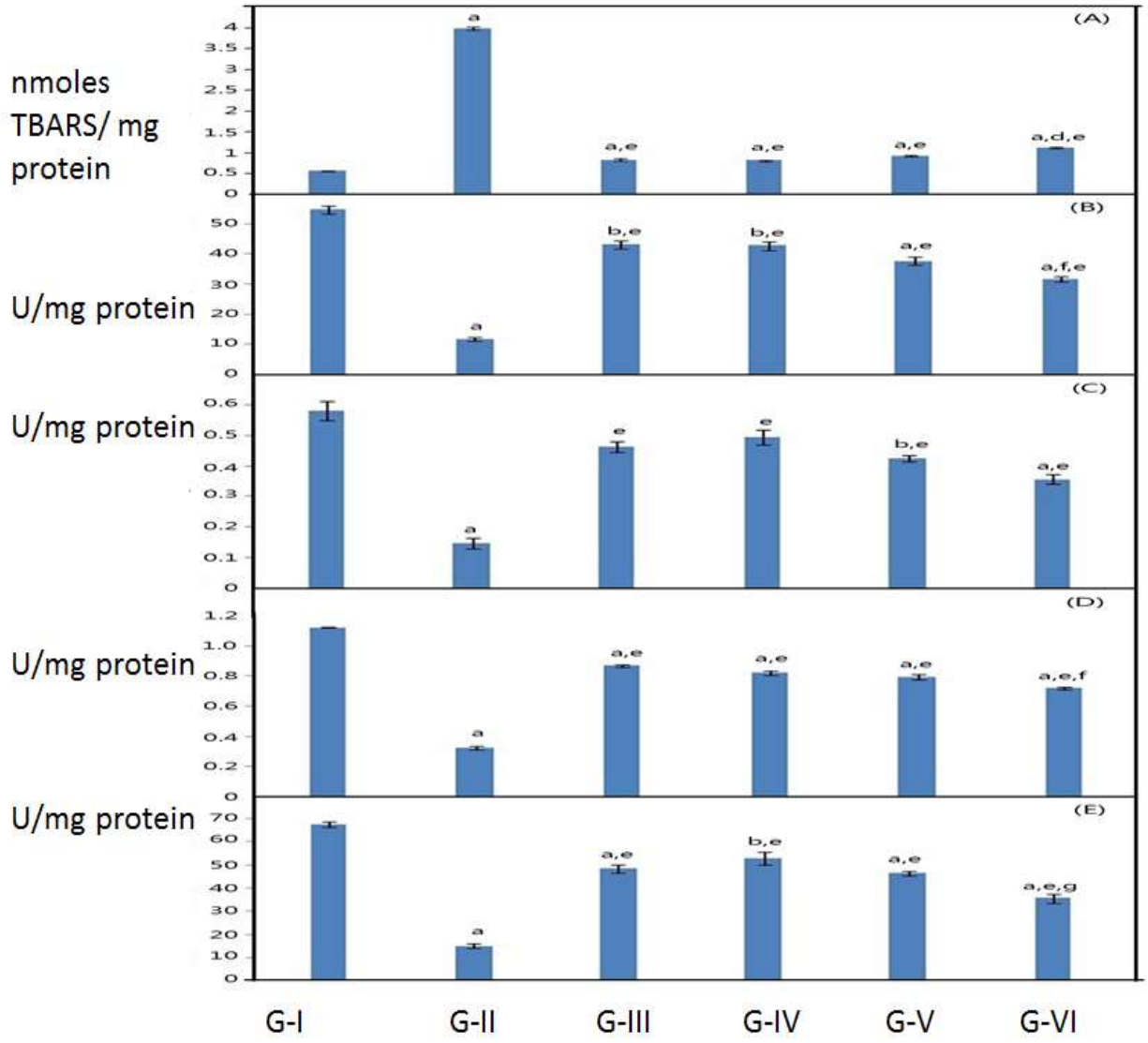


Fig. 12. Antioxidant efficacy offered by 2,3,6 trimethyloct-6-enal at different dose concentration manner on tissue enzymatic and non enzymatic levels of Swiss albino mice against CCl_4 induced hepatic damage. (A) changes in LPO level, (B) changes in GSH level, (C) changes in CAT level, (D) changes in SOD level, (E) changes in GPx level

a- compared with control P<0.001, b- compared with control P<0.01,c- compared with control P<0.05, d- compared with standard P<0.001, e- compared with toxic P<0.001,

f- compared with standard P<0.01, g - compared with standard P<0.001

Group I-control, Group II- toxic (CCl_4), Group III- standard (Silymarin), Group IV- 100 mg/kg b.w.p.o. dose of 2,3,6,trimethyloct-6- enal, Group V-200 mg/kg b.w.p.o. dose of 2,3,6,trimethyloct-6- enal, GroupVI- 300 mg/kg b.w.p.o. dose of 2,3,6,trimethyloct-6-enal.

Histopathology of treated mice livers after treatment with 2,3,6 trimethyloct-6-enal at different dose concentration manner:

The protective efficacy of isolated compound F5A (100, 200, 300 mg/kg b.w.p.o.) from bark of *Pajanelia longifolia* (Willd.) K. Schuman were further analysed by histopathological study where it was found that 100 mg/kg b.w.p.o. dose of the 2,3,6-trimethyloct-6-enal exhibited a maximum level of healing of necrosis (Fig 13D) which was nearly similar to the control group where normal hepatocytes (Fig 13A) were found. Whereas the liver sections of CCl_4 intoxicated mice showed cellular degradation and necrosis (Fig 13B). On the other hand healing of necrosis was found to be similar in the case of 100 mg/kg b.w.p.o dose of 2,3,6-trimethyloct-6-enal and 50 mg/kg b.w.p.o. dose of standard drug Silymarin (Fig 13D and 13C respectively).

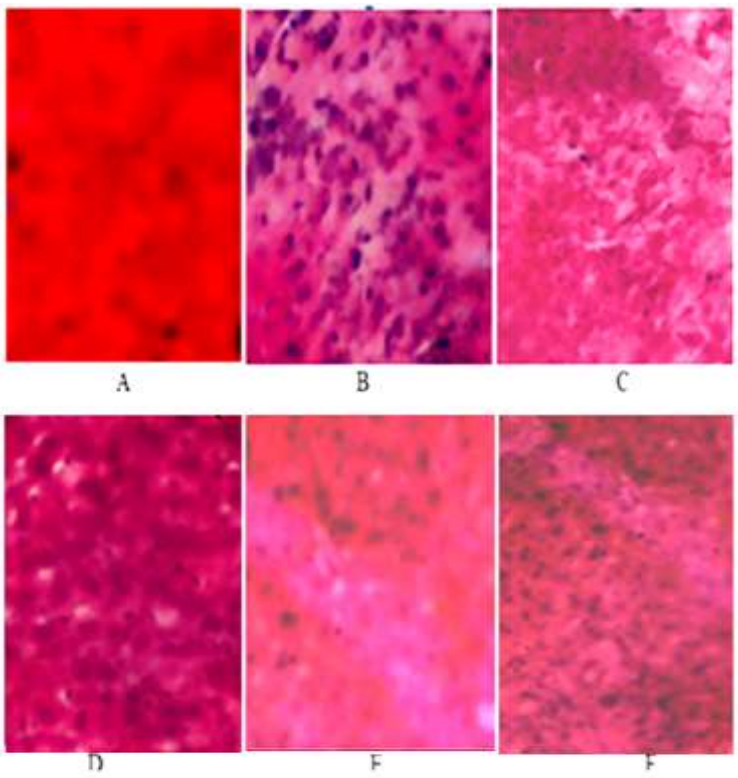


Fig. 13. Histopathological studies of sections of mice liver on 6th day after treatment. (A) Control, (B) CCl_4 (0.5 ml/kg b.w.i.p.),(C) Silymarin (50 mg/kg b.w.p.o.), (D) 100 mg/kg b.w.p.o. dose of 2,3,6 trimethyloct-6-enal, (E) 200 mg/kg

b.w.p.o. dose of 2,3,6 trimethyloct-6-enal, (F) 300 mg/kg b.w.p.o. dose of 2,3,6 trimethyloct-6-enal.

Enclosures – **Appendix – I and Appendix – II**

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