



A COMPARATIVE STUDY ON HEPATOPROTECTIVE ACTIVITY OF BOERHAAVIA DIFFUSA AND SILYMARIN IN CCl₄ INDUCED HEPATOTOXICITY IN ALBINO RABBITS



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Abstract

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Objective:To compare the hepatoprotective activity of Boerhaavia diffusa and Silymarin in CCl₄ induced hepatotoxicity in albino Rabbits. **Materials and Methods:** The study was conducted on 18 healthy albino rabbits of either sex weighing 1.5-2.0 kg , divided into 3 groups. Hepatotoxicity was induced in rabbits by carbon tetra chloride (CCl₄) 0.05 mg /kg ,intraperitonealy. Alcoholic extracts of roots of **Boerhaavia diffusa** and Silymarin were administered orally for 20 days from 1 day to day 20 in the doses of 100mg/kg/day with the help of syringe. **Results: Group I:** The rise of serum transaminase(p<0.001), serum alkaline phosphatase(p<0.001), serum bilirubin (p<0.001) and decrease in serum albumin (p<0.001)due to hepatotoxic effect of CCl₄ when compared to zero day of same group (p<0.001). **Group II:** Boerhaavia diffusa extract caused fall in the level of serum transaminase , serum alkaline phosphatase ,serum bilirubin and increased in serum albumin in a statistical highly significant amount (p<0.001), when compared with 11th day of rabbits receiving CCl₄ (group I). **Group III:** Silymarin was decrease the level of SGOT (p<0.0001), SGPT (p<0.0001), serum alkaline phosphatase (p< 0.0001), serum bilirubin (p<0.0001)and increased in serum albumin (p<0.0001) in a statistical highly significant amount , when compared with11th day of Group I . There is no significance difference (p>0.10) found in SGOT, SGPT, ALP, S. bilirubin, S. Albumin respectively when we compared group II & group III.

Conclusion: Boerhaavia diffusa extract had similar efficacy in reducing SGOT, SGPT, ALP, S.bilirubin in comparison to Silymarin. Boerhaavia diffusa extract had similar effect in increasing S.Albumin as Silymarin. However, Boerhaavia diffusa extract had shown higher protection in restoration of liver function and regeneration of liver cells than Silymarin as observed on histopathology.

INTRODUCTION

The liver is the largest internal organ in the body contributing about 2 percent of total body weight¹, which plays an essential role in the metabolism of foreign substances entering the body. They are known as **Xenobiotics**. The liver has considerable reserve capacity, can often maintain function in spite of significant disease and is one of the few human organs capable of regeneration¹.

More than 1000 xenobiotics substances are potentially hepatotoxic². The ability of the chemical to produce liver damage in vivo often results from interaction of a series of complex process involved in the uptake, biotransformation and elimination of these potentially toxic compounds.

Conventional drugs used in the treatment of liver disease are often inadequate. It is therefore necessary to search for **alternative drugs** for the treatment of liver

diseases to supplement the currently used drugs of limited efficacy and safety.

Much interest exists in the possibility that, antioxidants reduce the risk of degenerative diseases by inhibiting free radical induced oxidative damage³. Antioxidant properties present in the **herbs** can be used in the treatment of disease like hepatitis, jaundice and loss of appetite. Antioxidants property is claimed to be one of the mechanism of hepatoprotective effect of **Indigenous drugs**⁴. **Silymarin** is a hepatoprotective principle of a plant Silybum marinum. It is known in the traditional system of medicine for its use in liver disease.

Hence, the present study is designed to study the hepatoprotective effect of Boerhaavia diffusa and Silymarin in CCl₄ induced hepatotoxicity in experimental animals, supported by histopathological evidences.

MATERIALS AND METHODS

The present study was conducted in the department of Pharmacology and therapeutics in collaboration with department of Pathology, G.S.V.M. Medical college, Kanpur, after the clearance from Institutional Animal Ethical Committee for Prevention of cruelty and supervision of experiments on animals.

ANIMALS:

The study was conducted on 18 healthy albino rabbits of either sex weighing 1.5-2.0 kg, divided into 3 groups. The animals were made available in the animal house of Department of Pharmacology & Therapeutics. Rabbits, also have metabolism similar to human beings. Hepatotoxicity induced in rabbit by carbon tetra chloride (CCl₄) simulate the symptoms of drug induced hepatitis in human being without the development of concurrent infections. So, experiment on rabbits correlate well with human subjects.

All the animals were fed normal stock diet for 7 days. During this time the animals got acclimatized to the new environment. All the animals were housed individually in clean cage and maintained under standard conditions (12 hr light and dark cycle, at

room temperature 25± 3° C and 35-60% humidity).

DRUGS USED:

Alcoholic extracts of roots of **Boerhaavia diffusa** and **Silymarin**

All drugs were administered orally for 20 days from 1 day to day 20 with the help of syringe. Carbon tetra chloride (CCl₄) was administered intraperitoneally for 10 days from 11th day to day 20.

Preparation of extract:

Roots of Boerhaavia diffusa were obtained from market and extract was prepared in 70% alcohol using cold percolation method. After 7 days extract of each drug was collected .The alcohol free extract was weighed and preserved in a refrigerator at 4°C.

Silymarin was obtained from market as tablets. The Silymarin tablets were crushed using Mortor and Pestle ,The powder so obtained was dissolved in 1 ml of distilled water and was administered per orally through a syringe followed by 1 ml of water.

Carbon tetra chloride was obtained from market. Since CCl₄ is a hepatotoxic agent, it

induces hepatitis in the animals. Hepatitis produces anorexia and decreases in the body weight, Therefore assessment of weight loss was done in all the groups.

1. 60 grams of diet was given to each rabbit. Diet was provided between 11 A.M and 1.00 P.M daily. Weighed diet was given and the amount consumed was calculated from difference between the left over amount of diet 24 hours later. Water was given ad libitum.

2. Weight of the animals: Weight was recorded daily from first day to 20th day. Any increase or decrease in the weight of rabbit during drug administration was recorded

3. SGOT, SGPT, Alkaline phosphatase, Serum bilirubin, Serum albumin estimation was done, blood samples were collected on zero day, 11th day, 21st day.

4. Liver weight: At the end of the study rabbit were sacrificed and liver was taken out. It was weighed and preserved in 10% buffered formalin for histopathological study.

PROCEDURE

Rabbits were divided into 3 groups with 6 rabbits in each group.

GROUP I: Animals of this group were treated with hepatotoxic agent ie carbon tetrachloride (CCl₄) for 10 days in the dose of 0.05ml /Kg/day intraperitoneally from 1th day to day 10 along with normal feed. On 11th day blood samples were collected and rabbits were sacrificed.

GROUP II: Animals of this group were given extract of roots of Boerhaavia diffusa 100 mg/Kg/day orally for 20 days along with normal feed, from 11th day onward carbon tetrachloride(CCl₄) 0.05mg/kg, i.p. was also given followed by herbal drug ie Boerhaavia diffusa .

GROUP III: Animals of this group were given Silymarin 100 mg/Kg/day orally for 20 days along with normal feed, from 11th day onward carbon tetrachloride (CCl₄) 0.05mg/kg, i.p. Was also given followed by drug.

Blood samples were collected on zero day before giving any drug to see the control value of liver function tests (L.F.T.), on 11th day to see the per se effect of herbal drug on L.F. T. and on 21th day to see the protective effect of herbal drug on L.F.T. The value obtained were compared .Blood

samples were drawn from the marginal vein of pinna using 22 gauge needle, after the ear hairs were shaved off. 3 ml blood was collected in the vial, for the liver function test.

Body weight was measured daily. The animals of group I were sacrificed on 11th day and the animals of group II & III were sacrificed on 21th day. They were made unconscious, by giving ketamine. The abdomen was exposed and liver was excised, weighed and was preserved in 10% buffered formalin for histopathological study.

ASSESSMENT OF LIVER INJURY

Assessment of liver injury was done by biochemical estimation and histopathological study of liver under light microscope.

BIOCHEMICAL ESTIMATION:

Serum bilirubin, SGOT, SGPT, Alkaline phosphatase, serum albumin levels were estimated by Olympus autoanalyser in the department of Pathology.

HISTOPATHOLOGY

Histopathological study of the rabbit's liver was done to assess the extent of toxicity. Liver was taken out after sacrificing the

rabbit. It was weighed and preserved in 10% buffered formalin. Tissue sectioned to prepare slides. Staining was done with Hematoxylin and Eosin. Then slides were examined under light microscope and these slides were photographed⁵.

STATISTICAL CALCUCATIONS: Mean, standard deviation and standard error of mean was calculated and results were analyzed by using paired t test and Student't' test. P Value of < 0.05 was considered significant.

RESULTS

Effect on diet intake, body weight, liver weight (Table no: 1)

In Rabbits of **group I**, who were administered CCl₄ (0.05 mg/kg/day, intraperitonealy) along with normal feed, the diet intake was found to be **39.13 ±0.77 gm/day**. The decrease in food intake has lead to a decrease in body weight. The mean decrease in body weight in group I was considerably more than when compared to the group II, group III. The mean weight of liver was **28.38±0.18 gms**.

In Rabbits of **group II** (received Boerhaavia diffusa extract), average diet intake was

decreased by 15.2% when compared to average diet intake during first 10 days of same group and increased by 12.23% when compared with group I (CCl₄ administered group). Mean weight of the liver was measured to be **42.66±0.24**.

In Rabbits of **group III** (Received Silymarin) the average diet intake was **60.44 ± 0.54 gm/day** during first 10 days, on adding CCl₄ 11th day onward, average diet intake was **49.17 ± 0.13 gm/day** in last 10 days, which is decreased by 18.3% when compared to average diet intake during first 10 days of same group and increased by 16.6% when compared with group I. The mean weight of the liver was measured to be **38.47±0.22**. This suggests that Silymarin was able to arrest the decrease in weight of liver when compared to CCl₄ administered group.

In **group I**, there was a highly significant ($p < 0.001$), increase in the levels of serum transaminases, serum alkaline phosphatase, serum Bilirubin and significant decrease in serum albumin with $p < 0.001$ compared to zero day of same group (self control).

Administration of Boerhaavia diffusa extract and Silymarin to Rabbits, feed on normal, diet did not alter the level of serum

transaminase ($p > 0.10$), serum alkaline phosphatase ($p > 0.10$) serum bilirubin and serum albumin ($p > 0.10$), when compared to zero day of same group.

In group II, The rise of serum transaminase, serum alkaline phosphatase, serum bilirubin and decrease in serum albumin in a statistical highly significant amount ($p < 0.001$) due to hepatotoxic effect of CCl₄ when compared to zero day of same group (table 2,3,4,5,6).

Boerhaavia diffusa extract caused fall in the level of serum transaminase (Table No.2,3), serum alkaline phosphatase (Table No.4), serum bilirubin (Table No.5) and increased in serum albumin (Table No.6) in a statistical highly significant amount ($p < 0.001$), when compared with 11th day of rabbits receiving CCl₄ (group I).

In **group III**, Silymarin was able to bring down the level of serum transaminase (Table No.2,3), serum alkaline phosphatase (Table No.4), serum bilirubin (Table No.5) in a statistical highly significant amount ($p < 0.001$), when compared with 11th day of rabbits receiving CCl₄ (group I). Further, silymarin significantly increased the serum albumin level comparable to zero day of

same group ,that has been decreased with CCl₄ administration.

There is no significance difference (p>0.10) found in SGOT, SGPT , ALP, S.bilirubin, S.Albumin respectively when we compared group II & group III .

Histopathological Assessment

Rabbits administered carbon tetrachloride

Grade III fatty changes and hydropic degeneration was present in 75% of rabbits and Grade II fatty changes was present in 25% of rabbits. Centrilobular (perivenular) and periportal inflammation was found in 75% and 25% of rabbits respectively, chiefly infiltrated with monocytes .Grade II inflammation was present in all rabbits.Grade II necrosis and loss of cord pattern was found in all rabbits **(Photograph 1)**.

Rabbits administered Boerhaavia diffusa extract and CCl₄

When rabbits on CCl₄ were compared with rabbits receiving Boerhaavia Diffusa extract and CCl₄ , there was sub maximal protection of hepatic lobules from the damage induced by CCl₄. Grade I fatty

changes in 70% of rabbits & grade II fatty changes in 30% of rabbits were present .Portal inflammation of grade I , infiltrated by monocytes were present in 50% of rabbits and necrosis of grade I was found in 20% of rabbits in centrilobular zone. Cord pattern was maintained **(Photograph 2)**.

Rabbits administered Silymarin and CCl₄

When rabbits on CCl₄ were compared with rabbits receiving Silymarin and CCl₄ , there was some protection of hepatic lobules from the damage induced by CCl₄. Grade I fatty changes in 70% of rabbits were present. Foamy hepatocytes and fine vacuole was present. Portal inflammation of grade I , infiltrated by lymphocytes are present in 70% of rabbits and necrosis of grade I in centrilobular zone was found in 20% of rabbits .Cord pattern was maintained **(Photograph 3)**.

The result in this study suggest that administration of Boerhaavia diffusa extract and Silymarin, singly to the rabbits received CCl₄ from 11th day to 20th day caused a decline in hepatotoxicity induced by CCl₄. This is evidenced in the marked decrease in serum SGPT and SGOT level relative to the group treated with CCl₄ alone.

Comparatively Boerhaavia diffusa extract appears to exhibit higher protection of liver than Silymarin

DISCUSSION

Today, most xenobiotics to which humans are exposed come from sources that include environmental pollution, food additives, cosmetics products, agro-chemicals, processed food and drugs. In general, these chemicals in the absence of metabolism would not be eliminated from the body efficiently, and thus would accumulate in the body resulting in toxicity. Hepatic injury is a common sequel of exposure to toxic agents.

The CCl₄ is one of the most commonly used hepatotoxins in the experimental study of liver diseases⁶. Plant derived natural products such as flavonoids, terpenoids and steroids etc have received considerable attention in recent years due to their diverse pharmacological properties including hepatoprotective and antioxidant activity^{7,8}. Realizing the fact this study was carried out to evaluate the hepatoprotective of Boerhaavia diffusa extract and Silymarin in this direction.

Our findings regarding weight of liver, however, are different from earlier reports. **Simmon's et al**, 1995 in their study have reported that increased organ weight (whether absolute or relative) is a sensitive indicator of organ toxicity⁹. Also, **Sodhi et al** in their study have reported an increase in the specific liver weight in experimental animals given protein restricted diet administered INH+R (causing hepatotoxicity)¹⁰.

In this study, CCl₄ was able to produce hepatic damage which is manifested by increase in serological marker and abnormal histopathology. These changes are similar to previous studies.

The serum level of marker enzymes: SGOT, SGPT and ALP reflect the physiological state of the liver. The levels of these enzymes change accordingly to the distortion of liver resulting from cellular injury of the organ caused by toxic metabolites and diseases. Serum and plasma enzymes levels have been used as a marker for monitoring chemically induced tissue damages¹¹⁻¹².

The toxicity of CCl₄ to the liver of mammal is largely as a result of the active metabolite, trichloromethyl radical¹³. The above radical

bind to tissue macro- molecule and thus induce peroxidative degradation of membrane lipids of Endoplasmic Reticulum (ER), which are rich in polyunsaturated fatty acids. **Shenoy et al 2001**¹⁴, postulated, that such development would ultimately lead to the formation of lipid peroxides. The increase enzyme level in the plasma of CCl₄-treated rabbits suggests that the toxicant was able to reach the liver and induce a detectable damage .

The increase in levels of serum bilirubin reflects the depth of jaundice and increase in transaminases and alkaline phosphatase indicates the cellular leakage and loss of functional integrity of cell membrane¹⁵. Liver enzymes are usually raised in acute hepatotoxicity, but tend to decrease with prolonged intoxication due to damage to liver cells¹⁶.

The result of our experiment suggest that degree of hepatic damage was of lesser magnitude in Boerhaavia diffusa extract treated group. Boerhaavia diffusa extract not only prevent free radical injury but also suppresses the proinflammatory response of CCl₄ induced liver damage.

Boerhaavia diffusa extract was found to be devoid of any teratogenic effect¹⁸. A strong choleric action was observed which resulted in an increase in bile flow¹⁹

Animal studies¹⁴ and clinical trials¹⁷ suggests that silymarin can be useful in the management of early or progressive liver damage, when given for 3-6 months period. The active substances are 3 potent bioflavonoids ; Silybin, Silydianin and Silychristin known collectively as Silymarin. The proposed mechanism of action of Silymarin involves altering the membranes of the hepatic cells to inhibit passage of toxin, increasing cellular regeneration by stimulating protein synthesis, and antioxidant activity²⁰.

Silymarin treatment protected completely against harmful increase in the membrane ratio of cholesterol: phospholipids and sphingomyelin: phosphatidylcholine in rats with CCl₄ induced cirrhosis²¹. Silymarin when compared with various polyherbal formulations in CCl₄ induced hepatotoxicity in rats, has led to complete normalization of elevated transaminase level²².

We observed that roots of Boerhaavia diffusa and Silymarin protect the liver

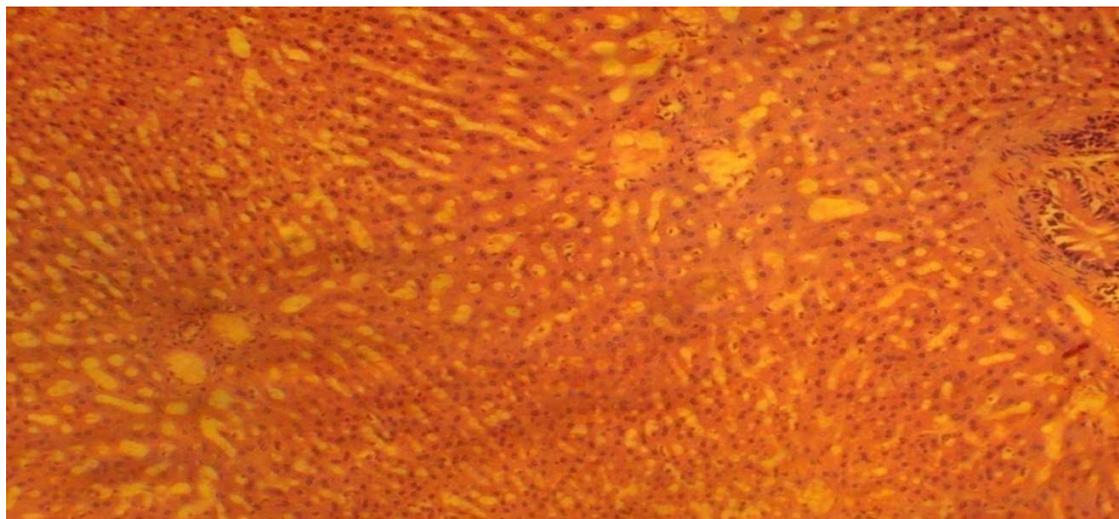
against CCl_4 induced hepatotoxicity. Boerhaavia diffusa extract had similar efficacy in reducing SGOT, SGPT, ALP, S.bilirubin and cause increase in S.albumin level as Silymarin. However, Boerhaavia diffusa extract has shown higher protection in restoration of liver function and regeneration of liver cells than Silymarin as observed on histopathology.

This study was done on small scale and for short duration so further research needs to be done to confirm the above results and to find the active principle and mechanism of action responsible for their hepatoprotective activity.

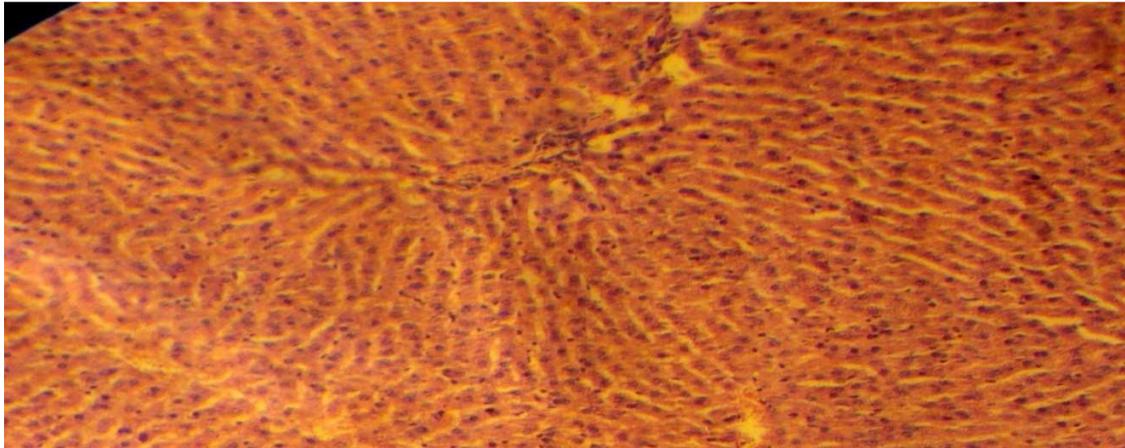
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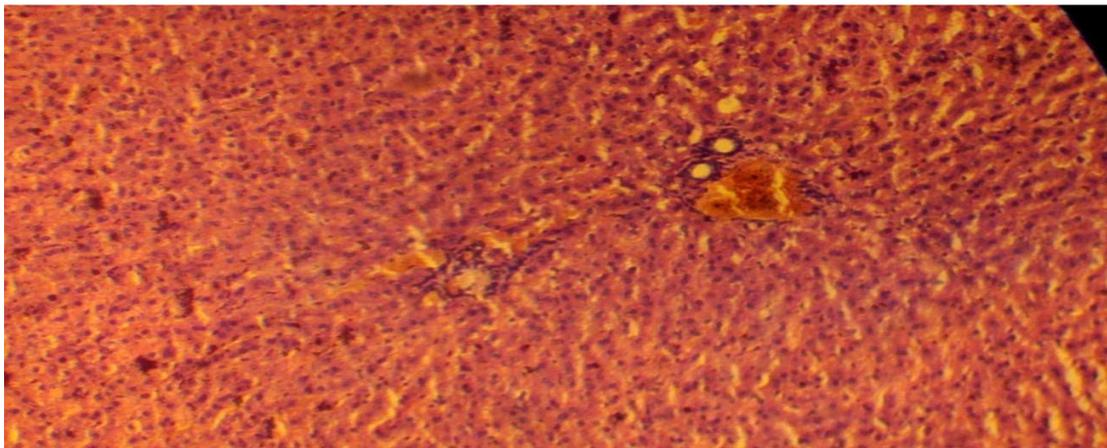
I am highly thankful to Dr. Priyamvada Sharma, M.D. Professor, Department of Pharmacology & Therapeutics S. N. Medical College, Agra, whose wealth of knowledge and vast experience has been instrumental at all stages of this study.



Photograph 1: A section of Rabbit Liver treated with CCl_4 alone showing marked fatty Changes & Grade II inflammatory changes in 100% area and loss of cord pattern



Photograph 2: A Section of rabbit Liver treated with Boerhaavia diffusa extract and CCl₄ showing protection from damage induced by CCl₄. (Grade I fatty changes in 70% , Grade II fatty changes in 30% and Grade I Portal inflammation in 50% area



Photograph 3: A section of Rabbit Liver treated with Silymarin and CCl₄ showing Protection from damage induced by CCl₄. (Grade I fatty changes in 70% and Grade I Portal inflammation in 70% area) .

Table 1

Showing average diet intake per day, Mean Liver weight (gm) of Rabbits, Mean body weight (Kg) of different groups.

Group	Average Diet Intake (gm/kg) 1 st to 10 th day	Average Intake (gm/kg) 11 th to 20 th day	Diet Mean Weight(gm)	Liver Average Body weight (in kg) 1 st to 10 th day	Average Body weight(in kg) 11 th to 20 th day
I (CCl ₄)	39.13 ± 0.77	-\$	28.38±0.18	1.04± 0.11	-\$
II (Boerhaavia diffusa extract)	60.51 ± 0.55	51.36 ± 0.25	42.66±0.24	1.56±0.24	1.46±0.27
III (Silymarin)	60.44 ± 0.54	49.17 ± 0.13	38.47±0.22	1.60± 0.40	1.51±0.42

All values are in MEAN ± SE, \$ group I rabbits were sacrificed on 11th day .

Table 2

Showing Mean Aspartate transaminase (AST,SGOT) in IU/L of Rabbits in different groups

GROUP	Mean SGOT ± SE (IU/L) At zero day	Mean SGOT ± SE (IU/L) At 11th day	Mean SGOT ± SE (IU/L) At 21th day
I (CCl ₄)	30.67 ± 0.88	87.17±1.58*	-\$
II (Boerhaavia diffusa)	28.83±1.30	28.50±1.38**,&	47.67±2.17* ,#,&

extract)

III (Silymarin)	29.00±1.53	29.67±1.69**,&	51.00±1.45*,#,&
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*P-Value<0.001, **P-Value>0.10,\$ group I rabbits were sacrificed on 11th day

*,** values are compared with zero day of same group (self control)

P-Value<0.0001,# compared with 11th day of group I (CCl₄)

& P-Value>0.10 when we compared group II and group III at respective day

Table 3

Showing Mean Alanine transaminase(ALT,SGPT) in IU/L of Rabbits in different groups

Group	Mean SGPT ± SE (IU/L) At zero day	Mean SGPT ± SE (IU/L) At 11th day	Mean SGPT ±SE (IU/L) At 21th day
I (CCl ₄)	30.33±1.49	132.67±3.77*	-\$
II (Boerhaavia diffusa extract)	28.50±1.18	29.67±1.33**,&	66.50±1.57*,#,&
III (Silymarin)	29.67±1.71	29.50±1.17**,&	71.17±0.87*,#,&

*P-Value<0.001, **P-Value>0.10,\$ group I rabbits were sacrificed on 11th day

*,** values are compared with zero day of same group (self control)

P-Value<0.0001, # compared with 11th day of group I (CCl₄)

& P-Value>0.10 when we compared group II and group III at respective day

Table 4

Showing Mean Alkaline phosphatase(ALP) in IU/L of Rabbits in different groups

Group	Mean ALP ± SE (IU/L) At zero day	Mean ALP ± SE (IU/L) At 11th day	Mean ALP ±SE (IU/L) At 21th day
I (CCl ₄)	36.50±2.53	121.83±3.07*	-\$
II (Boerhaavia diffusa extract)	39.50±1.34	40.50±1.08**,&	63.50±0.92*,#,&
III (Silymarin)	33.17±2.41	36.00±2.37**,&	63.17±1.38*,#,&

*P-Value<0.001, **P-Value>0.10

\$ group I rabbits were sacrificed on 11th day

*,** values are compared with zero day of same group(self control)

P-Value<0.0001,# compared with 11th day of group I (CCl₄)

& P-Value>0.10 when we compared group II and group III at respective day

Table 5
Showing Mean Serum Bilirubin (mg/dl) of Rabbits in different groups

Group	Mean Serum Bilirubin (mg/dl) ± SE At zero day	Mean Serum Bilirubin (mg/dl) ± SE At 11th day	Mean Serum Bilirubin (mg/dl) ± SE At 21th day
I (CCl ₄)	0.33±0.04	1.07±0.07*	- \$
II (Boerhaavia diffusa extract)	0.33±0.04	0.32±0.04***,&	0.57±0.03* ,#,&
III (Silymarin)	0.35±0.04	0.37±0.04**,&	0.55±0.04* ,#,&

*P-Value<0.001, **P-Value>0.10, *** P-Value >0.05

\$ group I rabbits were sacrificed on 11th day

*, **, *** values are compared with zero day of same group(self control)

P-Value<0.0001,# compared with 11th day of group I (CCl₄)

& P-Value>0.10 when we compared group II and group III at respective day

Table 6
Showing Mean Serum Albumin (gm/dl) of Rabbits in different groups

Group	Mean Albumin (gm/dl) ± SE At zero day	Serum Albumin (gm/dl) ± SE At 11th day	Mean Albumin (gm/dl) ± SE At 21th day
I (CCl ₄)	4.00±0.10	2.33±0.07*	-\$
II (Boerhaavia diffusa extract)	4.12±0.18	4.13±0.18**, &	3.13±0.12***, ##, &
III (Silymarin)	4.17±0.10	4.20±0.07**, &	4.17±0.09**, #, &

*P-Value<0.001, **P-Value>0.10,*** P-Value <0.02

\$ group I rabbits were sacrificed on 11th day

*, **, *** values are compared with zero day of same group(self control)

P-Value<0.0001, ## P-value <0.001

,# compared with 11th day of group I (CCl₄)

& P-Value>0.10 when we compared group II and group III at respective

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