



HEPATOPROTECTIVE AND ANTIOXIDANT ACTIVITY OF THE ETHANOLIC EXTRACTS OF *Boerhaavia Diffusa* L AND *Cichorium Intybus* L CCl₄ – INDUCED HEPATIC DAMAGE IN RATS

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ABSTRACT

In traditional medicine *Boerhaavia diffusa* and *Cichorium intybus* are used as a hepatoprotective and antioxidant drugs. The aim of the present study is designed to evaluate the hepatoprotective, and antioxidant properties of *Boerhaavia diffusa* and *Cichorium intybus* leaf powder (drug) in experimental albino rats study and access toxicity related drugs through histopathology studies in drug treated animals. The LD₅₀ value of the ethanolic extract of two plants are found out by acute toxicity studies. It is found out that both plants have greater than 2000 mg/kg as none of the animals has experienced any signs of toxicity nor death. The ethanolic extract of *Boerhaavia diffusa* and *Cichorium intybus* leaf powder (100 mg / kg and 200 mg / kg individually and combination of two plant extract 100 mg / kg and 100 mg / kg) are administered respectively to the animals. The drug CCl₄, used to induce the hepatotoxicity and the drug silymarin (25.0 mg / kg) is given as the reference standard. The both plant extracts are effective in protecting the liver against the hepatotoxicity induced by the CCl₄ in rats. This is evident from the significant reduction in Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), Acid Phosphatase (ACP), and total bilirubin levels and also effective against free radical scavenging activity by significant reduction in enzyme parameters like Glutathione peroxidase, Super Oxide Dismutase (SOD), Catalase, Thiobarbituric Acid Reactive Substance (TBARS). It is concluded from the result that the ethanolic extract of *Boerhaavia diffusa* and *Cichorium intybus* leaf powder possesses good hepatoprotective activity and antioxidant against CCl₄ induced hepatotoxicity in albino rats. Further studies are required to isolate and characterize the active principles which are responsible for the hepatoprotective efficacy of this medicinal plants.

KEY WORDS

Antioxidant Activity, *Boerhaavia diffusa* L, CCl₄, *Cichorium intybus* L, Hepatoprotective

INTRODUCTION:

The liver is the largest organ in the body weighing nearly one and half kilograms. Protected by ribs and it lies upper right part of abdomen. It is a chief site for intense metabolism. It has a surprising role in the maintenance, performance and regulating homostatices of the body [1-2]. Nowadays, liver diseases are one of the major issues of all medical community due to higher rate of mortality and morbidity [3]. Allopathy drugs used in the treatment

of liver diseases are inadequate and many times can cause serious side effects. The increasing cost of treatment and non-availability of effective drugs in the modern system of medicine led to several studies possible hepatoprotective action of traditional drugs [4-5]. In the traditional system of medicine there are numerous plants and polyherbal formulation have been used in the liver diseases. But only a small portion has been pharmacologically evaluated for their efficacy. Still more number of medicinal plants is needed to be

investigated for their antihepatotoxic effect [6]. *Boerhaavia diffusa* is a perennial creeping weed, prostrate herb, up to 1m long or more, having spreading branches and belonging to the family. The stem is prostrate, woody or succulent, cylindrical, often purplish, hairy, and thickened at the nodes. The shape of the leaves varies considerably – ovate-oblong, round, or subcordate at the base and smooth above. The upper surface of the leaves is green, smooth, and glabrous, whereas it is pinkish white and hairy beneath. Tribals eat this plant as vegetable. Its roots are used in the treatment of piles by the inhabitants of the Garhwal Himalaya (Uttaranchal). The root paste is used to cure bloody dysentery. The root juice is used in treating asthma, scanty urine, and internal inflammation disorders [7-9]. *Cichorium Intybus* L is a diploid species belonging to the family Asteraceae with blue, lavender or occasionally white flowers is also known as blue sailors, (or) Kasini. It is native to the mid Asia and northern Africa [10]. The tuberous root of this plant contains number of medicinally important compounds such as inulin, bitter sesquiterpene lactones, Coumarins, flavonoids and vitamins. Above two plants are widely used by the traditional healers to cure many diseases including to cure hepatotoxic effect in native system of medicine.

MATERIALS AND METHODS

Healthy, disease free entire plants of *B.diffusa* are collected from near palliaghrakaram, Thanjavur Dt, Tamil Nadu, India. *C.Intybus* plants are collected from local market, Trichirappalli, Tamil Nadu, India. The collected specimens are authenticated by Dr. S.John Britto, The Director, The Rabinat Herbarium and Centre for Molecular Systematics, St. Joseph's College (Campus), Tiruchirappalli 620002 Tamil Nadu, India. The fresh leaves are washed in tap water for 5 min and are dried using blotting papers. The washed plants leaves are air and shade dried for two weeks and pulverized to powder using mortar. The dried and powdered leaves material (150g) are extracted using ethyl alcohol using soxhlet extractor for 18 hrs at a temperature below the boiling point of the ethanol. The extracts are concentrated in vacuum at 40 degree using rotatory evaporator. The residues obtained are stored in a freezer until further test.

The present study is under taken to evaluate, "The hepatoprotective effect of ethanolic extract of

Boerhaavia diffusa and *Cichorium intybus* against CCl₄ induced liver damage in rats".

Selection of animals:

Adult male albino rats of wister stain 4-6 weeks, weighing 180-230g are used either sex for the present study. The animals are housed in large spacious cages, maintained in controlled environment of temperature, humidity and 12h light/dark cycles. They are fed with standard pelleted diet obtained from Hindustan lever limited, Bangalore and water adlibitum. All the experimental procedures and protocols used in the study were reviewed by the Institutional Animal Ethics Committee (Approved by 59 / CS / Pharma / CPCSEA / 12 / 16, Sri Kripa Institute of Pharmacological Studies, Telangana).

Experimental protocol for hepatoprotective and antioxidant activity

The treatment protocol are planned to study the role of both preventive and causative aspects of CCl₄ induced hepatotoxicity. Male wistar albino rats 4-6 weeks, 180-230 g are divided into eight groups of six. Group I served as a normal-control group where no treatment is given. CCl₄ (3 ml/kg 50% in Olive oil) is administered to animals of the other seven groups by intraperitoneal injection. Group II served as hepatotoxicity induced control for which only CCl₄ (3 ml/ kg 50% in olive oil) is administered to animals.

Similarly Group III to Group VIII CCl₄ is induced through intraperitoneal injection as before. Group III in addition to CCl₄, *Cichorium intybus* Ethanolic extract 100 mg / kg is given. Group IV in addition to CCl₄, *Cichorium intybus* Ethanolic extract 200 mg/kg is given. Group V in addition to CCl₄, *Boerhaavia diffusa* Ethanolic extract 100 mg / kg is given. Group VI in addition to CCl₄, *Boerhaavia diffusa* Ethanolic extract 200 mg/kg is given. Group VII in addition to CCl₄, *Cichorium intybus* and *Boerhaavia diffusa* of each Ethanolic extract 100 mg / kg is given.

Group VIII in addition to CCl₄, the reference drug silymarin (25.0 mg/kg) is administered. The CCl₄ is administered intraperitoneally and the drugs are given orally [11-12].

Assessment of hepatoprotective activity and antioxidant activity

In the present study, the hepatoprotective activity is evaluated biochemically. After 7th day of drug treatment the animals are dissected under ether anesthesia. Blood is drawn from the carotid artery and collected and labeled centrifuging tubes and allowed to clot for 30 min at room temperature. Serum is separated by

centrifugation at 3500 rpm for 10 min. The separated serum is used for the estimation of some biochemical parameters like Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), Acid Phosphatase (ACP), and total bilirubin according to standard methods ^[13-15] for hepatoprotective activity and Glutathione peroxidase, Super Oxide Dismutase (SOD), Catalase, Thiobarbituric Acid Reactive Substance (TBARS) are estimated in serum for antioxidant activity.

Histological evaluation method

The hepatative tissues are fixed in 10 per cent buffered formaline and embedded in paraffin by employing the standard technique, 5 μ in thick sections are cut and stained with hematoxylin-eosin for histological examination ^[16,17].

RESULTS AND DISCUSSION

Hepatoprotective activity in albino rats is studied by inducing hepatotoxicity using CCl₄ is shown in Table 1 and figures 1-5, Aspartate Amino Transference (AST) for normal control is 99.5 units/litre whereas in induced control the value is 181.3. The drugs are dose dependent because 100 mg/kg drug and 200 mg/kg drug are given different results. Among this two plant drugs *Cichorium intybus* shows better result that is 109.4 units/litre for 200 mg whereas for *Boerhaavia diffusa* at dose level 200 mg, 123.2 units/litre. The combination of two drugs *Cichorium intybus* and *Boerhaavia diffusa* each 100 mg/kg is shown as 103.2 units/litre. This value is lesser than standard silymarin which is 104.9 units/litre. Similar results are seen for all parameters like Alanine Transaminase (ALT), Alkaline Phosphatase (ALP), Acid Phosphatase (ACP) and Bilirubin. For all the biochemical parameters *Cichorium intybus* 200 mg/kg shows better result than *Boerhaavia diffusa*. The combination of these two drugs are given still more better results which is nearly equal to standard silymarin (25 mg/kg). All datas are statistically within limits which are seen by student 't' test.

In table 2 and figures 6-9, the various *in vivo* antioxidant enzyme parameters like Glutathione Peroxidase ^[18],

Superoxide Dismutase (SOD^[19], Catalase^[20] Thiobarbituric Acid Reactive Substance (TBARS)^[21] are given. All the parameters are compared, it is clearly seen that in controlling scavenging activity, *Cichorium intybus* is more effective than *Boerhaavia diffusa*^[22]. The drugs are dose dependent in this case also. The combination of these two plant drugs is proven to be more effective than individual drugs. In certain case, the combination drugs is more potent than standard silymarin. The results are statistically significant which are identified by student 't' test.

CONCLUSION

Hepatoprotective, and antioxidant are confirmed through experimental albino wistar rats' study and it is compared with standard silymarin in different dose levels and in combination of two drugs. Various biochemical parameters AST, ALT, ALP, ACP, Bilirubin are near to the standard value individually and specially in combination of drugs. Similarly, antioxidant potential are seen for various enzyme markers Glutathione, SOD, Catalase, and TBARS. Enzyme markers results are very close to the standard drug silymarin. All the animal model data values studies are found to be statistically significant. Histopathological examination of the liver tissues are shown in figures(10a-10g). A close up look at these figures revealed the regeneration changes brought out by the study extracts on the liver tissue which are damaged due to hepatic toxicity. . Futher studies are required to isolate and characterize the active constituents like flavonoid, alkaloid, Tannins etc., to find out the mechanism responsible for its hepatoprotective activity.

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Table 1. Effect of *Boerhaavia diffusa* and *Cichorium intybus* extracts on AST, ALT, ALP, ACP, Bilirubin levels of CCl₄ induced hepato-toxicity in experimental Albino rats

Groups	Treatment	AST (U/L)	ALT (U/L)	ALP (U/L)	ACP (U/L)	Bilirubin (mg/100 ml of blood) Total
I	Normal - Control	99.5 ± 1.3	36.07 ± 0.7	16.32 ± 0.92	10.2 ± 0.061	0.39 ± 0.02
II	Induced Control CCl ₄ (3 ml/kg ip)	181.3 ± 3.92	139.7 ± 5.91	97.9 ± 4.9	39.6 ± 1.9	0.92 ± 0.76
III	CI (100 mg/kg) + CCl ₄ (3ml/kg ip)	153.6* ± 7.32	86.3* ± 4.45	63.9* ± 5.1	25.3* ± 1.3	0.74 ± 0.051
IV	CI (200 mg/kg) + CCl ₄ (3ml/kg ip)	109.4** ± 2.3	69.8** ± 4.7	43.6** ± 2.4	18.7* ± 0.9	0.63* ± 0.01
V	BD (100 mg/kg) + CCl ₄ (3ml/kg ip)	147.3* ± 1.18	91.08* ± 0.2	69.92* ± 0.72	27.5 ± 0.064	0.76 ± 0.030
VI	BD (200 mg/kg) + CCl ₄ (3ml/kg ip)	123.2* ± 7.21	73.1** ± 4.3	49.6** ± 3.4	21.0* ± 1.2	0.67* ± 0.22
VII	CI + BD (100 + 100 mg/kg) + CCl ₄ (3ml/kg ip)	103.2** ± 6.50	52.4** ± 5.1	36.6** ± 2.9	16.9* ± 0.12	0.45* ± 0.19
VIII	Silymarin + CCl ₄ (3ml/kg ip)	104.9* ± 12.4	49.7** ± 3.6	34.8** ± 2.9	16.2* ± 1.2	0.41* ± 0.03

Data are expressed as mean ± S.E., n=6. * P < 0.01 Vs Control, ** P < 0.001 Vs Control by Student 't' test.

Table 2. Effect of *Boerhaavia diffusa* and *Cichorium intybus* extracts on Glutathione peroxidase, Superoxide dismutase, Catalase, Thiobarbituric Acid Reactive Substance levels of CCl₄ induced free radical study in experimental Albino rats

Groups	Treatment	Glutathione peroxidase (mg liver protein) ⁻¹	SOD (mg liver protein) ⁻¹	Catalase (mg liver protein) ⁻¹	TBARS (nmol malondialdehyde mg/liver protein)
I	Normal – Control	0.973 ± 0.05	77.81 ± 1.94	293.73 ± 10.05	1.31 ± 0.395
II	Induced Control CCl ₄ (3 ml/kg ip)	0.63 ± 0.03	49.64 ± 3.30	177.93 ± 9.73	1.79 ± 0.19
III	CI (100 mg/kg) + CCl ₄ (3ml/kg ip)	0.83* ± 0.06	67.74* ± 0.59	265.5* ± 8.79	1.43 ± 0.18
IV	CI (200 mg/kg) + CCl ₄ (3ml/kg ip)	0.96* ± 0.07	84.25* ± 0.84	281.3* ± 9.46	1.33* ± 0.16
V	BD (100 mg/kg) + CCl ₄ (3ml/kg ip)	0.84* ± 0.07	67.73* ± 0.54	269.27* ± 8.74	1.45 ± 0.08
VI	BD (200 mg/kg) + CCl ₄ (3ml/kg ip)	0.92* ± 0.06	86.97* ± 0.75	281.8* ± 9.92	1.33* ± 0.05
VII	CI + BD (100 + 100 mg/kg) + CCl ₄ (3ml/kg ip)	0.96* ± 0.07	88.25* ± 0.84	283.8* ± 16.46	1.33* ± 0.03
VIII	Silymarin + CCl ₄ (3ml/kg ip)	0.93* ± 0.05	88.34* ± 2.54	269.7* ± 12.4	1.29* ± 0.14

Data are expressed as mean ± S.E., n=6. * P < 0.01 Vs Control, ** P < 0.001 Vs Control by Student 't' test.

Fig1. Effect of *Boerhaavia diffusa* and *Cichorium intybus* extracts on AST levels of CCl₄ induced hepato-toxicity in experimental Albino rats

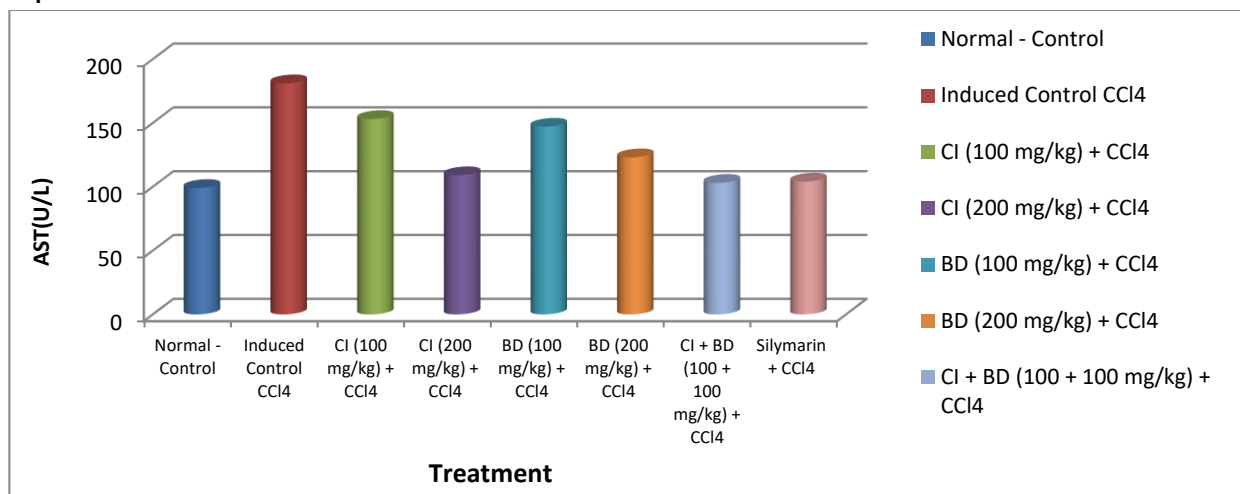


Fig2. Effect of *Boerhaavia diffusa* and *Cichorium intybus* extracts on ALT levels of CCl₄ induced hepato-toxicity in experimental Albino rats

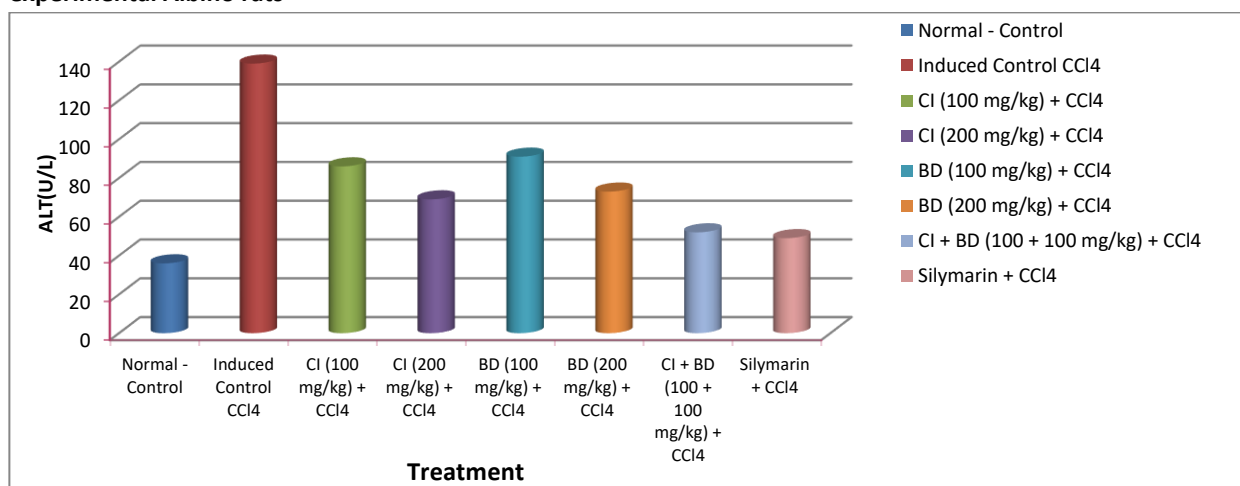


Fig3. Effect of *Boerhaavia diffusa* and *Cichorium intybus* extracts on ALP levels of CCl₄ induced hepato-toxicity in experimental Albino rats

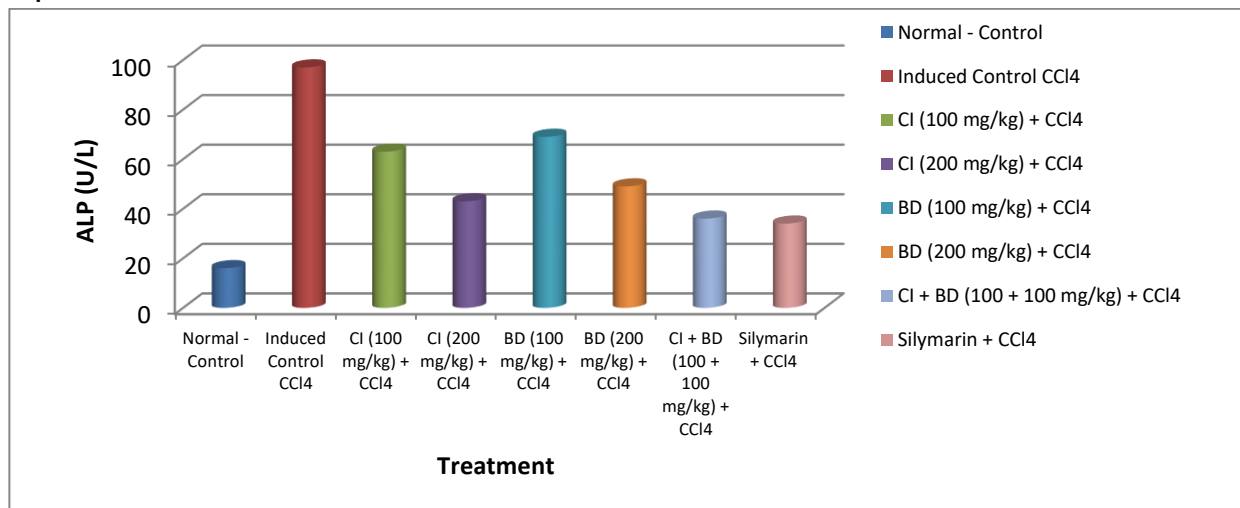


Fig4. Effect of *Boerhaavia diffusa* and *Cichorium intybus* extracts on ACP levels of CCl₄ induced hepato-toxicity in experimental Albino rats

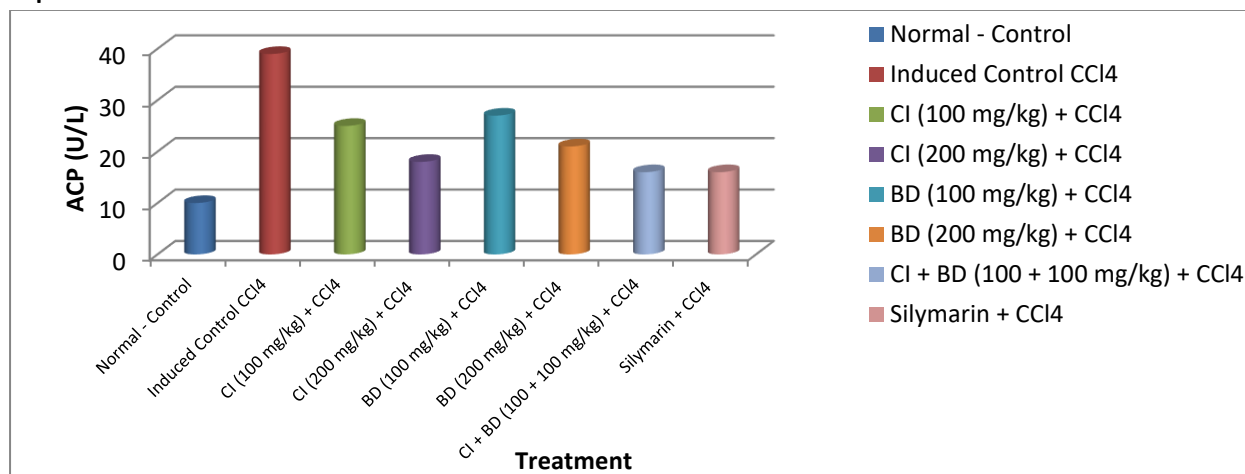


Fig5. Effect of *Boerhaavia diffusa* and *Cichorium intybus* extracts on Bilurubin Levels of CCl₄ induced hepato-toxicity in experimental Albino rats

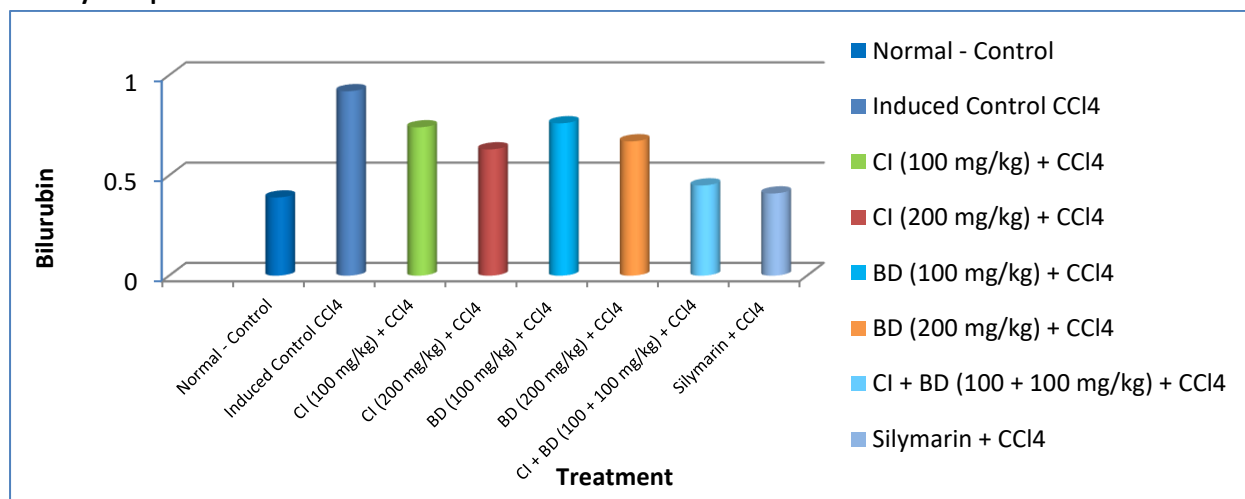


Fig.6 Effect of *Boerhaavia diffusa* and *Cichorium intybus* extracts on Glutathione peroxidase levels of CCl₄ induced free radical study in experimental Albino rats

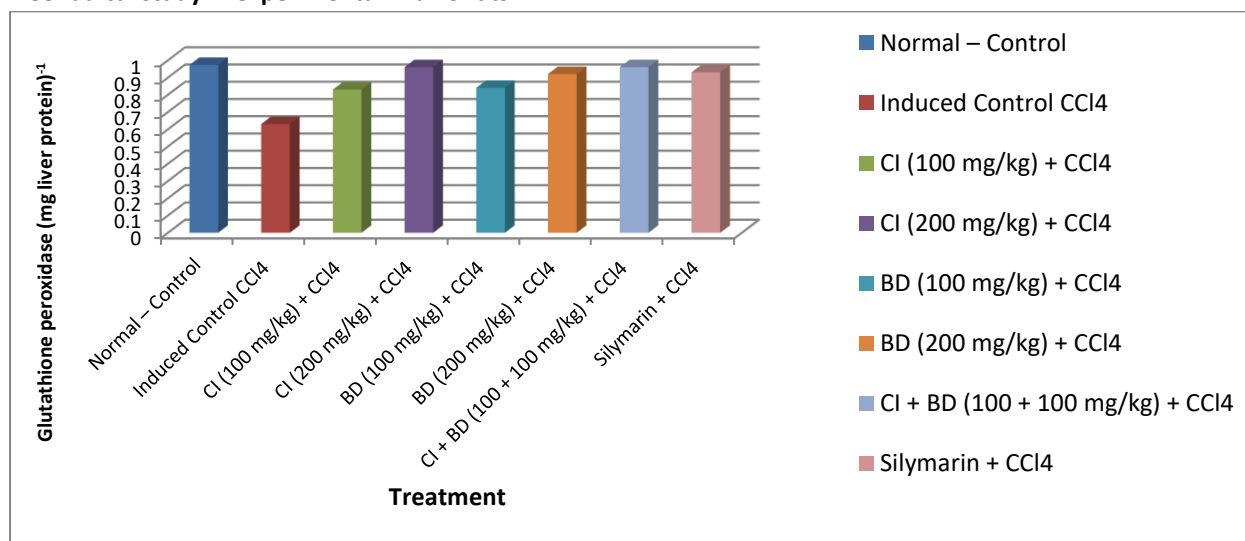


Fig.7 Effect of *Boerhaavia diffusa* and *Cichorium intybus* extracts on Superoxide Dismutase levels of CCl₄ induced free radical study in experimental Albino rats

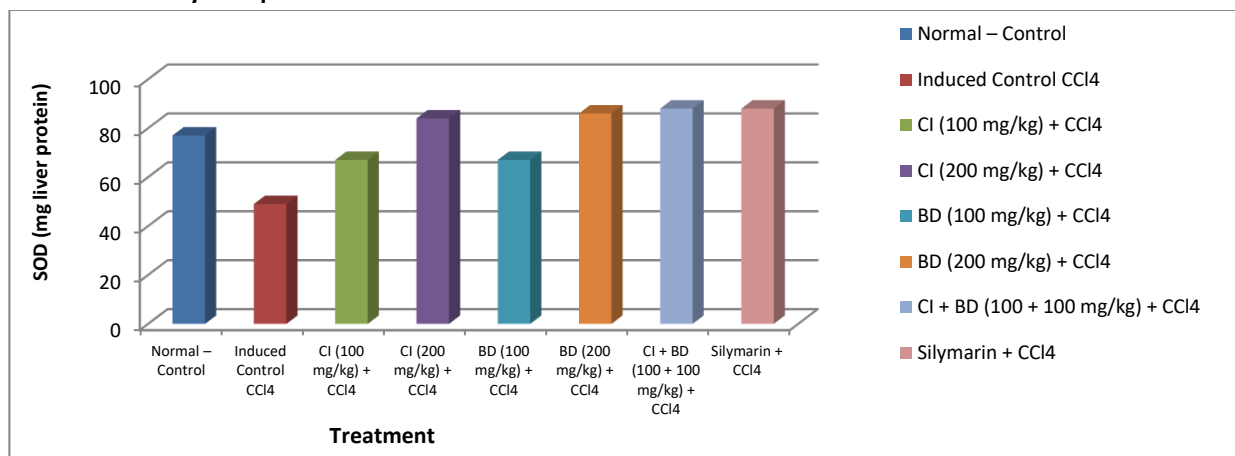


Fig.8 Effect of *Boerhaavia diffusa* and *Cichorium intybus* extracts on Catalase levels of CCl₄ induced free radical study in experimental Albino rats

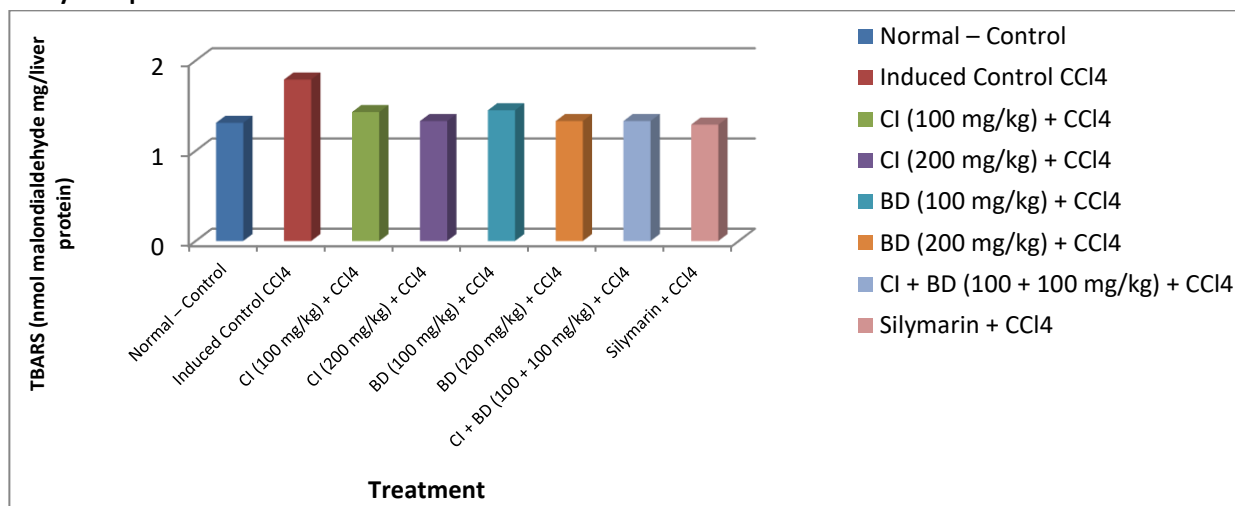
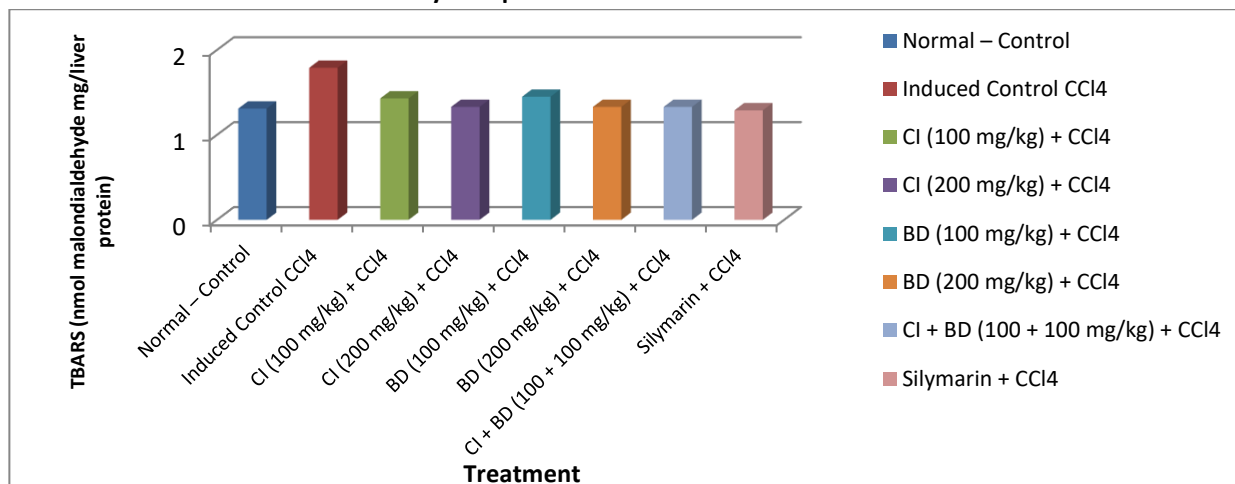


Fig. 9 Effect of *Boerhaavia diffusa* and *Cichorium intybus* extracts on Thiobarbituric Acid Reactive Substance levels of CCl₄ induced free radical study in experimental Albino rats



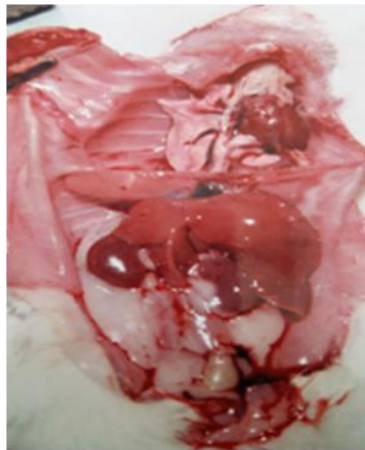


Fig.10a. Exposed liver of normal Albino rats.

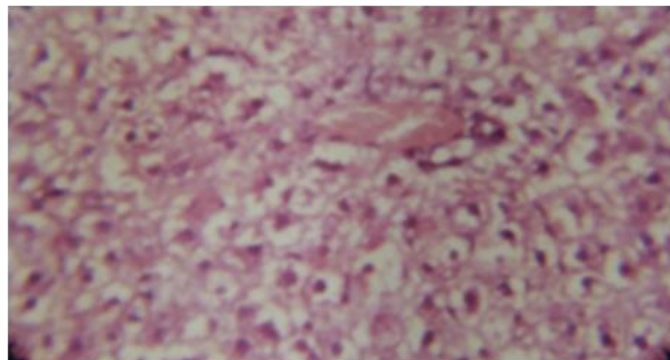


Fig. 10b. Liver section showing normal architecture and structure.

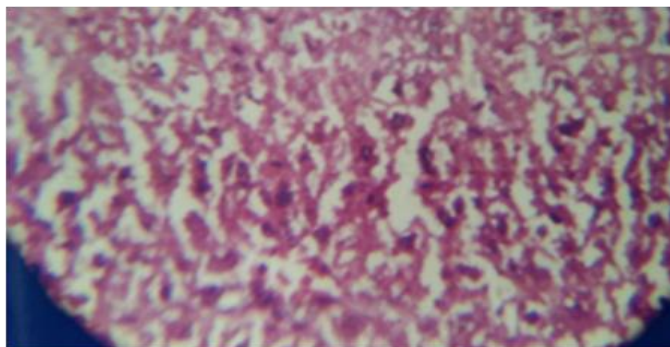


Fig.10c. CCl₄ treated showing extensive area of congestion, profound inflammation, portal infiltration, hyperplasia and necrosis.

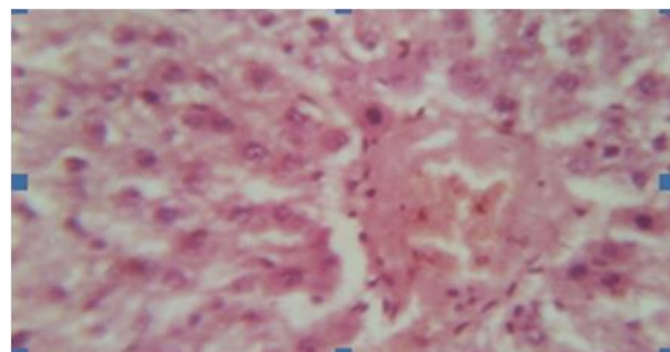


Fig.10d. CCl₄ + Cichorium intybus extract treated showing reduced inflammation and congestion.

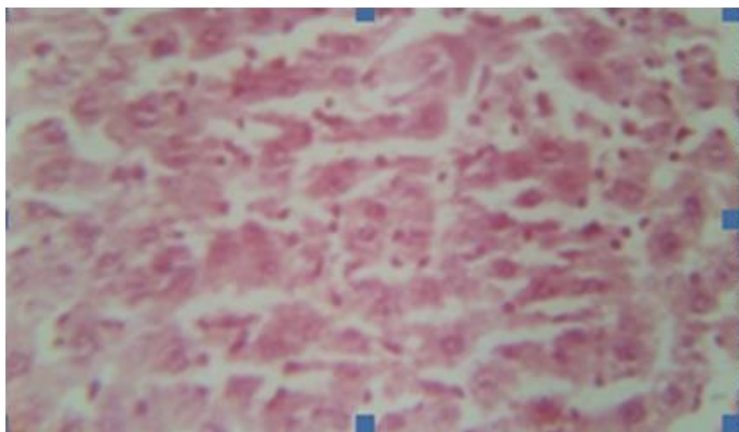


Fig.10e. CCl₄ + Boerhaavia diffusa extract treated showing less congestion and necrosis.

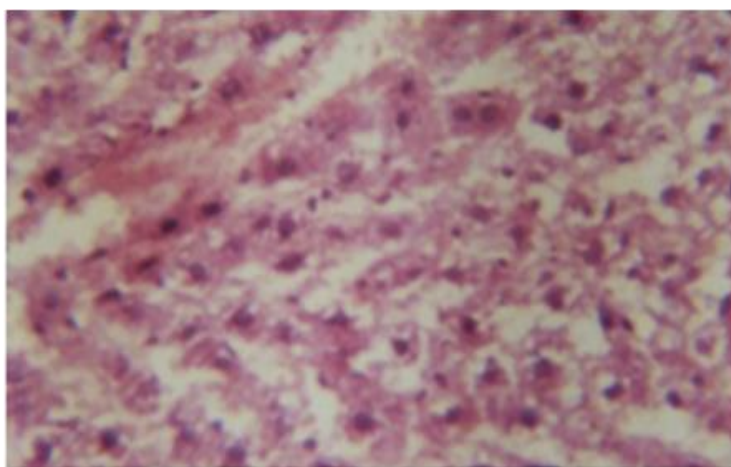


Fig.10f. CCl₄ + Boerhaavia diffusa treated showing decreased degeneration and congestion.

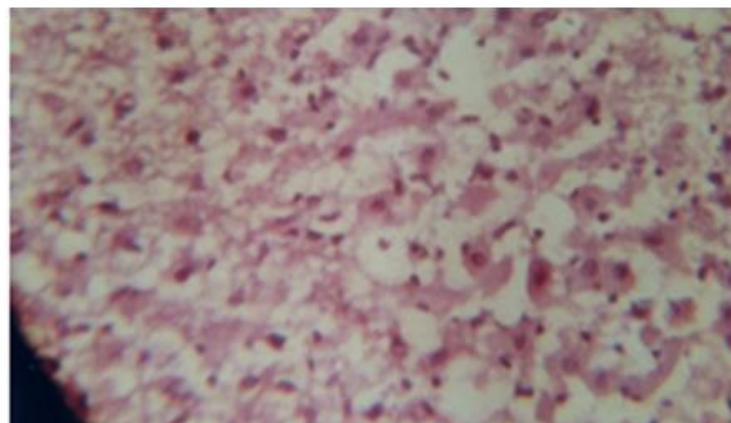


Fig.10g. CCl₄ + Silymarin treated showing limited inflammation and degeneration.

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