CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

http://doi.org/10.5281/zenodo.1307365

Available online at: http://www.iajps.com Research Article

EVALUATION OF ANTIOXIDANT AND HEPATOPROTECTIVE ACTIVITY OF METHANOL EXTRACT OF *BEGONIA CRENATA* ROOTS

Ambujakshi H R[1], Seru ganapaty[2], Ganga Rao Battu[3]

[1]Department of pharmacognosy, Acharya & B M Reddy College of Pharmacy, Bengaluru
[2]GITAM University Visakhapatnam-530 045 INDIA
[3]A U College of pharmacy, Andhra University, Visakhapatnam-530 003 INDIA

Abstract:

The objective of the present study is to evaluate antioxidant and hepatoprotective effects of methanol extract of Begonia crenata roots. Antioxidant activity was evaluated by using Diphenyl picryl hydrazyl (DPPH), superoxide and hydroxyl radical scavenging methods. The hepatoprotective activity of the extract was studied using wistar rats. The radical scavenging activity of the extract was found to be concentration dependant and comparable with ascorbic acid. In the hepatoprotective activity study, CCl_4 significantly increased the level of serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), serum alkaline phosphatase (SALP) and total bilirubin (T.BILL). The rats were treated with methanol extract of Begonia crenata at doses of (100, 200 and 400 mg/kg p. o.) exhibited a significant reduction of SGOT, SGPT, SALP and T.BILL and the inhibition was comparable with silymarin (50 mg/kg p.o). The studies revealed that Begonia crenata extract showed significant antioxidant and hepatoprotective activity.

Key words: *Antioxidant, hepatoprotective, Begonia crenata, methanol, biochemical estimation, CCl_4.*

Correspondin author:

H.R. Ambujakshi,
Asst.Prof. & Dean Administration
Acharya & B.M Reddy College of Pharmacy,
Acharya Dr. Sarvepalli Radhakrishnan Road,
Acharya Post Office,Soldevanahalli,
Hessarghatta Road,Bangalore - 560 107,
Karnataka, India
E- mail: ambujakshi@acharya.ac.in

QR code

Please cite this article in press Ambujakshi ***H R*** *et al.,* ***Evaluation Of Antioxidant And Hepatoprotective Activity Of Methanol Extract Of Begonia Crenata Roots,*** *Indo Am. J. P. Sci, 2018; 05(07).*

INTRODUCTION:

Plant medicine has been utilized successfully for thousands of years and is of great value in the field of treatment and cure of disease. In recent times, demand for herbal drugs is increasing throughout the world. As per the World Health Organization (WHO), 80% of the population of developing countries relies on traditional medicines for their primary health care needs. It is necessary to investigate the rationality of folklore use of medicinal plants for various ailments in modern scientific method in spite of sound traditional back ground and information available in the ancient literature [1,2].

Free radicals are molecules containing unpaired electrons are highly reactive and react with other molecules by taking or giving electrons, involved in wide variety of pathological effects such as DNA damage, carcinogenesis and various degenerative disorders such as cardiovascular diseases, aging and neuro-degenerative diseases [3]. There is a dynamic balance in the amount of free radicals generated in the body and antioxidant to quench them and protect the body against the harmful effects. It necessitates maintaining balancing of antioxidants in the body. Many plant extracts and plant products known to have significant antioxidant activity to scavenge free radicals [4].

Begonia crenata Dryand. belongs to the family, Begoniaceae is a small, herbaceous, 1-4 leaved plants; root sub-tuberous stems usually red, smooth slender. The root decoction is given for liver diseases and fever [5,6]. However the, literature survey indicated no published reports on the antioxidant and hepatoprotective activities of the *Begonia crenata* root. In the view of the lack of study on selected plant, the author planned to give scientific proof by studying *in vitro* antioxidant activity by DPPH, superoxide and hydroxyl radical scavenging assay and hepatoprotective activities by carbon tetrachloride induced liver toxicity model.

MATERIALS AND METHODS:

Plant material:

The roots of Begonia crenata (Begoniaceae) collected from north Karnataka and were authenticated by V Chelladurai, Research officer (Retired)-Botany, Central Council for Research in Ayurveda and Siddha, Government of India.

Preparation of the extract:

Freshly collected roots were shade dried, coarsely powdered and exhaustively extracted with methanol in Soxhlet apparatus for 48 hours. The extracts were concentrated under controlled temperature to dryness in rotary flaks evaporator and percentage of yield was found to be 29.31% w/w.

Drugs and chemicals:

Ascorbic acid (Sigma Aldrich Chemie, Germany), Riboflavin (S.D chemicals, India), Silymarin (Himalaya Drug Company), SGOT, SGPT, SALP and Bilirubin estimated kits were purchased from Span Diagnostics, Surat, India. All others reagents and chemicals used in this study were of analytical grade purchased from local source.

Phytochemical analysis:

Phytochemical screening was carried out by qualitative test for the presence of alkaloids, flavonoids, glycosides, phytosterols, tannins and triterpenoids, carbohydrates, proteins and aminoacids [7,8].

***In-vitro* antioxidant activity**

The methanol extract of *Begonia crenata* root *was* screened for antioxidant activity against DPPH, superoxide radical and hydroxyl radicals. The percentage inhibition and 50% inhibition concentrations (IC_{50}) were calculated.

Calculation of percentage inhibition:

The percentage inhibition was calculated using the formula:

% Inhibition =

DPPH-Radical-Scavenging activity:

The evaluation of the DPPH radical scavenging activity was performed according to methodology described by Alessandra B et al [9]. Methanol extract of *Begonia crenata* at various concentrations (40, 80, 120, 160, 200, 240, 280, 320 and 360 µg/ml) were reacted with 3 ml of 0.004% DPPH solution in methanol. The mixture was shaken vigorously and allowed to reach a steady state at room temperature for 30 min. The change in color from dark blue to yellow was determined by measuring the absorbance at 517 nm. A control was prepared using 0.1 ml of vehicle in the place of Methanol extracts of *Begonia crenata* /ascorbic acid.

Superoxide radical scavenging activity:

Superoxide radical scavenging activity of the extract was measured according to Riboflavin photo reduction method [10]. The reaction mixture in a tube contained EDTA (6 µM): NaCN (3 µg); riboflavin (2 µM); NBT (50 µM); KH_2PO_4-Na_2HPO_4 buffer (67 µM, pH 7.8) and 0.1 ml of different concentrations (40, 80, 120, 160, 200, 240, 280, 320 and 360 µg/ml) of methanol extract of *Begonia crenata*, in a final volume of 3 ml of phosphate buffer. The optical

densities were measured at 560 nm after uniformly illuminating assay tubes with an incandescent light (40 Watt) for 15 minutes. The percentage inhibition of superoxide production was evaluated by comparing the absorbance values of control and experimental samples.

Screening for Hydroxyl Radical Scavenging Activity:

The screening of hydroxyl radical scavenging activity of methanol extracts of *Begonia crenata is based on* Deoxyribose degradation method [11]. Hydroxyl radicals generated from Fe^{3+}-ascorbate-EDTA-H_2O_2 system (Fenton reaction) was estimated by its degradation of deoxyribose that resulted in thiobarbituric acid reactive substance (TBARS). Fenton reaction mixture consisting of 200 µl of 10 mM ferrous sulphate , 200 µl of 10 mM EDTA and 200 µl of 10 mM 2-deoxyribose and was mixed with 1.2 ml of 0.1 M phosphate buffer (pH 7.4) and 200 µl of plant extract. Thereafter 200 µl of 10 mM H_2O_2 was added before the incubation at 37°C for 4 h. Then 1 ml of this Fenton reaction mixture was treated with 0.2 ml of 8.1% sodium dodecyl sulphate, 1.5 ml of 0.8% thiobarbituric acid and 1.5 ml of 20 % acetic acid. The total volume was then made to 5 ml by adding distilled water and kept in an oil bath at 100°C for 1 hour. After the mixture had been cooled, 5 ml of 15:1 v/v butanol-pyridine mixture was added. Following vigorous shaking, the tubes were centrifuged at 4000 rpm for 10 min and the absorbance of the organic layer containing the thiobarbituric acid reactive substances was measured at 532 nm. The percentage inhibition of hydroxyl radicals by the extract was determined by comparing the absorbance values of the control and the experimental samples.

Animals:

Wistar albino rats of either sex weighing between 200–250 g were housed under standard environmental conditions (temperature of 22 ±1°C with an alternating 12 hrs light/dark cycle and relative humidity of 60 ± 5%). The study protocol was approved by the Institutional Animal Ethics Committee (IAEC/ABMRCP/2012-2013/25).

Acute toxicity study:

The acute toxicity study was conducted for methanol extract of the *Begonia crenata* root as per Organization for Economic Co-operation and Development (OECD) guidelines 423 (OECD 2001) [12] using female albino rats. Mortality rate were observed at the dose levels of 5 mg, 50 mg, 300 mg to 2 g/kg.

Hepatoprotective activity:

The animals were fed with standard diet and water *ad libitum* for two weeks before and during the experimental period. The animals were divided into 6 groups containing 6 animals each. Group I served as positive control received 5% gum acacia suspension. Group II served as negative control received CCl_4 (1 ml/kg p. o.). Groups III-VI was treated with Silymarin (50 mg/kg) and methanol extract of *Begonia crenata* root with 100, 200 and 400 mg/kg respectively for 5 days. On 6^{th} day, CCl_4 was administered to all groups except group-I one hour after the administration of drug. On the 7^{th} day blood samples were collected from retro orbital puncture and serum was separated by centrifugation for estimation of biochemical markers (SGOT, SGPT, SALP and T.BILI.) using autoanalyzer. Then animals were anaesthetized using ether, liver was collected for histopathological analysis.

Histopathological studies:

Liver tissue section was fixed in 10% buffered neutral formalin for 24 h. Hepatic tissues were stained with haemotoxylin and were examined for histopathological studies.

Statistical analysis:

All data were represented as mean ± SEM. The results were analyzed by one way ANOVA followed by Dunnet's test. P values <0.05 were considered as statistically significant.

RESULTS:

The phytochemical analysis of methanol extract of *Begonia crenata* root

The preliminary phytochemical tests revealed the presence of alkaloids, flavonoids, glycosides, triterpenoids and steroids **[Table No1]**

Table 1: Phytochemical analysis of methanol extract of *Begonia crenata* root

Sl No	Phytoconstituents	Methanol extract
1	Alkaloids	+
2	Carbohydrates	-
3	Flavonoids	+
4	Glycosides	+
5	Phytosterols	+
6	Proteins & amino acids	-
7	Saponins	-
8	Tannins	-
9	Triterpenoids	+

'+' Present, '-' Absent

Effect of *Begonia crenata* extract against DPPH radicals

The free radical scavenging activity of *Begonia crenata* extract against DPPH radicals was shown in **Figure 1.** *Begonia crenata* extract and ascorbic acid showed antioxidant activity in a dose-dependent manner in the range of 40-360 µg/ml and produced maximum scavenging activity at a dose of 360 µg. The IC_{50} values for *Begonia crenata* and ascorbic acid were 214.33 and 201.75 µg/ml respectively.

Fig. 1: DPPH radical scavenging activity

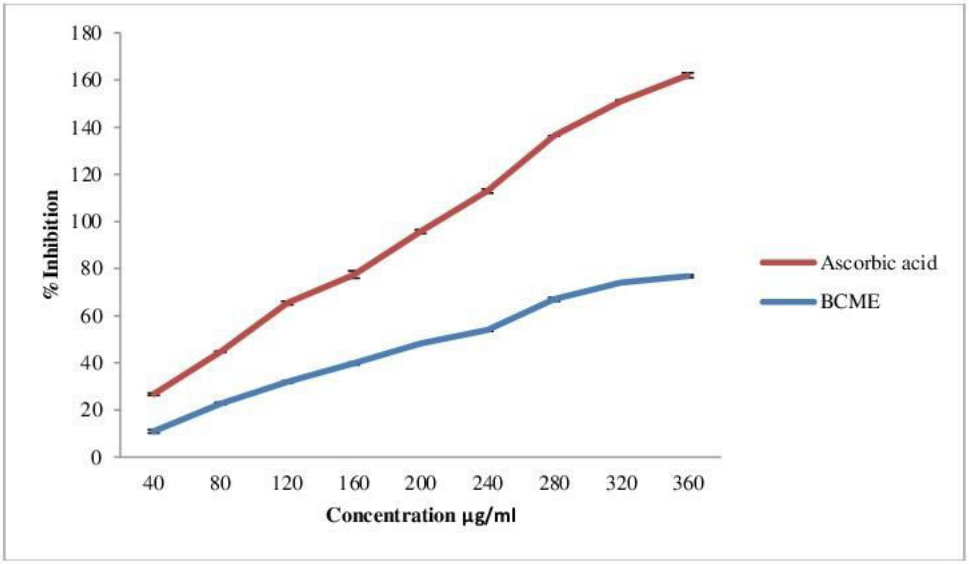

Fig. 1: DPPH radical scavenging activity

Effect of *Begonia crenata* extract on the superoxide scavenging activity

The free radical scavenging activity of *Begonia crenata* extract against superoxide radical was shown in **Figure 2**. *Begonia crenata* extract and ascorbic acid standard showed antioxidant activity in a dose dependent manner (40-360 µg/ml) and showed significant scavenging activity at a dose of 360 µg. The IC_{50} values for extract and ascorbic acid were 246.97 and 176.95µg/ml respectively.

Fig. 2: Super oxide radical scavenging activity

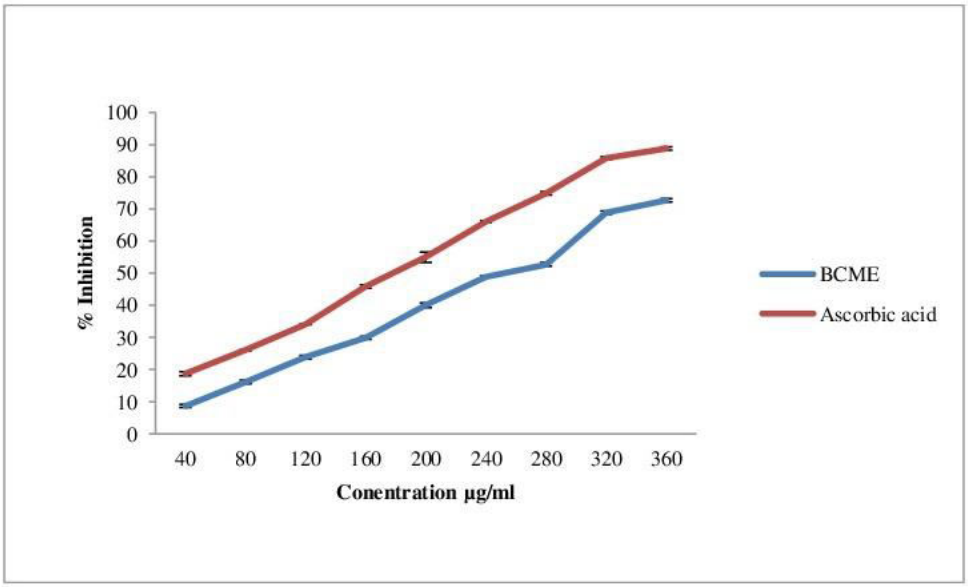

Fig. 2: Super oxide radical scavenging activity

Effect of *Begonia crenata* extract against the hydroxyl radicals

Begonia crenata extract and ascorbic acid showed antioxidant activity in a dose dependent manner in the range of 40-360 µg/ml. The IC_{50} values for *Begonia crenata* extract and ascorbic acid were 199.36 and 211.61 µg/ml respectively (**Figure 3**).

Fig.3: Hydroxyl radical scavenging activity

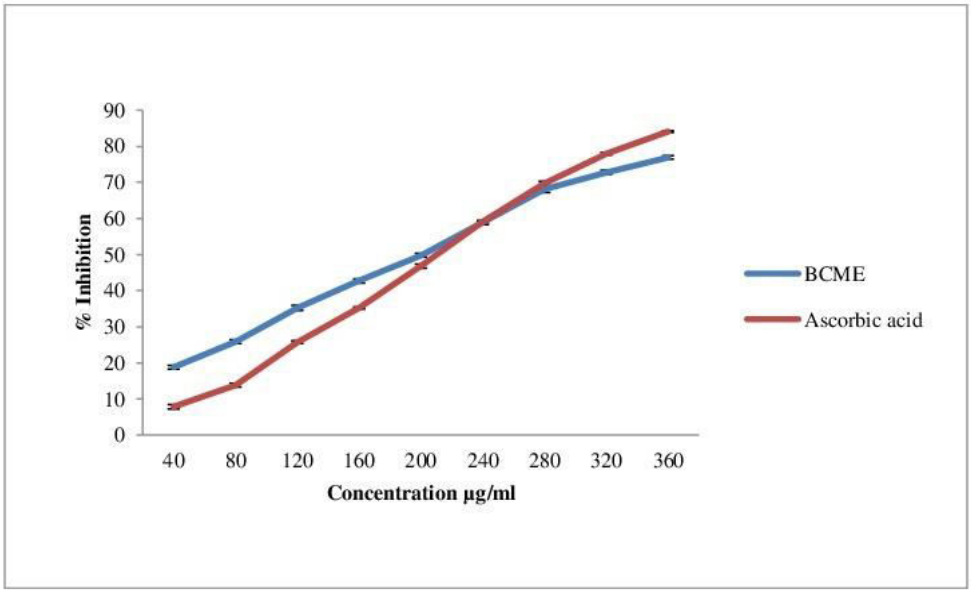

Fig.3: Hydroxyl radical scavenging activity

Biochemical parameters

Serum enzymes, namely SGOT, SGPT, ALP and T.BILI, were significantly ($P < 0.01$) increased in CCl_4 treated control group when compared with normal group. However, levels of serum enzymes, were significantly ($P < 0.001$) decreased in extract treated groups and silymarin in compared with CCl_4 treated rats (**Tables 2).**

Table 2: Effect of methanol extract of *Begonia crenata* root against CCl4 induced hepatotoxicity in albino rats

Serum biochemical parameters					
S. No	**Treatment group**	**SGOT (IU/L)**	**SGPT (IU/L)**	**SALP (IU/L)**	**T. BILI. (mg/dl)**
1	Control (5% gum acacia 1ml/kg p.o.)	78.99±0.45	65.77±0.47	144.84±0.45	0.47±0.06
2	Hepatotoxin- CCl_4 (1ml/kg p. o.)	526.06±0.70**	412.85±0.60***	719.11±0.66***	4.80±0.44***
3	Standard-Silymarin (50 mg/kg)	118.15±0.52***	90.20±0.37***	200.91±0.34***	1.21±0.01**
4	BCME (100 mg/kg)	331.76±0.37***	203.87±0.72***	412.79±0.18***	3.85±0.26***
5	BCME (200 mg/kg)	197.57±0.45***	123.79±0.77***	317.97±0.56***	2.46±0.04**
6	BCME (400 mg/kg)	128.68±0.43***	98.91±0.80***	221.74±0.50***	1.63±0.02**

Data were analyzed by one way ANOVA followed by Dunnet's test. n=6 values are expressed as mean ± SEM. *P<0.05, **P<0.01, ***P<0.001.

Effect of *Begonia crenata* extract on histopathological studies

The results of light microscopy examination of the liver section of normal control, CCl_4 treated, silymarin and extract treated groups were showed in **Figure43. Figure 4A** representing control group liver cells. From that image, it can be observed that the liver cells are normal architecture with distinct hepatic cells, sinusoidal spaces and clear central vein (CV), a well-preserved cytoplasm with prominent nucleus. Overall, a healthy set of cells can be observed. **Figure 4B** shows the liver section of CCl_4 intoxicated rats, it indicated the disarrangement of normal hepatic cells with intense centrilobular necrosis across the cells. The liver sections of these rats indicate vacuolization, fatty changes, sinusoidal hemorrhages and dilation. **Figure 4C** shows liver section of silymarin treated rats showing a normal hepatic architecture with normal hepatocytes, sinusoidal spaces, less vacuole formation, absence of necrosis and less visible changes as compared to control group.

The histopathological examination of rats treated with methanol extract of *Begonia crenata* roots at doses of 100 mg/kg , 200 mg/kg and 400 mg/kg are present in **Figure 4D** , **Figure 4E** and **Figure 4F** respectively demonstrated recovery from CCl_4 induced liver damage as evident from normal hepatocytes and with higher dose of 400 mg/kg showed significant attenuation of inflammatory and necrotic changes and cellular architecture of liver rats was preserved indicating a marked protective activity similar to that observed in silymarin treated rat liver sections and the effect was found to be dose dependant**.**

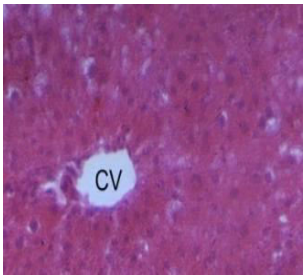

Fig. 3A: Normal control

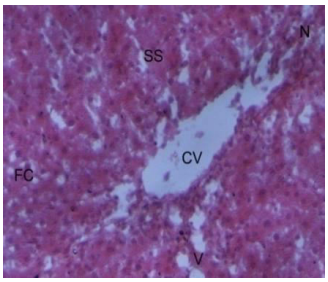

Fig. 3B: Negative control (CCl_4treated)

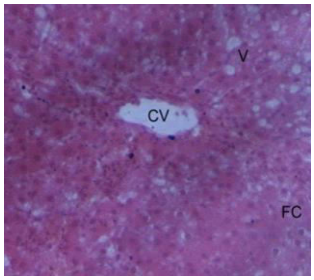

Fig. 3C: Positive control (Silymarin treated)

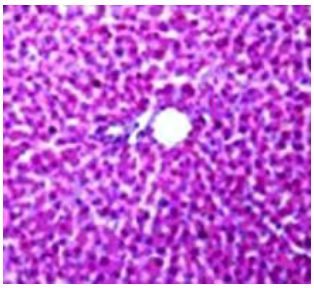

Fig.3D: BCME100 mg/kg

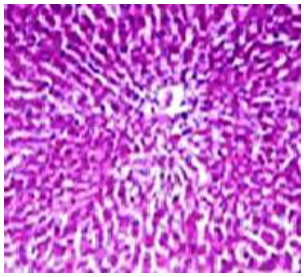

Fig. 3E: BCME200 mg/kg

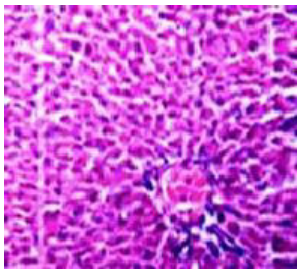

Fig.3F: BCME 400 mg/kg

Fig. 3: Effects of methanol extract of *Begonia crenata* against CCl4-induced liver histopathological changes in mice:

Fig. 3A- Control group; Fig.3B- CCl_4 treated group; Fig. 3C Silymarin + CCL_4; Fig. 3D- *Begonia extract* (100 mg/kg) + CCL_4; Fig. 3E- *Begonia extract* (200 mg/kg) + CCL_4; Fig. 3F- *Begonia extract* 400 mg/kg + CCL_4.

DISCUSSION:

The DPPH assay is based on the reduction of alcoholic DPPH solution in the presence of a hydrogen donating antioxidant converted to the non radical form diphenyl picryl hydrazine. The DPPH scavenging activities of antioxidants are attributed to their hydrogen donating abilities. The method helps to determine the antiradical power of an antioxidant by measuring of a decrease in the absorbance of DPPH.

Superoxide anion plays an important role in the formation of more reactive species such as hydrogen peroxide, hydroxyl radical, and singlet oxygen, which induce oxidative damage in lipids, proteins, and DNA [13]. Therefore, studying the scavenging activity of plant extracts on superoxide radical is most important. At low pH value superoxide will protonate to form the perhydroxyl radical (HO_2 .), a more reactive oxidizing species but at physiological pH less than 1%will be in protonated form [14] .The reactive oxygen radicals are unstable and likely to elicit many of the tissue damages and human diseases [15]. The hydroxyl radicals attack adjoining biological macromolecules and cause oxidative damage to DNA, lipids and proteins, resulting in oxidative stress-originated diseases. Consequently, the removal of hydroxyl radical is most likely to be one of the most effective defenses of a living body against various diseases [16]. The hydroxyl scavenging` activity of *Begonia crenata* was assessed by its ability to compete with deoxyribose for hydroxyl radical generated from the Fe^{2+}/EDTA/H_2O_2 system.

CCl_4 is a hepatotoxin most widely used for the production of experimental liver toxicity. The trichloromethyl radical and trichloromethyl peroxy radical are generated from CCl_4 by the action of the mixed function of cytochrome P-450 oxygenase system are capable of binding with proteins/lipids or abstracting a hydrogen atom from an unsaturated lipid, thus initiating lipid peroxidation. This process of lipid peroxidation can significantly damage hepatic plasma membranes. SGOT, SGPT, SALP and T. BILI. are increased during liver injury. The *Begonia crenata* extract were reduced the injurious effects on hepatic tissue and physiological mechanisms that have been altered by a hepatoxin is the manifestation of its protective effect [17].

Phytoconstituents like flavonoids, glycosides, steroids, triterpenoids and alkaloids were reported to

exhibit hepatoprotective activity [18-21]. The preliminary phytochemical studies of the *Begonia crenata* revealed presence of alkaloids, flavonoids, glycosides, triterpenoids and steroids in the extract. The due to the presence of these active constituents in *Begonia crenata* extract were responsible for antioxidant and hepatoprotective activity.

CONCLUSION:

The present study provides experimental evidence for the hepatoprotective effect of *Begonia crenata* against CCl_4-induced hepatotoxicity in rats appears to be related to its antioxidant property of its phytoconstituents present in the root. Hence the present study justified the traditional use in the treatment of liver diseases. However additional investigations are required to determine the active constituents responsible for hepatoprotective activity.

REFERENCES:

1. Vaidya AB. The Status and Scope of Indian Medicinal Plants Acting on Central Nervous System. Indian J Pharmacol 1997; (29): S340-343.
2. Dahanukar SA, Kulkarni RA. Pharmacology of Medicinal Plants and Natural Products. Indian J Pharmacol 2000; (32): S81- S118.
3. DL Madhavi, SS Deshpande, DK Salunkhe. Food antioxidants: Technological, Toxicological. Health perspectives, Marcel Dekker, New York, 1996, 267-359.
4. S Khlifi, YE Hachimi, A Khalil, N Es-Safi, A Belahyan, R Tellal, E Abbouyi. *In vitro* antioxidant properties of *Salvia verbenaca* L. hydromethanolic extract. Indian J Pharmacol 2006; (38):276-280.
5. Frodin DG. History and concepts of big plant genera**.** Taxon 2004; 53(3): 753-76.
6. Khare CP. Indian Medicinal Plants; An illustrated dictionary, New york: ed .London: Dorling Kindersley 2008.
7. Khandelwal KR., Practical Pharmacognosy techniques and experiments, 8th (Ed.) Nirali Prakashan publications 2001.
8. Kokatae CK. Practical Pharmacognosy, Vallabha Prakashan publications, New Delhi 2002, 107-103.
9. Alessandra B, Gelsomina F, Ivano M, Francesco De S, Franca T, Nunziatina D T. Antioxidant and free radical scavenging activity of flavonols glycosides from different Aconitum species. J Ethnopharmacol 2003; 86: 63-67.
10. Mc Cord JM, Fridovich I.Antioxidant and free radical scavenging activity of flavonol glycosides from different Aconitum species. J Biol Chem 1969; 244(2): 6049-6055.
11. Elizabeth K, Rao MN. Superoxide dismutase. A peroxide dismutase. An enzymic function for erythrocuprein (hemocuprein). Int J Pharm1990; (58): 237-240.
12. OECD. Organization for Economic Co-operation and Development Guidelines for the Testing of Chemicals, Test No 423: Acute Oral Toxicity-Acute Tociz Class Method. 2001
13. Dahl MK, Richardson T. Photogeneration of superoxide anion in serum of bovine milk and in model systems containing riboflavin and amino acids. J Dairy Sci 1978; (61): 400–407.
14. Yun-Zhong Fang, Shang Yang, Guayaouru. Protective effect of liver, cabbage and yeaston radiation damage. Acta Nutrimenta Sinica1987; 2.
15. Halliwell B, Gutteridge JMC. Free radicals in biology and medicine, 2nd edition, Clarendon Press, Oxford University, UK 1989, 301-310.
16. Aruoma OI, Halliwell B, Hoey BM, Butler. The antioxidant action of N-acetylcysteine: its reaction with hydrogen peroxide, hydroxyl radical, superoxide, and hypochlorous acid. Free Radic Biol Med 1989; 6(6): 593–597.
17. Boll M (1), Weber LW, Becker E, Stampfl A. Reactivity of hydroxyl and hydroxyl-like radicals discriminated by release of thiobarbituric acid reactive material from deoxy sugars, nucleosides and benzoate. Z Naturforsch 2001; 56(7-8):649-59.
18. Sarg TM, Abdel Salman NA, EI-Domiaty M, Khafagy SM. Intense oxidative DNA damage promoted by L-DOPA and its metabolites, implications for neurodegenerative disease. Science and Pharmacy 1981 ;(49):262-64.
19. Singh B, Saxena AK, Chandan BK, Agarwal SG and Anand KK. Mechanism of carbon tetrachloride-induced hepatotoxicity. Hepatocellular damage by reactive carbon tetrachloride metabolites. Indian J. Physiol.Pharmacol 2001; 45(4): 435-41.
20. Girish C And Pradhan SC.: The steroid triterpenoids and flavonoids constituents of Eclipta alba (L) Hassk**.** J Pharmacol. Pharmacother 2012; 3(2): 149-55.
21. Handa S S and Sharma A. In vivo hepatoprotective activity of active fraction from ethanolic extract of Eclipta alba leaves. Indian J Med Res 1990; 92: 276-83.