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# Protective Effect of the Mixture of Stem bark, Leaf and Root Extract of *Bauhinia acuminata* L. against Carbon tetrachloride induced Liver Damage in *Wistar Albino Rats*

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## ABSTRACT

**Objective:** The objective of the present study was to evaluate the hepatoprotective effect of mixture of stem bark, leaf and root extract of *Bauhinia acuminata* L. using carbon tetrachloride induced hepatotoxicity model.

**Method:** Powdered mixture materials were extracted with distilled water using soxhlet apparatus. The dried extracts were subjected to preliminary phytochemical analysis and the extracts were evaluated for acute oral toxicity by OECD guidelines 423.The aqueous extract at a dose level 200 mg/kg and 400 mg/kg were selected and administered to albino rats for hepatoprotective screening. Hepatotoxicity was induced by Carbon tetrachloride (1 ml/kg with CMC). Silymarin (100 mg/kg p. o) was used as the standard.

**Result:** The preliminary phytochemical examination showed the presence of Alkaloids, Glycosides, Saponins, Flavanoids, Tannins, Aminoacids, Tryptophan, Quinones, Terpinoids, Starch, Vitamin C and Carbohydrates. Furthermore, the acute toxicity study results showed that the extracts were found to be safe up to 2000 mg/kg b. wt. The extract dose dependently shows hepatoprotective potential by restoring the elevated biochemical parameters.

**Conclusion:** The results obtained from the study indicates that mixture of stem bark, leaves and root extract of *Bauhinia acuminata* L. possess promising hepatoprotective activity. The activity might be due the presence of the phytoconstituents including Alkaloids, Glycosides, Saponins, Flavanoids, Tannins, Aminoacids, Carbohydrates Terpinoids, Starch, Vitamin C, Tryptophan and Quinones in the extract. Further studies are required to identify the active principle responsible for the hepatoprotective activity.

## ARTICLE DETAILS

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**KEYWORDS:** Hepatoprotective activity, Bauhinia acuminata L, Carbon tetrachloride

## 1. INTRODUCTION

Hepatotoxicity refers to liver dysfunction or liver damage that is associated with an overload of drugs or xenobiotics. Chemicals that cause liver injury are called hepatotoxins. There are literally thousands of chemicals that could be toxic to the liver and a few examples of these chemicals include: Carbon tetrachloride, Thioacetamide, Galactosamine, Alcohol, Paracetamol, Anti-tubercular drugs (Rifampicin, Isoniazid and Pyrazinamide), Azathioprine, Ranitidine etc. Herbal treatment has been used to alleviate disorders related to liver and other internal organs for many centuries in the eastern world, and have currently become a favourable therapy internationally for pathological liver conditions. Among these, the current study discusses a compilation of herbal medicines used for liver protection, such as Silymarin (milk thistle), Liv-52, *Camellia sinensis* (green tea), Glycyrrhizaglabra (Liquorice) and *Fuzheng Huayu.* The increasing use of herbal medicines reflects their perceived effectiveness in the treatment and prevention of disease, and the belief that these treatments are safe because they are

'natural'. Chronic administration or exposure causes liver cirrhosis, liver tumor, and also kidney damage. The reason for the liver being the major target is that the toxicity of carbon tetrachloride is dependent on metabolic activation by $CYP2E_1$ [1]. The liver is a major inflammatory organ and inflammatory processes contribute to a number of pathological events after exposure to various hepatotoxins. Kupffer cells release pro inflammatory mediators either in response to necrosis or in direct response to an activated hepatotoxin; these pro-inflammatory mediators are believed to aggravate $CCl_4$-induced hepatic injury. Although carbon tetrachloride was originally thought to be resistant to metabolic attack, it is now clear that it is metabolized by cytochromes P-45027 [2].

## 2. MATERIALS AND METHODS

### Chemicals and Instruments

The dried powdered mixture of stem bark, root and leaf of *Bauhinia acuminata* L. is extracted with distilled water by soxhlet extraction. The filtrates obtained are distilled and concentrated under reduced pressure at low temperature and finally freeze dried and stored in a refrigerator until further use.Then the extract was weighed and the yield was recorded. All the chemicals and reagents used in the research work were analytical or synthetic grade.

### Plant material and Extraction

**Fig 1. *Bauhinia acuminate* L. (Stem bark, Leaf, and Root)**

*Bauhinia acuminata* L. is widely cultivated for its ornamental value require very less space to grow. This species occurs widely in deciduous forests and scrub [3] The specimen of *Bauhinia acuminate* L. was collected from Manjeri, Malappuram District, Kerala. The plant specimen (No.148220) was authenticated by Dr. A K. Pradeep, Department of Botany, University of Calicut. The specimen voucher was deposited in the Department of Botany, University of Calicut, itself. The dried powdered mixtures of stem bark, root and leaf of *Bauhinia acuminate* L.is extracted with distilled water by soxhlet extraction. The filtrates obtained are distilled and concentrated under reduced pressure at low temperature and finally freeze dried and stored in a refrigerator until further use.Then the extract was weighed and the yield was found to be 12.72%w/w [4-7].

### Preliminary Phytochemical Analysis

The aqueous extract of *Bauhinia acuminata* L. was subjected to preliminary phytochemical screening by using standard procedure.

### Pharmacological Evaluation

### *In-vitro* Method

HEP $G_2$ (Human Hepatocellular Carcinoma) cell line was purchased from NCCS Pune was maintained in Dulbecco's modified eagles media from National Centre for Cell Sciences (NCCS), Pune, India and maintained Dulbecco's Modified Eagles medium(DMEM). The cell line was cultured in 25 $cm^2$ tissue culture flask with DMEM supplemented with 10% FBS, L-glutamine, sodium bicarbonate and antibiotic solution containing: Penicillin (100 U/ml), Streptomycin (100 µg/ml), and Amphotericin B (2.5 µg/ml). Cultured cell lines were kept at 37ºC in a humidified 5% $CO_2$ incubator. The viability of cells were evaluated by direct observation of cells by Inverted phase contrast microscope and followed by MTT assay method. [8].

### Cells seeding in 96 well plate

Two days old confluent monolayer of cells were trypsinized and the cells were suspended in 10% growth medium, 100 µl cell suspension ($5x10^4$ cells/well) was seeded in 96 well tissue culture plate and incubated at 37ºC in a humidified 5% $CO_2$ incubator.

### Preparation of Compound stock

1 mg of the AEBA was weighed and completely dissolved in 1 mL DMEM using a cyclomixer. The extract solution was filtered through 0.22 µm Millipore syringe filter to ensure the sterility. Carbon tetrachloride (20 mM) was added to induce toxicity.

**Cytotoxicity Evaluation**

After attaining sufficient growth, Acetaminophenol (20 mM) was added to induce toxicity and incubated for one hour, prepared extracts in 5% DMEM were five times serially diluted by two fold dilution (100 µg, 50 µg, 25 µg, 12.5 µg, 6.25 µg in 500 µl of 5% DMEM) and each concentration of 100 µl were added in triplicates to the respective wells and incubated at 37ºC in a humidified 5% $CO_2$ incubator. [9].

**Cytotoxicity Assay by Direct Microscopic observation**

Entire plate was observed at an interval of each 24 hours; up to 72 hours in an inverted phase contrast tissue culture microscope and microscopic observation were recorded as images. Any detectable changes in the morphology of the cells, such as rounding or shrinking of cells, granulation and vacuolization in the cytoplasm of the cells were considered as indicators of cytotoxicity. [10].

**Cytotoxicity Assay by MTT Method**

15 mg of MTT was reconstituted in 3 ml PBS until completely dissolved and sterilized by filter sterilization. After 24 hours of incubation period, the sample content in wells were removed and 30 µl of reconstituted MTT solution was added to all test and cell control wells, the plate was gently shaken well, then incubated at 37ºC in a humidified 5% $CO_2$ incubator for 4 hours. After the incubation period, the supernatant was removed and 100 µl of MTT Solubilization Solution (DMSO was added) and the wells were mixed gently by pipetting up and down in order to solubilize theformazan crystals. The absorbance values were measured by using microplate reader at a wavelength of 540 nm. [11].

***In-vivo* Method**

**Animals**

Albino mice of Swiss strain and Albino rats of Wistar strain were used for pharmacological and toxicological studies. These animals were purchased stock maintained in the animal house of Devaki Amma Memorial College of Pharmacy, Chelembra, Malappuram district of Kerala, India. Female mice selected were nulliparaus and non-pregnant. Female mice weighing 25 to 30 g and rats of either sex weighing 125 to 150 g were used for the study. Each animal, at the commencement of its dosing, was between 8 and 12 weeks old and their weight variation was within ± 20% of the mean weight of any previously dosed animals. The temperature in the experimental animal room was 22ºC (± 3ºC) and the relative humidity was between 50-60%. These animals were fed with pellet diet manufactured by Amrut laboratory, Animal Feed Company, Sangli, Maharashtra and drinking water ad libitum. They were kept in 12 h/12 h light/dark cycle and maintained for at least 5 days prior to dosing to allow for acclimatization to the laboratory conditions. The animal experimental protocol has been approved by our Institutional Animal Ethics Committee (IAEC) vide reference no: 1527/PO/Re/S/11/CPCSEA.

**Evaluation of Acute oral toxicity study of aqueous extract of *Bauhinia acuminata* L.**

Acute toxicity of aqueous extract of *Bauhinia acuminata* was carried out as per OECD guideline 423. The test procedure minimizes the number of animals required to estimate the acute oral toxicity. The test allows the observation of signs of toxicity and can also be used to identify principles that are likely to have toxicity. Animals were fasted (food but not water was withheld overnight) prior to dosing. The fasted body weight of each animal was determined and the dose was calculated according to body weight.

**Limit test at 2000 mg/kg**

Female Swiss albino mice will be used for acute toxicity studies. The assay will be done as per OECD guidelines (No.423). A total of 3 animals are required for this acute toxicity study. All animals will be fasted overnight before the acute toxicity studies. After oral administration of a single dose of plant extract 2000mg/kg, animals are observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and daily thereafter, for a total of 14 days.

**Carbon tetrachloride induced Hepatotoxicity**

**Experimental design**

The Wistar albino rats (150 - 200 g) are divided into five groups, each group comprising of six animals. All the animals except control group were intoxicated with carbon tetrachloride (0.1 ml/kg i. p) for 10 days.

The rats are randomly divided in to five groups with six animals in each.

**Group 1:** Normal control –Received the vehicle (1 ml / kg /day) for 14 days.

**Group 2:** Negative control – Received 0.1 ml / kg of $CCl_4$ i.p for 10 days.

**Group 3:** Positive control – Received 100 mg / kg of standard drug Silymarin through oral for 14 days.

**Group 4:** Extract (lower dose, 200mg/kg p .o) for 14 days.

**Group 5:** Extract (higher dose, 400mg/kg p. o) for 14 days.

Group 3, 4, 5 are intoxicated by $CCl_4$ (0.1 ml / kg / day, i.p) for 10 days 1 hour before administration of standard drug and extract respectively.

On day 15$^{th}$, blood was collected by retro orbital puncture from the inner canthus of the eye under mild Thiopentone sodium anaesthesia using capillary tubes in fresh vials and serum separated. Serum was used for the estimation of hepatic biochemical markers. Immediately after the blood collection, the animals were sacrificed by cervical dislocation. Liver of each animal is separated, get dried and immediately weight these organ and further use for histopathological examination [12-14].

**Blood collection**

Blood will be collected after 48 hours Rats of all groups are anesthetized with (Thiopentone sodium 40 mg/kg, i. p.)Blood samples were withdrawn from the animals by puncturing the

retro-orbital venous plexus with a fine sterilized glass capillary in fresh vials. The blood samples were then left to clot in clean dry tubes and centrifuged at 5000 rpm for 10 minutes to separate serum.

**Preparation of liver sample**

After blood collection, rats were killed by cervical dislocation and livers were dissected out immediately, washed with ice cold saline, cleaned and weighed. Small pieces of liver were cut and transferred into 10% neutral formalin solution for histological studies. Other portions of liver washed with ice cold saline and homogenized in 10% (w/v) ice cold phosphate buffer (0.1 M pH 7.4) and centrifuged at 10,000 x g for 15 minutes. The supernatants obtained were kept in deep freezer until used for the assays.

**Estimation of serum biochemical parameters**

Biochemical parameters like Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT), Alkaline phosphatase (ALP), Total bilirubin (TB), oxidative stress markers (lipid peroxidation, catalase, glutathione peroxidase) were estimated by commercial kits (Agappe Diagnostic) as per the manufacture instructions. [15-17].

**Histopathology of liver**

The animals were sacrificed by cervical dislocation. Dissect the liver store in 10% formalin then observed for histopathological studies

**Statistical analysis**

The data were expressed as mean ± standard error of the mean (SEM). For paired comparisons, student's t-test analyses were performed. Different groups were assessed by one-way analysis of variance (ANOVA) for multiple comparisons followed by Dunnett's test (Graphpad software Inc, La Jolla, CA. Trial version 5). The criterion for statistical significance was set at $P < 0.05$.

## 3. RESULT AND DISCUSSION

**Preliminary phytochemical analysis**

Preliminary phytochemical analysis shows the presence of Alkaloids, Glycosides, Saponins, Flavanoids, Tannins, Aminoacids, Carbohydrates Terpinoids, Starch, Vitamin C, Tryptophan and Quinones in the extract.

**Pharmacological Evaluation**

***In –vitro* methods**

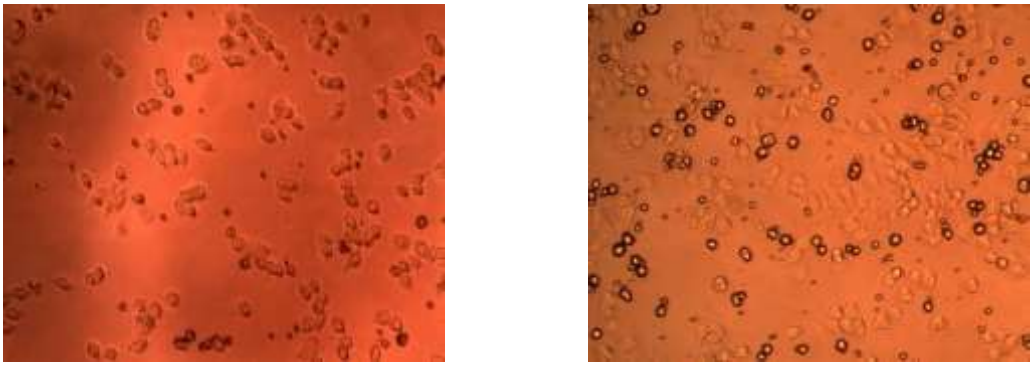
**MTT Assay**

$CCL_4$ has a toxic effect on the cell lines and the percentage viability of the cells was about (41.20 ±0.0560 %). AEBA at a concentration of 100 µg/ml showed highest protection (81.37±0.0296) and showed least protection (42.97±0.0202 %) at a concentration of 6.25 µg/ml. The $EC_{50}$ value of the extract in cell viability was found to be as 11 µg/ml. The extract produced a significant increase in protective effect on cell lines in a dose dependent manner. The morphological assessment of cell lines treated with Carbon tetrachloride indicate the presence of condensed nuclei, cell shrinkage and presence of apoptotic bodies. Cell lines showed a prominent effects after treatment with different concentration of AEBA. The microscopic observations revealed that the extract has a protective effect on cell lines against carbon tetrachloride induced toxicity. At lower extract concentration, cell shrinkage and membrane blebbing was observed but decreased gradually on treatment with increasing concentrations of AEBA.

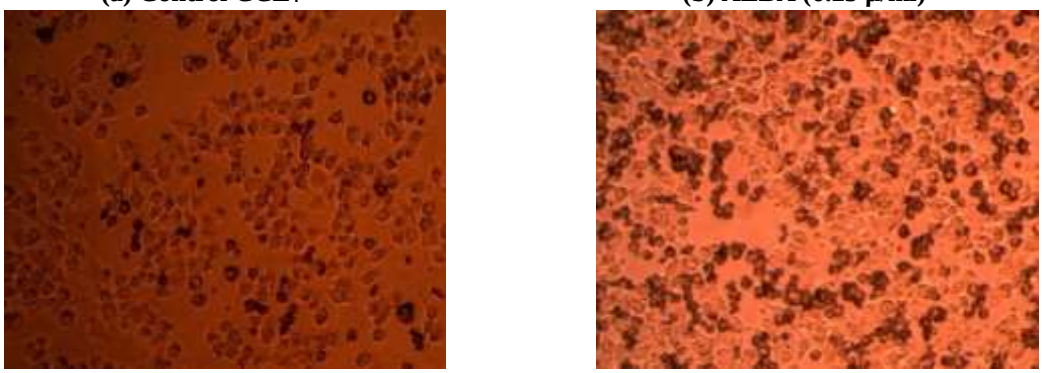
**Table 1. Hepatoprotective activity of AEBA against $CCl_4$ on Hep $G_2$ cell line**

| **Sample Concentration (µg/ml)** | **Percentage Viability (%) ± SEM** |
|---|---|
| **Control** | 100±0.0112 |
| **Carbon tetrachloride** | 41.20 ±0..0560 |
| **AEBA** | |
| 6.25 | 42.97±0.0202 |
| 12.5 | 52.73±0.0220 |
| 25 | 61.79±0.0809 |
| 50 | 71.34±0.0375 |
| 100 | 81.37±0.0296 |
| **$EC_{50}$ of AEBA = 11 µg/ml** | |

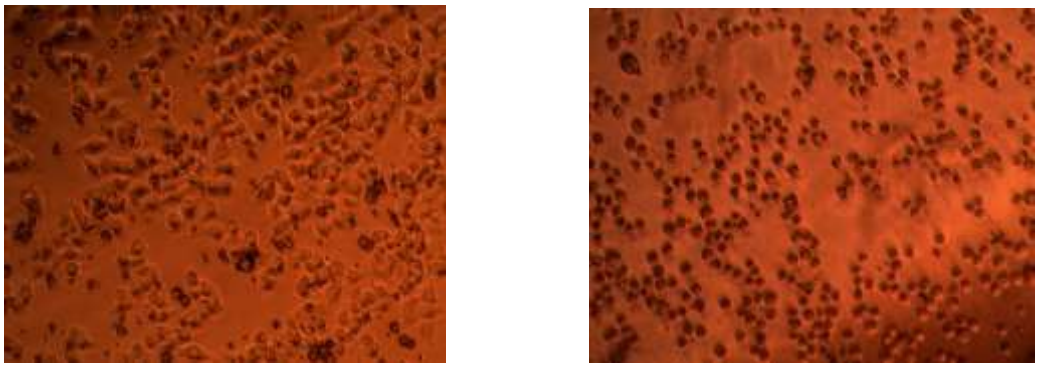
**Values are expressed as mean ± SEM**



**(a) Control $CCL_4$** **(b) AEBA (6.25 µ/ml)**



**(c) AEBA 12.5 µ/ml** **(d) AEBA (25 µg/ml)**



**(f) AEBA (50 µg/ml)** **(h) AEBA (100 µg/ml)**

**Fig 2. Cell viability of Control and$CCl_4$ induced on $HepG_2$ cell lines by MTT assay**

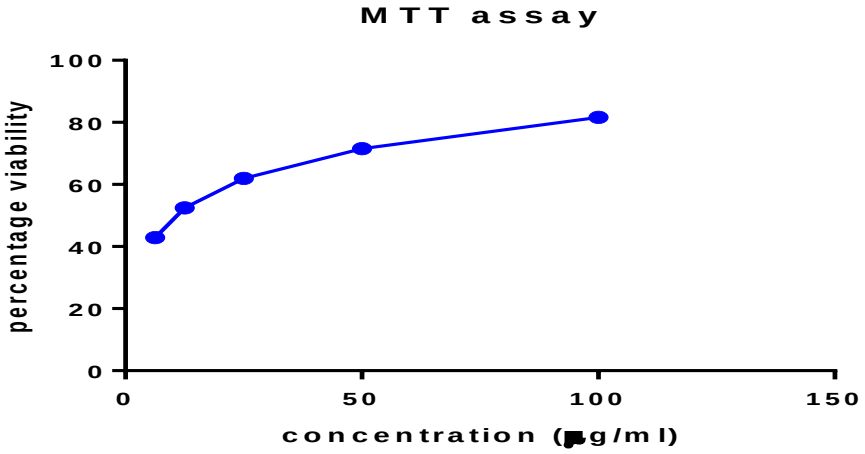



**Fig 3. Effect of different concentrations of AEBA on MTT assay**

**Toxicity evaluation**

**Acute oral toxicity study**

No mortality at a dose of 2000 mg/kg.

All the animals were found to be normal.

No behavioural changes were found on animals till the end of testing.

No observable differences in body weight till the end of testing.

The extract was found to be safe up to 2000 mg/kg.

From the study, $1/5^{th}$ and $1/10^{th}$ of 2000 mg/kg of ethanol extract were selected for Further pharmacological screenings.

***In-vivo* Methods**

**Evaluation of hepatoprotective effect of AEBA on $CCL_4$ induced toxicity**

Hepatoprotective activity of aqueous extract of *Bauhinia acuminata* L. on Carbon tetrachloride induced hepatotoxic rats was evaluated by determining various biochemical parameters. Parameters were determined using diagnostic kit as per the manufacture instruction and results.

**Table 2. Effect of AEBA on liver enzyme markers against $CCL_4$ induced hepatotoxicity**

| GROUP | SGOT (U/L) | SGPT (U/L) | ALP (U/L) | TOTAL BILIRUBIN |
|---|---|---|---|---|
| CONTROL | 47.9±2.81 | 25.62±0.92 | 122.22±1.75 | 0.48±0.02 |
| NEGATIVE | 74.61±0.31*** | 45.41±1.02*** | 188.64±2.20*** | 1.12±0.05*** |
| STANDARD | 53.22±1.26*** | 29.73±1.25*** | 129±0.71*** | 0.50±0.03*** |
| AEBA (200 mg/kg) | 66.71±1.11* | 39.53±39.53* | 168.20±1.37*** | 0.69±0.03*** |
| AEBA (400 mg/kg) | 58.22±3.01*** | 35.26±0.49*** | 154.90±2.24*** | 0.50±0.05*** |

**Values are expressed as mean ± SEM. One way ANOVA comparison between negative group and control group and between negative group and treatment groups (Tukey's Method). The data are considered significant if *p<0.05, **p<0.01, ***p< 0.001, ns-not significant.**

**Table 3. Effect of AEBA on oxidative stress enzyme markers against $CCL_4$ induced hepatotoxicity**

| GROUP | LPO (n mol/g tissue) | CATALASE (U/mg) | REDUCED GLUTATHIONE |
|---|---|---|---|
| CONTROL | 8.18±0.30 | 46.65±0.45 | 18.96±0.49 |
| NEGATIVE | 39.76±0.27*** | 18.01±0.45*** | 11.444±0.27*** |
| STANDARD | 11.10±0.37*** | 41.97±0.35*** | 17.7±0.24*** |
| AEBA (200 mg/kg) | 17.61±0.36*** | 36.05±0.17*** | 14.21±0.21*** |
| AEBA (400 mg/kg) | 13.15±0.26*** | 36.97±0.48*** | 15.27±0.23*** |

**Values are expressed as mean ± SEM. One way ANOVA comparison between negative group and control group and between negative group and treatment groups (Tukey's Method). The data are considered significant if *p<0.05, **p<0.01, ***p< 0.001, ns-not significant**

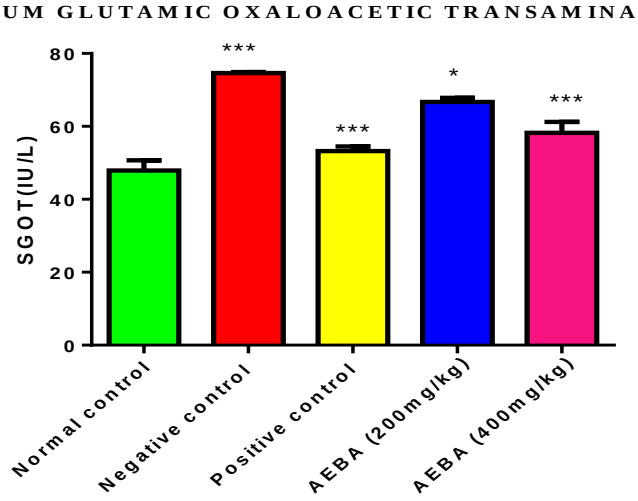



**Fig 4. SGOT variation observed in different group of rats administrated with aqueous extract of *Bauhinia acuminata* L. (AEBA) *p<0.05, **p<0.01**

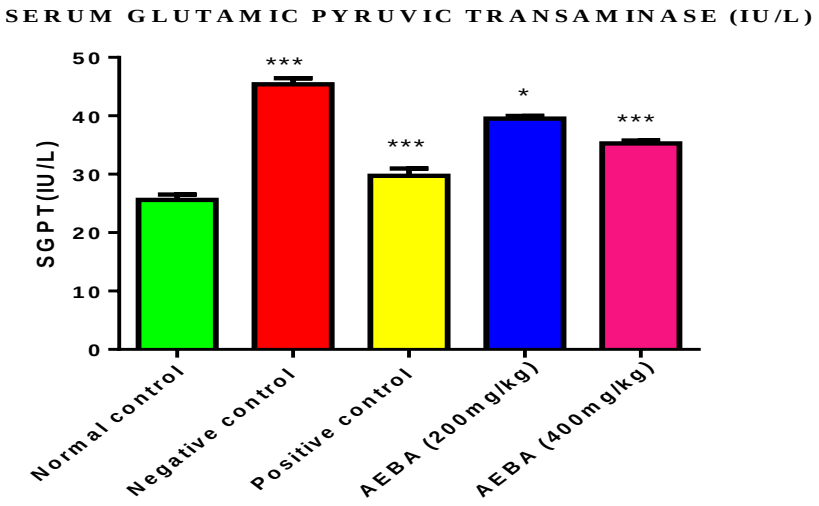



**Fig 5. SGPT variation observed in different group of rats administrated with aqueous extract of *Bauhinia acuminata* L. (AEBA) *p<0.05, **p<0.01**

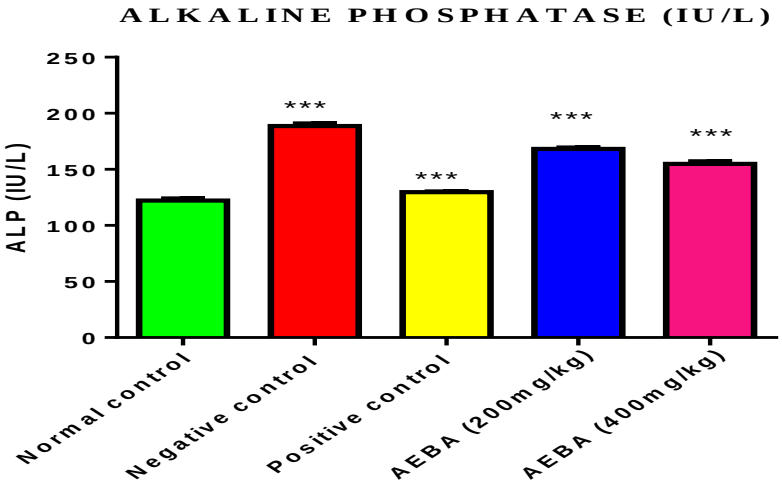



**Fig 6. ALP variation observed in different group of rats administrated with aqueous extract of *Bauhinia acuminata* L. (AEBA) *p<0.05, **p<0.01**

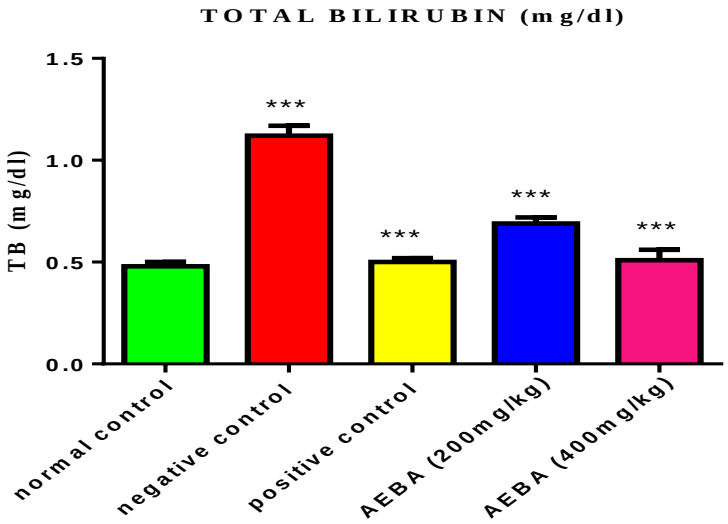



**Fig 7. Total Bilirubin variation observed in different groups of rats administrated with aqueous extract of *Bauhinia acuminata*, L. (AEBA) *p<0.05, **p<0.01**

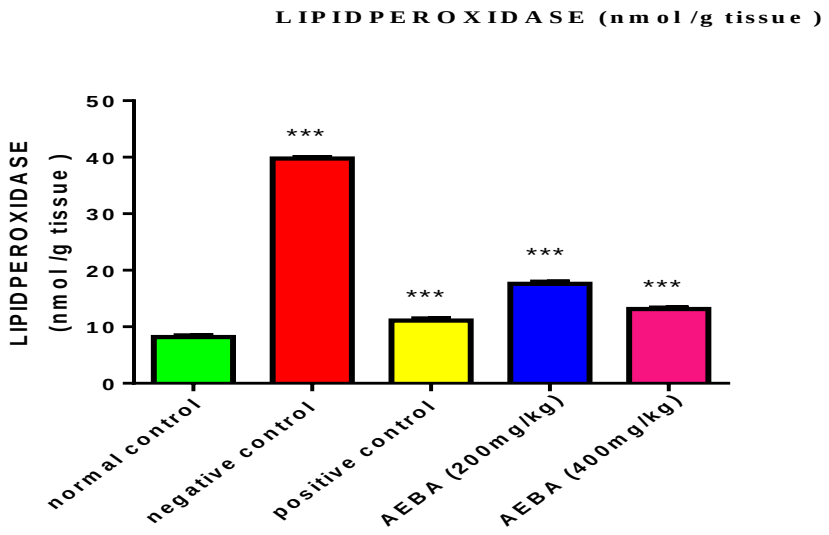



**Fig 8. LPO variation observed in different group of rats administrated with aqueous extract of *Bauhinia acuminata* L. (AEBA) *p<0.05, **p<0.01**

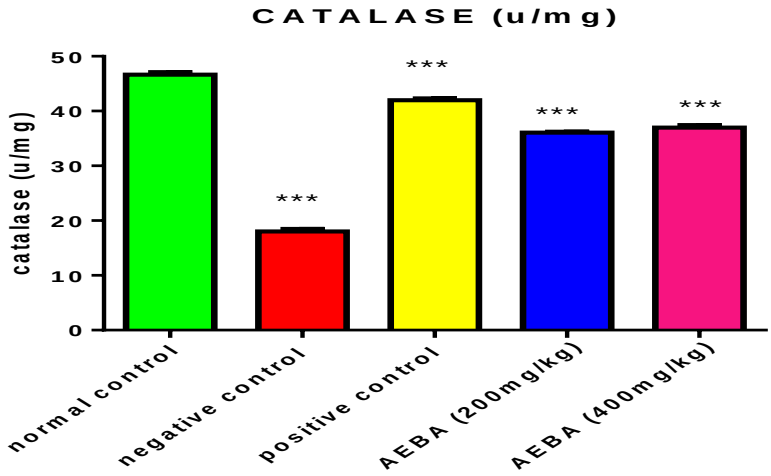



**Fig 9. Catalase variation observed in different group of rats administrated with aqueous extract of *Bauhinia acuminate* L. (AEBA) *p<0.05, **p<0.01**

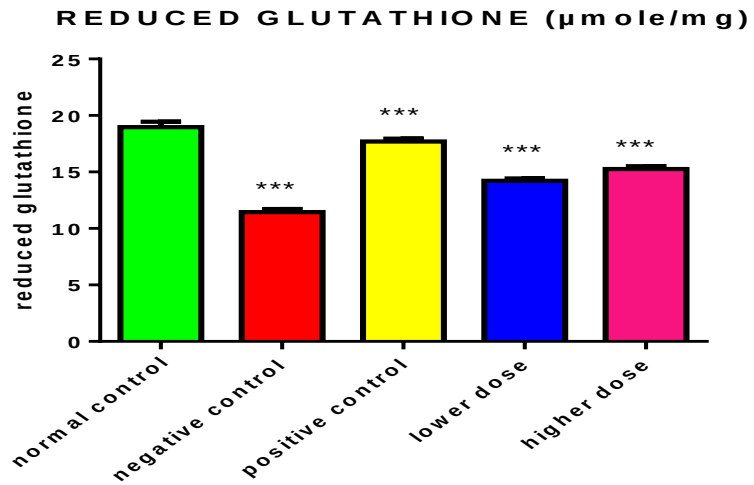



**Fig 10. Reduced glutathione variation observed in different group of rats administrated with aqueous extract of *Bauhinia acuminata* L. *p<0.05, **p<0.01**

**Determination of relative liver weight**

**Table 4. Determination of relative liver weight**

| Sl. No. | Group | Relative liver weight (Mean ± SEM) |
|---|---|---|
| **1** | Control | 2.576±0.1750 |
| **2** | $CCl_4$ (1 ml/kg) | 6.495±0.5255** |
| **3** | $CCl_4$ (0.1 ml/kg) + Silymarin (100 mg/kg) | 3.667±1.155** |
| **4** | $CCl_4$ (0.1 ml/kg) + AEBA (200mg/kg) | 5.993±0.2142** |
| **5** | $CCl_4$ (1 ml/kg) + AEBA (400mg/kg) | 4.154±0.2234** |

**Values given are mean ±SEM. All the groups are compared with the control group (by Dunnett method). The data are considered significant if *p<0.05, **p<0.01**

**Histolopathology of Liver**

In the microphotographs of liver damage was done after staining slides of liver tissue with Haematoxylin and Eosin.

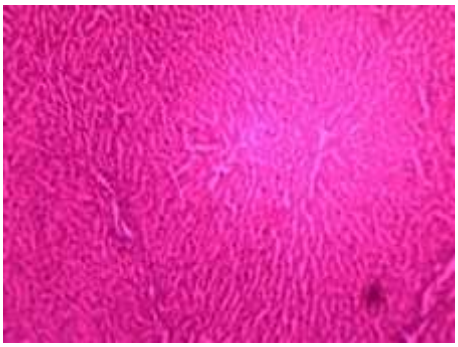


**Fig 11. Microphotographs showing histopathology of liver in normal group**

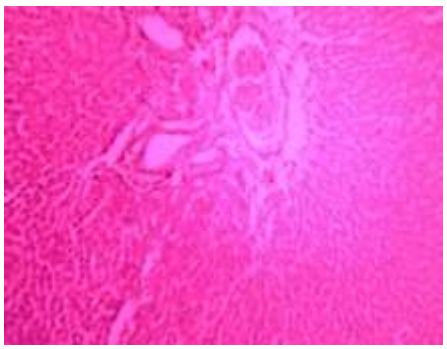


**Fig 12. Microphotographs showing histopathology of liver in $CCl_4$ group**

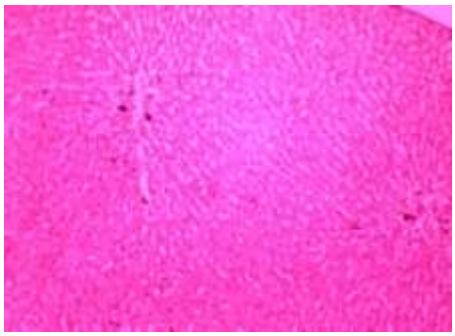


**Fig 13. Microphotographs showing histopathology histopathology of liver in AEBA (200mg/kg) group**

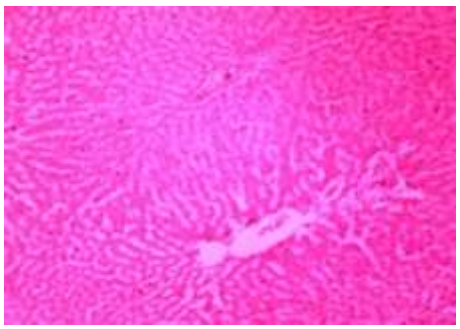


**Fig 14. Microphotographs showing of liver in Silymarin group**

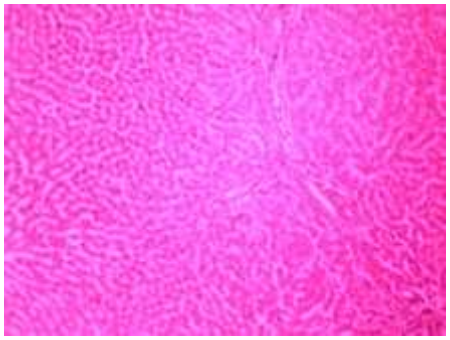


**Fig 15. Microphotographs showing histopathology of liver in AEBA (400mg/kg) group**

The histopathological studies observed in the photomicrographs of liver for treated and control groups. Treatment with the extract decreased the extent of fatty liver and necrosis caused by the hepatotoxic agent $CCl_4$. Based on

the results obtained from the histopathology, the mixture of AEBA shows a significant hepatoprotective activity.

Liver has a prominent role in the regulation of physiological processes. It involved in varieties of vital functions such as metabolism, secretion and storage. Furthermore, detoxification of a variety of drugs and xenobiotics occurs in liver. Hence liver diseases are among the most serious health ailments. The conventional drugs used in the treatment of liver diseases are sometimes inadequate and may lead to serious adverse effects. In India, numerous medicinal plants and their formulations are used for liver disorders in traditional systems of medicine.[18]

*In-vitro* hepatoprotective activity by MTT assay was performed. The morphological assessment of cell lines treated with Carbon tetrachloride indicate the presence of condensed nuclei, cell shrinkage and presence of apoptotic bodies. Cell lines showed a prominent effects after treatment with different concentration of AEBA. The microscopic observations revealed that the extract has a protective effect on cell lines against $CCl_4$ induced toxicity. At lower extract concentration, cell shrinkage and membrane blebbing was observed but decreased garadually on treatment with increasing concentrations of AEBA.

Though *Bauhinia acuminata* L. has been reported to possess therapeutic activities such as anti-oxidant, anti-microbial etc., there exists no scientific evidence on its in vivo hepatoprotective activity. Thus, in the present work, is the mixture of stem bark, leaf and root of aqueous extracts of *Bauhinia acuminata* L. was evaluated as an alternative cure on $CCl_4$ induced hepatotoxicity in rats. [19]

Further the extracts possess significant antioxidant property. Experimental evidences show that free radicals are reported to be involved in the pathogenesis of liver injury. Also, it is established that plants having antioxidant property also exert hepatoprotective effect.

In the present study, the animals treated with the *Bauhinia acuminata* L. Linn extract exhibited reduced hepatic damage and reduced disturbance in the architecture of the liver indicating that it has the hepatoprotective activity. [20]

## 4. SUMMARY AND CONCLUSION

The present study was designed to evaluate the hepatoprotective activity of the mixture of stem bark, leaf and root extract of *Bauhinia acuminata* L. using $CCl_4$ induced hepatotoxicity model. The study revealed that SGOT, SGPT, GGTP, ALP levels were increased and total bilirubin, total protein, glucose, total cholesterol, HDL and LDL levels were decreased in the diseased group. These elevated biochemical parameters were brought back to the near normal level in the ethanol extract treated group dose dependently. These results were comparable to that of the standard drug Silymarin. The aqueous extract significantly reduced the elevated relative liver weight and wet liver volumes of animals as compared to negative control group and hence it possesses statistically significant hepatoprotective activity.

Histopathological evaluation of liver in the treatment group with ethanol extract of *Bauhinia acuminata* L. extract exhibits the prevention of hepatic damage due to $CCl_4$. Extract of high dose (400mg/kg) was showed almost equal effect to the standard drug Silymarin on prevention of hepatic toxicity.

**Compliance with ethical standard**

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***Statement of ethical approval***

All applicable International, National and / or Institutional guidelines for the care and use of animals were followed.

**REFERENCES**

1. De Pasquale, A., 1984. Pharmacognosy: *The oldest modern science, Journal of Ethnopharmacology* 11, 1 – 16.
2. Gerwick, W.H., Marquez, B., Milligan, K., Tan, L.T., and Williamson, T., 2001. Plant sources of drugs and chemicals, *Encyclopedia of biodiversity* 4, 711 – 722.
3. Robinson, M.M and Zhang, X., 2011. The World Medicines Situation, Traditional Medicines: *Global Situation, Issues and Challenges*, 3rd ed., *World Health Organization*, Geneva.
4. WHO, 1991. Guidelines for the assessment of herbal medicine, Program on Traditional Medicine, *World Health Organization*, Geneva.
5. Gerwick, W.H., Marquez, B., Milligan, K., Tan, L.T and Williamson, T., 2001. Plant sources of drugs and chemicals, *Encyclopedia of biodiversity* 4, 711 –722.
6. Sha li, Hor-yue Tan, Ning Wang, Zhang-jin zhang, Lixing Lao, Chi-Woon Wong and Yibin Feng., 2015. The role of oxidative stress and antioxidants in liver diseases, *International journal of Molecular sciences* 16 (11), 1 – 29.
7. Shamala.T, Krupanidhi.A.M, Kalleshappa C.M., 2015. Investigation on antioxidant activity activity of bioactive molecules of *Bauhinia purpurea* Bark, *International Journal of Engineering Research and Development* 11(11), 60 – 62.
8. Archana Dongray, Dr. Raghuvir Irrchariya, Dilip Chanchal and Saurabh Chaudhary., 2015. Phytochemical and pharmacological properties of *Bauhinia acuminate* L., *World journal of pharmaceutical research*, 5(1), 531 – 546.
9. Divya Sebastian and Albin T. Fleming., 2017. Biosynthesis of silver nanoparticles from aqueous extract of *Bauhinia acuminata* using various

physical parameters and its application. *World Journal of Pharmacy and Pharmaceutical Sciences* 6(4), 1889 – 1900.

10. Reyad-ul-Ferdousa, B. C, Farzana Lizab, Tamara Towshin Alamb, Fariha Tasnimb, Mohsina Muktib, Md. Eshak, Khan, Tazmel Haque., 2014. Evaluation of potential antioxidant activity of leaves of *Bauhinia acuminata,, Iranian Journal of Pharmaceutical Sciences* 10(1):55 – 60.
11. Anju Krishna S. R., Hafza S., Poorna Chandrika G., Lekhya Priya C. and Bhaskara Rao K. V., 2015. Pharmacological properties, phytochemical and GC-MS analysis of *Bauhinia acuminata* Linn. *Journal of Chemical and Pharmaceutical Research*, 7(4), 372 – 380.
12. Halina cichoz-lach and Agata Michalak., 2014. Oxidative stress as a crucial factor in liver diseases, *World journal of Gastroenterology* 20 (25), 8082 – 8091.
13. Sha li, Hor-yue Tan,Ning Wang,Zhang-jin zhang,Lixing Lao,Chi-Woon Wong and Yibin Feng., 2015. The role of oxidative stress and antioxidants in liver diseases, *International Journal of Molecular Sciences* 16 (11),1 – 29.
14. Reyad-ul-Ferdousa, B.C., Farzana Lizab, Tamara Towshin Alamb, Fariha Tasnimb, Mohsina Muktib, Md. Eshak, Khan, Tazmel Haque., 2014. Evaluation of potential antioxidant activity of leaves of *Bauhinia acuminate, Iranian Journal of Pharmaceutical Sciences* 10(1), 55 – 60.
15. Navarro VJ, 2006.Drug-related hepatotoxicity. The New England Journal of Medicine 354, 731 – 739.
16. Adewale O.B., Adekeye A.O., Akintayo C.O., Onikanni A., 2014. Carbon tetrachloride ($CCl_4$)-induced hepatic damage in experimental Sprague Dawley rats. Antioxidant potential of *Xylopiaaethiopica*, *The Journal of Phytopharmacology* 3(2), 118 – 123.
17. Mayuresh Rajaratnam, Andrzej Prystupa, Patrycja Lachowska-Kotowska, Wojciech Załuska., 2014. Herbal medicine for treatment and prevention of liver diseases, *Journal of Pre-Clinical and Clinical Research* 8(2), 55 – 60.
18. Archana Dongray, Dr. Raghuvir Irrchariya, Dilip Chanchal and Saurabh Chaudhary, 2015. Phytochemical and pharmacological properties of *Bauhinia acuminata'.*
19. Gilman, Edward F. and Dennis G. Watson., 2009. *Bauhinia purpurea* Purple Orchid – Tree." *Institute of Food and Agriculture Sciences*, University of Florida 5 Jul. 2011.
20. Kumari Nutan Sinha, and Tanuja Sinha, 2013. Phytochemical Screening and Electrophoretic Study of Seed Storage Proteins of *Bauhinia acuminata* and *Cassia occidentalis*. *International Journal of Scientific Research* 4(11), 1812 – 1816.