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RESEARCH ARTICLE

Evaluation of Hepatoprotective Activity of Ethanolic Extracts of Caesalphania Bonducella using In-vivo Models

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ABSTRACT

The present study has been designed to achieve the following aims and objectives. To evaluate the hepatoprotective activity of Caesalpinia bonducella on AZathioprine induced oxidative stress in mice. On the basis of our findings, it may be worthy to suggest that *Caesalpinia bonducella* has antioxidant activity against Azathioprine induced oxidative stress in mice by decreasing the oxidative stress biomarkers serum AST, serum ALT in liver *Caesalpinia bonducella* has antioxidant effect, elevated by measuring antioxidant enzymes. There is increase in superoxide dismutase in liver tissue in standard and test extracts treated mice. *Caesalpinia bonducella* has hepatoprotective effect against Azathioprine induced toxicity in liver by observing the histopathological changes in rat liver tissue.

Keywords: Caesalpinia bonducella, Azathioprine etc.

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1. Introduction

The liver is the largest gland in the human body. It is reddish-brown organ of unequal size and shape. It lies below the diaphragm in the abdominal-pelvic region of the abdomen. This organ plays a major role in metabolism and has a number of functions in the body, including glycogen storage, decomposition of red blood cells, plasma protein

synthesis, hormone production and detoxification. Its primary role is to control the flow and safety of substances absorbed from the digestive system before distribution of these substances into the systemic circulatory system[1]. Hepatotoxicity may be predictable or unpredictable. Predictable reactions typically are dose related and occur which are exposed shortly after some threshold for toxicity is reached. Chemicals such as carbon tetrachloride, phosphorus, and chloroform fairly predictable hepatotoxins that are no longer used as drugs. Unpredictable hepatotoxic reactions occur without warning, are unrelated to dose, and have variable latency periods, ranging from a few days to 12 months[13,14].Liver injury is defined as an alanine aminotransferase (ALT) level of more than three times the upper limit of the normal range, an alkaline phosphatase (ALP) level of more than twice the upper limit of normal, or a total bilirubin (TB) level of more than twice the upper limit of normal if associated with any elevation of the alanine aminotransferase or alkaline phosphatase level. Liver injury is further characterized as hepatocellular when there is a predominant initial elevation of the alanine aminotransferase level or as cholestatic when there is a predominant initial elevation of the alkaline phosphatase level a mixed pattern comprises elevations of both the alanine aminotransferase and alkaline phosphatase levels. Recognizing the pattern of liver injury helps to categorize it, since drugs tend to create injury predominantly in one or another pattern. The injury patterns are not mutually exclusive, and a mixed pattern of injury may occur in many instances of drug-related hepatotoxicity shown in Table.

Many herbal remedies have been employed in various medical systems for the treatment and management of different diseases. The plant Caesalpinia bonducella (syn: Caesalpinia Crista Linn.) has been used in different system of traditional medication for the treatment of diseases and ailments of human beings. It is reported to contain various Alkaloids, Glycosides, Terpenoids and Saponins. It has been reported as anti-asthmatic, antidiabetic, anti-inflammatory, anti-oxidant, anti-bacterial, anti-filarial, anti-tumor, anxiolytic, immunomodulatory, hypoglycemic, activity. This review attempts to encompass the available literature on Caesalpinia bonducella with respect to its pharmacognostic characters, chemical constituents, summary of its various pharmacological activities and traditional uses. Plants have played a significant role in maintaining human health and improving the quality of human life for thousands of years and have served humans well as valuable components of medicines, seasonings, beverages, cosmetics and dyes. Herbal medicine is based on the premise that plants contain natural substances that can promote health and alleviate illness. In recent times, focus on plant research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems. Today, we are witnessing a great deal of public interest in the use of herbal remedies. Furthermore many western drugs had their origin in plant extract. There are many herbs, which are predominantly used to treat cardiovascular problems, liver disorders, central nervous system, digestive and metabolic disorders. Given their potential to produce significant therapeutic effect, they can be useful as drug or supplement in the treatment / management of various diseases. Herbal drugs or medicinal plants, their extracts and their isolated compound(s) have demonstrated spectrum of biological activities. Such have been used and continued to be used as medicine in folklore or food supplement for various disorders. Ethno pharmacological studies on such herbs/medicinally important plants continue to interest investigators throughout the world. Caesalpinia bonducella is an Indian herb reported in Ayurveda, the ancient Hindi medicine system of India. Caesalpinia bonducella belonging to Family: Fabaceae. Found throughout India and tropical countries of the World. The plant was much confused with Caesalpinia bonducella (Syn. C. bonduc) and was described under the same. Beside this species like C. nuga and C. jayoba are also sometimes wrongly designated as synonyms for C. crista. In fact, C. jayoba is an adulterant of C. crista. The seed kernel powder was reported to have hypoglycaemic activity in experimental animals. Four extracts (petroleum ether, ether, ethyl acetate and aqueous) were prepared from the seed kernels and tested for their hypoglycaemic potentials in normal as well as alloxan induced diabetic mice.

In normal mice, only ethyl acetate and aqueous extracts showed a minimum significant hypoglycaemic effect, compared to that of glibenclamide. In diabetic mice, the non-polar extracts i.e. the ether extract showed a marginal anti diabetic activity, while the petroleum ether extract failed to showed significant hypoglycaemic effect, besides, reversing the diabetes induced changes in lipid and liver glycogen levels .But both the polar extracts (ethyl acetate and aqueous) as well as glibenclamide, showed. Since both the polar extracts were, chemically, found to contain triterpenoidal glycosides, we presume that they might be the active principles contributing to the ant diabetic actions.

2. Materials and methods

Materials used

Trichloroacetic acid (TCA), nitro blue tetrazolium (NBT), reduced nicotinamide adenine dinucleotide (NADH), Phenazine metho sulfate (PMS), Ferrozine, glutathione reduced, Batho phenanthroline sulfonate disodium salt, Thiobarbituric acid (TBA), 5,5 -dithiobis-2-nitrobenzoic acid (DTNB), Are bought from Sisco Research Laboratories Pvt. Ltd, Mumbai, India.Hydrogen peroxide, ammonium iron (II) sulfatehexahydrate, 1-chloro-2,4-dinitrobenzene (CDNB), chloramine-T, hydroxylamine hydrochloride, Dimethyl-4-aminobenzaldehyde,2,4-dinitro phenylhydrazine (DNPH) Are bought from Merck, Mumbai, India.

Collection and Authentification of Plant Material

The leaves of caesalpinia bonducella is collected and authenticated by Dr K Madhava Chetty, Department Of Botany, Sri Venkateswara University, Tirupati.

Extraction of Plant Material

The leaves are grinded in to a coarse powder with the help of suitable grinder.

Cold Extraction (Ethanol Extraction)[40,41]

In this work the cold extraction process was done with the help of ethanol. About 200gms of powdered material was taken in a clean, flat bottomed glass container and soaked in 750 ml of ethanol. The container with its contents were sealed and kept for period of 7 days accompanied by continuous shaking with the shaker. The whole mixture then went under a coarse filtration by a piece of a clean, white cotton wool.

Evaporation of Solvent (drying procedure)

The filtrates (ethanol extract) obtained were evaporated using Rotary evaporator in a porcelain dish. They rendered a gummy concentrate of greenish black. The extract was kept in vaccum dissecator for 7 days[42].

Preliminaryphytochemicalscreening[43,44]

Freshly prepared leaves extracts of plant were tested for the presence of phytochemical constituents by using reported methods.

Acute toxicity studies[45]

The Acute oral toxicity test of the extracts was determined prior to the experimentation on animals according to the OECD (Organization for Economic Co-operation and Development) guidelines no 423. Female Albino swiss mice (25-35 g) were taken for the study and dosed once with 2000 mg/kg. The treated animals were monitored for 14 days to observe general clinical signs and symptoms as well as mortality. No mortality was observed till the end of the study revealing the 2000 mg/kg dose to be safe. Thus, 1/10 and 1/20 doses of 2000 mg/kg i.e. 200 mg/kg and 100 mg/kg were chosen for subsequent experimentation.

Induction procedure[46,47,48]**:**

Induction of oxidative stress:

50mg/kg of Azathioprine solution was given as i.p shots to all the group of animals and the samples were collected from the animals through retro-orbital plexus root and the liver, kidney bio marker parameters were estimated like SGOT, SGPT.

Experimental design:

The animals were assigned to five groups, each group containing six mice:

Group I: Mice were orally administered with normal saline (1.2ml/day) for 21days as the normal control.

Group II: Mice administered with Azathioprine (50mg/kg) (i. p) for 21days.

Group III: mice were treated with azathioprine (50mg/kg) and treated with *Caesalpinia bonducella* (100mg/kg) by oral for 21days.

Group IV: mice were treated with azathioprine (50mg/kg) and treated with *Caesalpinia bonducella* (200mg/kg) by oral for 21days.

Group V: mice were treated with azathioprine (50mg/kg) and treated with ascorbic acid (100mg/kg) by oral for 21days.

Collection of blood samples and organs[16-18]:

Blood samples were collected from all the groups of animals 24hours after the 21st day of treatment through puncture of retro orbital plexus and were centrifuged at 3000 revolutions per minute (RPM) for 15 minutes. Serum was separated and stored at -20 c and used for estimating SGOT, SGPT, levels. Mice were killed by over anaesthesia. A midline abdominal incision is made to open up the abdominal cavity and access the liver. The liver are removed rapidly and washed with saline. Then fixed quickly in formaldehyde. The liver were homogenized in 0.25 M cold sucrose solution and centrifuged at 5000 rpm for five minutes. The supernatant which is store at -20^{0}C used for the quantitative estimation of superoxide dismutase within 48hours by using UV spectrophotometry.

Estimation of biochemical parameters:-

The following are the biochemical parameters estimated to evaluate the effect of the test materials against the experimentally induced oxidative stress in mice. They are SOD, ALT (SGPT), AST (SGOT), serum bilirubin.

Estimation of Superoxide Dismutase (SOD)[41,42]

Extraction procedure: 3ml of packed blood cells were lysed by the addition of equal volume of cold deionized water. Hemoglobin was then precipitated by the addition of chloroform: ethanol (1.5:1). This was diluted with 500µl of water and centrifuged for 15 minutes at 3000 rpm. The supernatant containing SOD was taken for the measurement of its activity.

Assay procedure:

0.88ml of riboflavin solution (1.3×10^{-5} M in 0.01M potassium phosphate buffer, pH 7.5) was added to 60µl of O-dianisidine solution (10^{-2} M in ethanol) and to this 100µl of clear separated SOD was added and optical density was measured at 460nm. Then the cuvette containing reaction mixture was transferred to the illuminating box, illuminated for 4min., and optical density was remeasured against blank containing ethanol in place of enzyme. The change in the optical density was determined. The SOD content was determined from the standard graph prepared using pure bovine SOD.

Serum Glutamate Pyruvate Transaminase (SGPT)[43,44]

SGPT converts L- Alanine and - ketoglutarate to pyruvate and Glutamate. The pyruvate formed reacts with 2,4, Dinitrophenyl hydrazine to procedure a hydrazone derivative, which in an alkaline medium produces a brown coloured complex whose intensity is measured. The reaction does not obey Beer's law and hence a calibration curve is plotted using a pyruvate standard. The activity of SGPT (ALAT) is read off this calibration curve

Table 5: Assay procedure for SGPT

Working Reagent	1ml
Sample	0.1ml

Mix and after 1minute incubation, measure the change of optical density per minute (OD/min). During 3 minutes.

Normal range : <40U/L.

Wave length: 340nm.

Calculation:

Activity (U/L) = OD/min × 1768

Serum Glutamate Oxaloacetic Transaminase (SGOT)

SGOT converts L- Aspartate and - ketoglutarate and Glutamate. The oxaloacetate formed reacts with 2,4, Dinitrophenyl hydrazine to procedure a hydrazone derivative, which in an alkaline medium produces a brown coloured complex whose intensity is measured. The reaction does not obey Beer's law and hence a calibration

curve is plotted using a pyruvate standard. The activityf SGOT (AST) is read off this calibration curve *(Excel diagnostics Pvt, Ltd, Hyd, India).*

Procedure:

Table 6: Assay procedure for SGOT

Working Reagent	1ml
Sample	0.1ml

Mix and after 1 minute incubation, measure the change of optical density per minute (OD/min). During 3 minutes.

Normal range : <40U/L.

Wave length: 340nm

Calculation:

$$\text{Activity (U/L)} = \frac{\text{OD}}{\text{Min}} \times 1768$$

Statistical analysis:

All the values were expressed as mean ±standard deviation (S.D). Statistical comparisons between different groups will be done by using one way analysis of variance (ANOVA) followed by dunnett's test. P <0.05 will be considered as statistically significant[36-38].

3. Results and discussion

% yield of ethanol extract = (weight of extract)/ (Powder taken for extraction) × 100 = 20 /200 ×100 = 10%.

% Yield of the *Caesalpinia bonducella* is found to be 10.0

Table 1: Results of Phytochemical Analysis Ethanolic Extract of *caesalpinia bonducella*

Name of the Phytochemical Constituents	Ethanol extract
Saponins	-
Alkaloid	+
Glycoside	_
Reducing sugar	+
Tannin	+
Flavonoid	++
Steroid	_
Anthocyanin	_
Phenol	+
Amino acid	_
Protein	++

+: Indicates the presence and -: Indicates the absence of phytoconstituents

***In- Vivo* Studies:** Evaluation of antioxidant activity of *Caesalpinia bonducella* (EECB) using azathioprine induced oxidative stress in mice

Superoxide Dismutase:

Superoxide dismutase is class of enzyme that catalyze the dismutation of superoxide into oxygen and hydrogen peroxide. It is an important antioxidant defense in nearly all cells exposed to oxygen. Superoxide dismutase activity was estimated in tissue homogenate with help of pure bovine superoxide dismutase standard. The values were shown in below table, and figure.

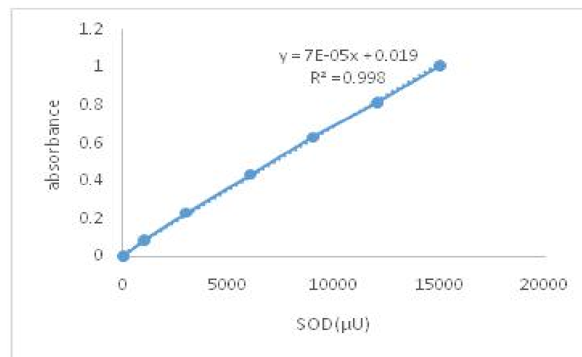

Figure 3: Standard graph of superoxide dismutase

Table 5: Superoxide dismutase levels in liver tissue homogenate

Group	SOD(U/mg) in liver
Normal group	8.75±0.17
Toxic control (50mg/kg)	1.58±0.05
(EECB) low dose(100mg/kg)	4.75±0.08**
EECB high dose (200mg/kg)	6.57±0.071**
Standard ascorbic acid(100mg/kg)	7.12±0.41***

All the values are expressed as mean ±SD (n=6); ** indicates p<0.001, *** indicates p<0.0001 vs toxic control.

In this study, we found that 50mg/kg dose of azathioprine causes significant (p<0.001) decrease in superoxide dismutase levels. This reduction indicates that oxidative stress and toxicity is produced with azathioprine. treated with EECB at the dose of 100mg/kg and 200 mg/kg after a 50 mg/kg dose of azathioprine administration, shown a significant (p<0.001, p<0.0001) dose dependent increase in levels compared to toxic control group.

Serum Alanine Aminotransferase (ALT):

Table 6: Effects of test compound on serum ALT levels in mice treated with azathioprine

Group name	ALT (IU/L)
Normal group	136.65± 1.28
Toxic control (50mg/kg)	195.7± 1.5
EECB low dose(100mg/kg)	157.9± 0.71**
EECB high dose (200mg/kg)	145.7± 0.55***
Standard ascorbic acid (100mg/kg)	139.1 ± 1.28***

All the values of mean ±SD; (n= 6), ** indicates p<0.001, *** indicates p<0.0001 vs toxic control.

Azathioprine and test compound effects on ALT in mice from various groups shown in following figure. Measurements of ALT levels in AZP intoxicated female albino mice, and treated mice with EECB indicate the effect of treatment. The normal control group ALT level show 136.65± 1.28 IU/L. After AZP treatment, the ALT level is 195.7± 1.5IU/L. This AZP treated group ALT level was increased compared to the normal control group in 21days. After 21days treatment, the test compound low dose ALT level was (157.9± 0.71IU/L) decreased compared to the toxic control group has shown significance (**p<0.001) and at high dose ALT level was (145.7±

0.55IU/L) decreased compared to the toxic control group has shown significance (***$p<0.0001$). On treatment standard ascorbic acid serum ALT level 139.1 ± 1.28, has shown significant (***$p<0.0001$).

Serum Aspartate Aminotransferase (AST):

Table 7: Effects of test compound on serum AST levels in mice treated with azathioprine

Group names	AST(IU/L)
Normal group	139.7±1.43
Toxic control (50mg/kg)	227.15±1.49
EECB low dose(100mg/kg)	159.0± 1.31***
EECB high dose (200mg/kg)	143.7± 0.92***
Standard ascorbic acid(100mg/kg)	140.3± 1.06***

All the values of mean ±SD; n= 6, *** indicates $p<0.0001$ vs toxic control.

The above table shows the effect of test compound on serum AST levels in mice intoxicated with AZP. After 21days, the normal control group shows the AST level is 139.7± 1.43IU/L. In AZP control group level is 227.15± 1.49IU/L, increased compared to the normal group. Treatment with EECB at low dose AST level was (159.0±1.31IU/L) decreased compared to the toxic control group has shown significance (***$p<0.0001$) and at high dose AST level was (143.7± 0.92IU/L) decreased compared to the toxic control group has shown significance (***$p<0.0001$). On treatment standard ascorbic acid serum AST level 140.3±1.06, has shown significant (***$p<0.0001$).

Histopathological evaluation:

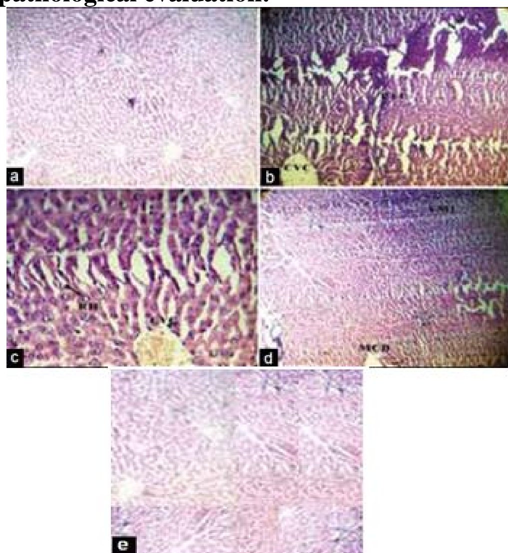

Figure 1: Effect of ethanolic extract of CB on histopathological examination of mice liver in azathioprine-induced hepatotoxicity. (a) Group 1 (normal): Showing normal histology of mice liver. (b) Group 2 (toxic control): N-Focal Necrosis, PTI-Extensive portal triad inflammation, CVC-Central vein congestion. (c) Group 3 (low dose): CVC-Central vein congestion, RH-Regenerating hepatocytes. (d) Group 4 (high dose): MCD-Mild central vein dilation, VMI-Very mild inflammation (e) group 5 (standard): nearly normal liver cells observed.

4. Conclusion

On the basis of our findings, it may be worthy to suggest that *Caesalpinia bonducella* has antioxidant activity against Azathioprine induced oxidative stress in mices by decreasing the oxidative stress biomarkers serum AST, serum ALT in liver. *Caesalpinia bonducella* has antioxidant effect, elevated by measuring antioxidant enzymes. There is increase in superoxide dismutase in liver tissue in Azathioprine induced oxidative stress in mices. *Caesalpinia bonducella* has hepatoprotective effect against Azathioprine induced toxicity in liver by observing the histopathological changes in mice liver tissue.

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