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Amelioration of CCl_4 Induced Liver Injury in Albino Rats by Antioxidant Rich Leaf Extract of *Biophytum sensitivum.* L

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Abstract
Objective: The present study was aimed to evaluate the antioxidant and hepatoprotective activity of *Biophytum sensitivum* L. plant extracts against CCl_4 induced liver damage in albino rats. Materials and methods: Hepatotoxicity was induced by using CCl_4.

Keywords
Antioxidant, Hepatoprotective activity, *Biophytum sensitivum* L

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INTRODUCTION:
For thousands of year's human beings are using plant sources to cure illness[1]. The active chemical compounds are synthesized from the plants. Various plant materials are exclusively used in pharmaceuticals, cosmetics, and food industry, and are recommended as efficient antioxidants[2]. Plants are the chemical factories of nature, producing many secondary metabolites, some of which have medicinal and insecticidal properties *Biophytum sensitivum* (L.) is an herbaceous plant belonging to oxalidaceae family[3]. The phytochemical constituents of the plant shown that amentoflavone[4], 3,8 – biepigenin[5], proanthocynidines[6] and Phenolic compounds[7]. It has several medicinal properties like antiseptic, the plant parts are used in the treatment of asthma, inflammatory diseases and diabetes[8-10]. The plant shows hypoglycemic[11], hypocholesterolemic, immunomodulatory[12], anti-inflammatory, chemoprotective[13], antitumor, cell-mediated immune response[14], and antibacterial activity.

MATERIALS AND METHODS:
Collection of plant material
The Leaves of *Biophytum sensitivum* L. (*Oxalidaceae*) plant was collected during the month of November 2013 from Tirumala Hills, Tirumal, Chittoore District, India. The plant was authenticated by Dr. Madhava Setty, Department of Botany, S.V University, Tirupathi.

Preparation of the extracts
After collection of leaves of *B.Sensitivum* the leaves were shade dried and powdered. The powder was subjected to successive solvent extraction by using methanol and water. Then the dried extract was obtained by evaporation of the solvent using a rotatory vacuum evaporator at 50 ºC and kept in

desiccator. The extract is further studied for phytochemical investigations and animal studies.

Pharmacological activities:

Experimental animals:

Wistar albino rats weighing 150-200g were used for experimental purpose they acclimatized for one week in experimental room. After acclimatization the animals were selected for final allotment of the study. Feed and water were given *ad libitum* throughout the study. All the animal experiments were conducted according to the ethical norms approved by the Institutional ethical committee of CPCSEA, New Delhi (Reg. No.: 1722/Ro/Ere/S/13CPCSEA).

Determination of Acute toxicity (LD_{50}):

Plant extracts of *B.sensitivum* L*.,* up to a higher dose of 2 g/kg were administered orally to normal rats. During the first four hours after the drug administration, the animals were observed for gross behavioral changes. The parameter such as hyperactivity, grooming, convulsions, sedation, hypothermia, body weight and mortality was observed up to 14 days. No mortality was observed with oral administration of all the extracts even at the highest dose of 2 g/kg, p.o. according to OECD guidelines.

Experimental design:

To evaluate the antioxident and hepatoprotective potential of *B sensitivum* L., in Carbon tetrachloride (CCl_4) - induced hepatic damage, 42 rats was randomly divided into seven equal groups. Group I served as a control. Group II received CCl_4 (1ml/kg b.w., p.o, in olive oil 1:1 ratio) once daily for 21 days Group III received daily oral dose of Silymarin (50mg/kg b.w.) along with CCl_4, Group IV and V received once daily oral dose of 200, 400mg/kg b.w. of MEBS respectively along with CCl_4, Group VI and VII received once daily oral dose of 200, 400mg/kg b.w. of AQEBS, respectively along with CCl_4, All groups were administered with their respective drugs for 20 days and blood was collected by retro orbital plexus and transferred to sterilized non-heparinized syringes to separate serum for biochemical analysis and liver was collected and preserved for histopathological examinations. The serum was stored at -10 ^{o}C until biochemical analysis which was carried out within 24 hrs.

Determination of serum biochemical parameters: The biochemical parameters includes serum enzymes SGPT, SGOT by (uv kinetic method)[15], ALP by by p-NPP method[16] by Schlebusch *et al.*, 1974., Total Bilurubin by taylor RLS *et al*, 1996[17] and Total Protein by Lowry's method by Dunn *et al*, 1992[18] were estimated.

Determination of anti-oxidant enzymes from liver homogenate: anti-oxidant parameters includes SOD by method of pyrogallol[19], CAT by the method of Aebi (1974)[20], GSH by the method of Ellman, (1959) [21]and lipid peroxidation by ohkawa *et al,* 1979 [22]by using liver homogenate.

Histopathological examination The sections of liver were processed for histopathological examination involving tissue fixation and were then mounted using DPX for microscopic examinations[23].

Statistical analysis Experiments were performed in triplicate and data analyzed are Mean ± SEM subjected to one-way ANOVA by using Graph Pad Prism 7. Means were separated by the Tukey's multiple range test when analysis of variance (ANOVA) was significant ($p<0.05$). Pearson correlation test was used to assess correlations between means.

RESULTS AND DISCUSSION:

Acute toxicity studies[1]

Acute toxicity studies for dried methanolic and aqueous extracts of *B. sensitivum* L., *was* carried out in different groups of mice (10 mice/group) with one group served as control group, at different graded doses (oral) of the dried plant extract of *B. Sensitivum* L., (200 – 4000 mg/kg, orally), diluted with 1% w/v of CMC suspension, showed no gross evidence of any toxicity and abnormalities in the mice up to 72 hr of the observation period. As no mortality signs had been observed even after administration of dose up to 4000 mg/kg of body wt, the extract might have LD50 value beyond 4000 mg/kg. Hence, further pharmacological investigation was carried at dose levels equivalent to 1/10th of LD50 (here maximum therapeutically safe dose) eqvalent to 400mg/kg and below. Acute toxicity study was done as per OECD Guidelines 423.

Rats treated with CCl_4 developed a significant hepatic damage observed as elevated serum levels of biochemical parameters like SGOT, SGPT, ALP, Total Protein and Total Bilrubin when compared to normal control. Pretreatment with Silymarin, and extracts of *Biophytum Sensitivum* L. leaves had showed good protection against CCl_4 induced toxicity to liver. Silymarin and extract treated animals showed significant reduction in elevated levels of biochemical parameters compare to toxic control animals which is evident in table no: 2

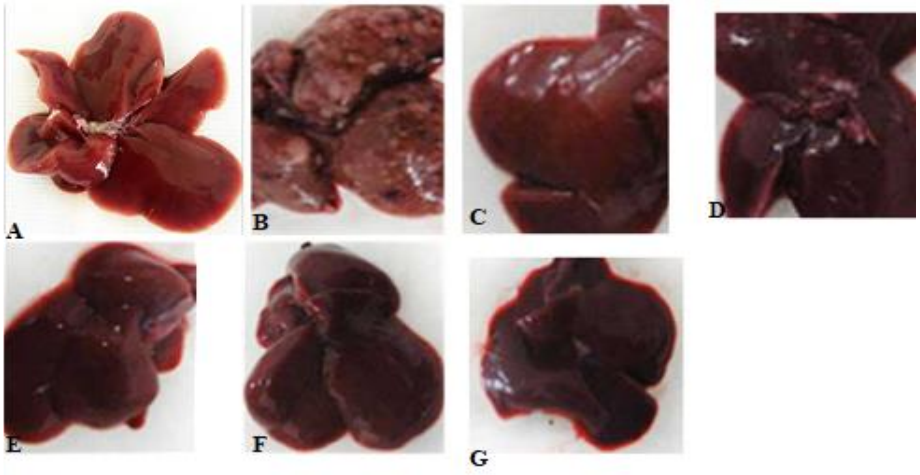
Antioxidant activity:

Effect of *B.sensitivum.L* leaf extracts on antioxidant parameters in CCl_4 induced hepatotoxic rats.

Rats treated with CCl_4 developed a significant hepatic damage observed by marked decreased in levels of antioxidant parameters like Superoxide dismutase, Catalase, Reduced Glutathione and increase in levels of lipid peroxidation when compared to normal control. Pretreatment with silymarin, and extracts of *B. Sensitivum.L* leaves had showed good protection against CCl_4 induced toxicity to liver. Silymarin and extract treated animals showed significant increase in levels of antioxidant enzymes and decrease in levels of lipid peroxidation compare to toxic control animals which is evident in table no: 3

Fig:1 Extraction by Roary Flask Evoparator

Photographs of isolated rat livers:



Effect of *Biophytum Sensitivum. L* on CCL_4 induced hepatotoxicity in Wister rats

A. Normal B. Disease control c. Standard D.Low dose of methanol extract E. High dose of methanol extract F. Low dose of aqouse extract G. High dose of aqouse extract

Fig: 2 Effect of *Biophytum sensitivum* L. on isolated rat livers

Histopathological studies:

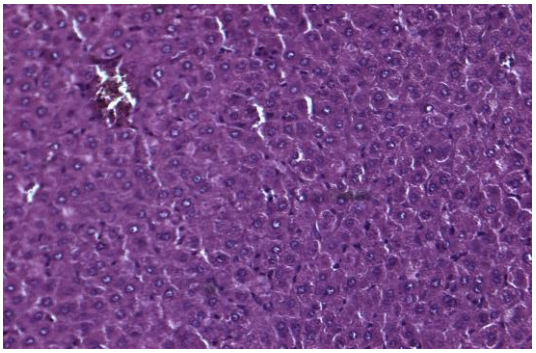


Fig: 3 Group: I Normal control

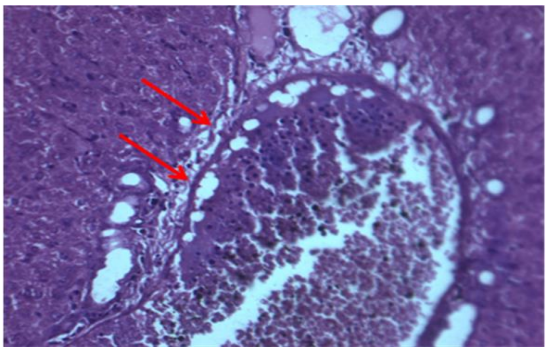


Fig: 4 Group: II Disease Control (CCL_4)

Fig.3 control shows hepatocytes appeared normal, no degeneration of necrosis and inflammation.
Fig.4 moderate to severe periportal fibrosis in which proliferation of connective tissue is noticed with red arrow

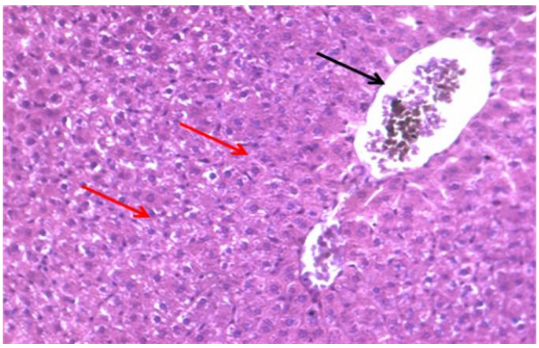


Fig: 5 Group: III Standard (Silymarin)

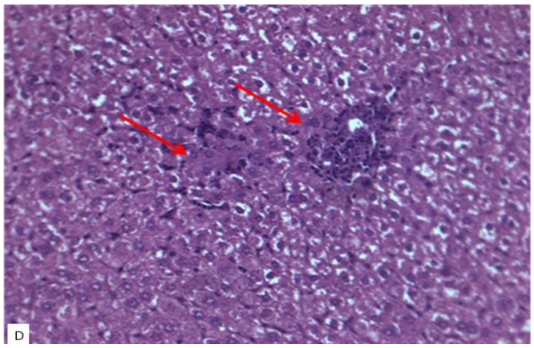


Fig: 6 Group: IV Methanol extract(200 mg/kg

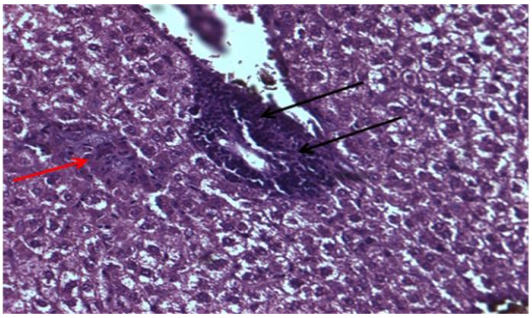


Fig: 7Group: V Methanol extract (400 mg/kg)

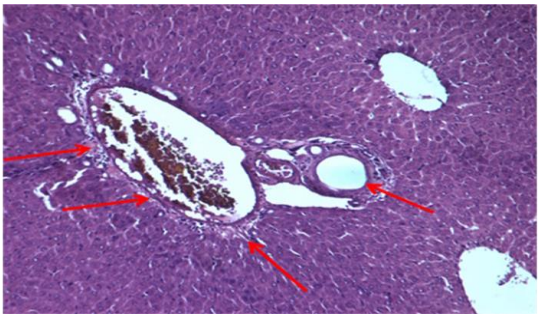


Fig: 8 Group: VI Aqueous extract e**xtract (200 mg/kg)**

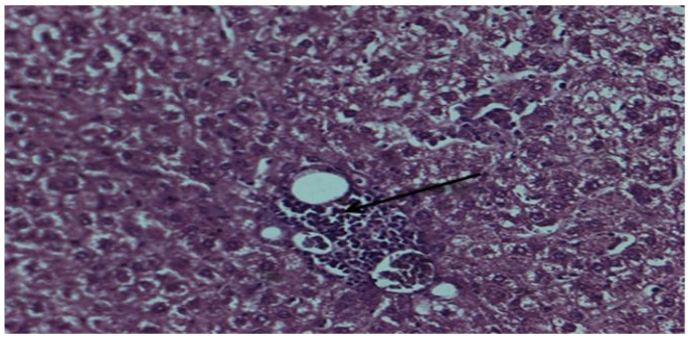


Fig: 9 Group: VII Aqueous extract (400 mg/kg)

Fig.5 sliymarin treated shows Central vein [portal vein], peri portal region appeared normal – black hepatocytes appeared normal – Red arrow Fig.6 200 mg/kg methanol extract treated shows multiple foci of inflammation surrounding central vein in liver – Red Fig: 7 400mg/kg methanol extract treated shows Mild periportal inflammation in which infiltration inflammatory cells particularly lymphocytes noticed in the liver – Black arrow

also, mild foci of necrosis in centri lobular region of liver – Red arrow. Fig.8 200 mg/kg aqueous extract treated shows Mild to moderate peri portal inflammation and fibrosis noticed in the liver – Red arrow.Fig.9 400 mg/kg aqueous extract treated shows Mild foci of inflammation in which infiltration inflammatory cells particularly lymphocytes noticed surrounding the central vein in liver – Black arrow

Table: 1The % yield of both methanol and aqueous extracts are as follows

S.NO	Extracts	Colour	Consistency	%Yield (w/w)
I	Methanol	Greenish black	Semisolid and non- sticky	17.4
II	Aqueous	Light dark green	Semisolid and nonsticky	21.1

Table no: 2 Effect of *Biophytum sensitivum* L. Extracts on biochemical parameters in CCL_4 induced hepatotoxic rats.

Groups	Dose (mg/kg)	AST(U/L)	ALT (U/L)	ALP (U/L)	TBL (mg/dl)	TP (U/L)	Liv.wt (g/100g)	liv.vol (ml/100g)
Normal control (1ml of CMC)	1ml	50.94±1.61	80.23±1.62	129.30±2.07	0.31±0.01	7.8±0.14	3.72±0.08	4.32±0.09
Disease control (CCL_4)	1ml	231.10±4.1$^{###}$	324.88±4.75$^{###}$	373.32±5.41$^{###}$	6.75±0.24$^{###}$	4.11±0.14$^{###}$	6.75±0.15$^{###}$	7.43±0.12$^{###}$
Standard (silymarin)	50	73.75±2.06	129.54±3.57	174.±2.12	0.71±0.02	6.89±0.12	4.28±0.03	4.82±0.03
MEBS low dose	200	170.45±3.87	185.94±3.13	177.07±3.57	2.9±0.15	4.47±0.12	4.27±0.03	5.23±0.02
MEBS high dose	400	121.26±3.96**	131.35±2.74**	121.97±5.28**	1.43±0.09**	5.37±0.06***	3.87±0.05***	4.85±0.06**
AEBS low dose	200	159.76±3.53	152.76±3.53	167.32±3.72	1.81±0.05	5.30±0.04	4.11±0.04	4.95±0.08
AEBS high dose	400	100.55±3.67***	110.60±4.31***	106.59±5.04***	1.14±0.05***	5.95±0.04***	3.72±0.06***	4.15±0.08***

Values are expressed in Mean ± SEM (n=6) Tukey's multiple range test when analysis of variance (ANOVA) was significant (p<0.05).

Graph: 1 Graphical representation of biochemical parameters of *Biophytum sensitivum* L. in CCL_4 induced hepatotoxicity rats.

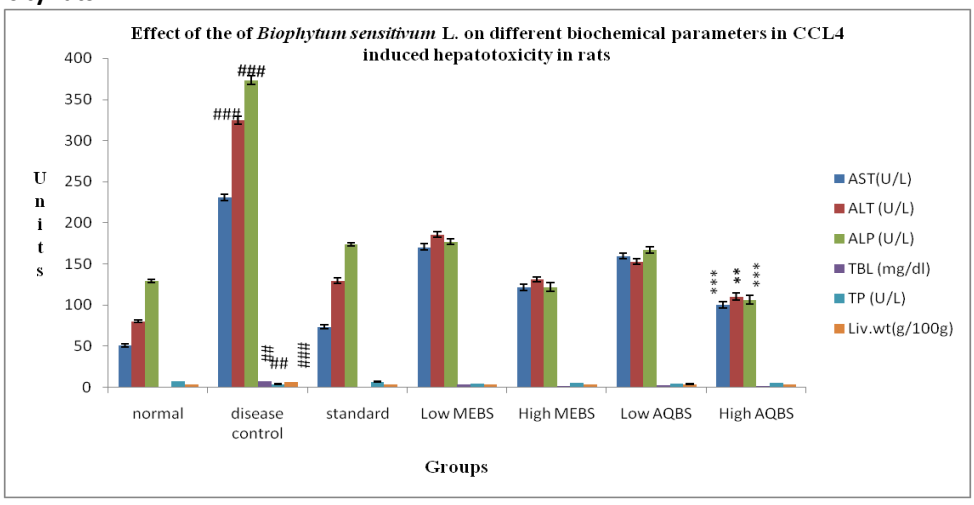
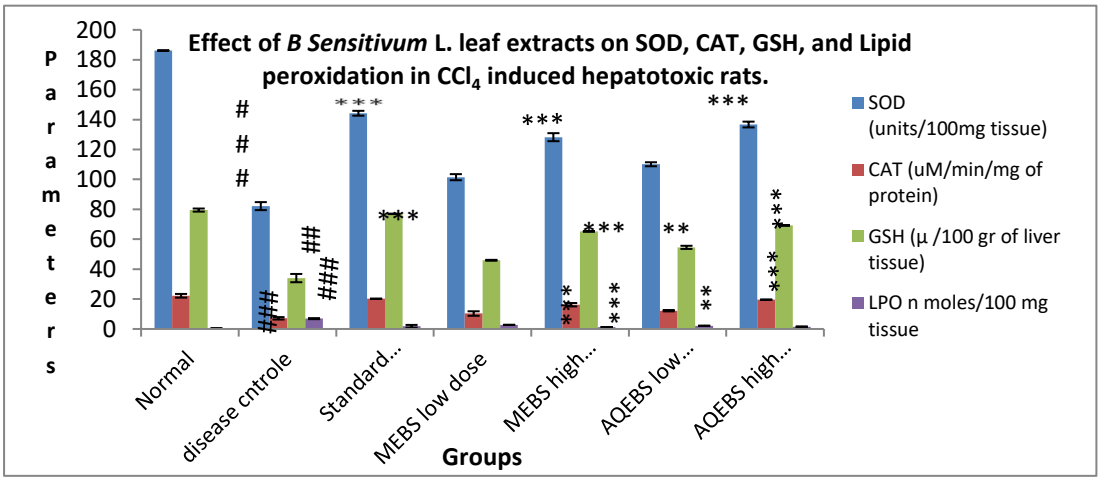


Table no. 3: Effect of *B Sensitivum* L. leaf extracts on SOD, CAT, GSH, and Lipid peroxidation in CCl_4 induced hepatotoxic rats.

Groups	Treatment	Dose (mg/kg)	SOD (units/100mg tissue)	CAT (uM/min/mg of protein)	GSH (µ moles/100 gr of liver tissue)	LPO n moles/100 mg tissue
I	0.9% Saline	1ml	186.15±0.11	22.19±1.23	79.5±1.04	0.29±0.2
II	CCL_4	1ml	82.14±2.68 ##	7.12±.81 ##	34.01±2.77 ##	6.9±0.34 ##
III	Standard (silymarin)	50	144.23±1.62***	20.19±0.1***	76.96±0.15***	1.8±0.9
IV	MEBS low dose	200	101.49±2.03	10.32±1.51	45.92±0.3	2.74±0.07
V	MEBS high dose	400	128.24±2.68	16.21±1.12	65.23±0.06	1.24±0.08
VI	AQEBS low dose	200	110.12±1.34**	12.21±0.46**	54.56±1.05**	2.12±0.06**
VII	AQEBS high dose	400	136.69±1.94***	19.56±0.2***	69.23±0.09***	1.52±0.21***

Values are expressed in Mean ± SD (n=6)
##indicate significance from the control group at P<0.01 probability level.
*****indicate significance from the CCl4 group at P<0.01 probability level.**

Graph: 2 Graphical representation of effect of *B.Sensitivum* L. leaf extracts on SOD, CAT, GSH and Lipid Peroxidation in CCL_4 induced hepatotoxicity rats



DISCUSSION

In the Present research work the MEBS and AQBS of dose (400 mg/kg) have shown better results of hepatoprotection against CCL_4 induced hepatic damage. The trichloromethyl radicals that are metabolites of CCL_4 binds to organs and cell membranes covalently and causes peroxidation of lipids that leads to hepatic damage by altering the functional integrity of mitochondria of liver. The phytoconstituents of extract induces enzymatic activity resulting in inhibited lipid peroxidation or accelerated excretion of CCL_4. The following phytoconstituents such as (biflavones, three flavonoids luteolin-7-methyl ether, isoorientin, 3'-methoxyluteolin 7-O-glucoside, as well as two acids, 4-caffeoylquinic acid and 5-caffeoylquinic acid) have been detected in plant extracts which were already proven for hepatoprotective activity.

The parameters referred as markers for liver damage were serum SGOT, SGPT, ALP and Total Bilurubin. There was significant change or decrease in the levels of above markers upon administration of high doses of MEBS and AQBS. Whereas no change was observed for low doses of extracts. Among the two extracts AEBS as shown dose dependent and high hepatoprotective activity and hence was selected to compare with positive control group (Silymarin).

Carcinogensis of tissue is a result of oxidative stress induced by generation of free radicals and decreased antioxidant levels in target cells and tissues. Leak

down of enzymes to the circulatory fluid and their assessment serves as markers in cell membrane damage. Lipid peroxidation can be inhibited by free radical scavenging. The groups pretreated with standard drug and high dose of extract have shown decreased lipid peroxidation by decrease in MDA levels. Elevation of GSH, SOD and CAT activities was found. In present study the rats treated with standard and high doses of extracts shown increase in catalase, superoxide dismutase and GSH and decrease in MDA levels indicating that they possess antioxidant and hepatoprotective activity.

The histopathology studies conducted shows that low doses of extracts have shown multiple foci of necrosis or mild to moderate inflammation in liver. Whereas the high doses of extracts show mild inflammatory changes by compared to disease control (CCL_4 induced).

CONCLUSION:

Based on above findings it can be concluded that antioxidant and hepatoprotective activities reported for high dose of aqueous extract shows significant protection against CCL_4 induced hepatic damage.

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