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Research Article

Assessment Of Hepatoprotective Activity Of Barleria Gibsoni And Cleome Gynandra Against Thioacetamide Induced Hepatotoxicity In Experimental Animals

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Abstract
Liver is a vital organ that plays a major role in the elimination of xenobiotics from the body. Diseases that affect the liver become major health problems and challenge health-care professionals as well as the pharmaceutical industry. Since the conventional treatment of liver diseases is associated with a wide range of adverse effects, botanical agents are commonly used. Among these agents, *barleria* species and *Cleome gynandra* are the most widely used herbs in Indian traditional medicine. In the current research rats were divided in to respective groups of six animals in each and hepatoprotective activity of EECG and EEBG was screened at 200, 400 and 500 mg dosages. Plant extracts were given one day following inducing agent administration and the treatment was proceeded for 21 days. Then, the levels of biomarkers of liver injury – such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) – and liver function such as total protein, albumin, and bilirubin were measured. Evaluation of the change in body weight and liver weight, and histopathologic examination against thioacetamide-induced hepatotoxicity were also carried out. The ethanolic extract of both plants decreased the absolute and relative weight of the liver of rats at the doses of 200 and 400 mg/kg ($p<0.01$ and $p<0.001$, respectively). It also suppressed the plasma levels of AST, ALT and ALP ($p<0.001$) in the aforementioned doses. Both palnts were endowed with hepatoprotective activity, probably mediated via its antioxidant activity. Thus, the both plants can be taken as one candidate for the development of hepatoprotective agents because of its good safety profile.

Key words: Cleome gynandra, Barleria gibsoni, Hepato protective, Thioacetamide

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Introduction:
Human liver is the most essential visceral organ in body concerned with synthesis, excretion, metabolism and detoxification of diverse exogenous and endogenous substances such as drugs [1]. Because of these multi-dimensional functions, it is prone to many diseases. Death of hepatocytes is the main feature of liver diseases and liver disease is a worldwide major health issue. Causative agents include diseases that interfere with liver functions; chemicals (ethanol,

CCl4, thioacetamide, D-galactosamine; environmental toxins) and drugs such as paracetamol Liver damage caused by inhalation or ingestion of hepatotoxin like drugs is increasing worldwide, and conventional drugs for this drug induced liver damage management are generally insufficient and show serious adverse effects. Drug- induced liver damage accounts for more than 50% of acute liver damage according to the United States Acute Liver Failure Study Group [2]. Drug-induced liver damage caused by overdose of paracetamol, PILD, accounts for about 13% of the hospitalization [3]. A 0.08% of the UK people each year are found in hospital with paracetamol poisoning and only 0.6% of them develop acute liver damage [4]. Paracetamol overdose is a significant clinical problem in USA, Denmark and Australia although less common than in the UK, and is the commonest cause of acute liver damage in USA [5]. Each year, approximately 0.02% of the Australian and 0.01% of the US population are found with paracetamol poisoning in hospital [6]. So, paracetamol toxicity is the second most common cause of liver transplantation worldwide. Several already approved drugs were withdrawn from the market due to common reason, the drug induced liver damage. It is, hence, necessary to explore the herbal drugs in the management of drug induced liver damage to replace the drugs of low safety and efficacy [7].

Natural resources such as medicinal plants are constantly being searched to find new molecules as drugs. Several plants and their formulations are being utilized for drug induced liver damage in traditional systems of medicine and in ethnomedical practice in India [8]. Plant-derived medicinal agents such as silymarin extracted from the seeds of milk thistle (*Silybum marianum*)) are utilized globally as hepatoprotective agent [9]. Commercially, about 40 polyherbal formulations reputed to possess hepatoprotective activity are being utilized in India. Extracts from 25 medicinal plants have been reported to treat drug induced liver damage [10]. Natural remedy by utilizing medicinal plants is considered a safe and an effective alternative management of drug induced liver damage. Heart wood, leaves, flowers and bark of *Pterocarpus marsupium*, the Indian Kino tree have useful medicinal properties [11]. *Pterocarpus marsupium* is also a rich source of flavonoids, polyphenolic compounds and their derivatives [12]. Flavonoids and phenolics are reported bioactives which decrease different destructive processes in human being due to their ability to scavenge free radicals and reduce its formation [13].

Barleria gibsoni Dalz. (Acanthaceae) is widely distributed throughout Africa, India, Sri Lanka and tropical Asia. It is commonly known as Neel koranti, the juice of the leaf is used in cataract and fever. The dried bark is used in cough treatment and the leaves chewed to relieve toothache. The paste of the root is applied to disperse boils and glandular swellings [14]. It exhibits several medicinal properties. Juice of the leaves is used in ulcer and fever. Paste of the roots is applied to disperse boils and glandular swellings. Leaves are also used by some tribal communities for the treatment of piles and to control irritation. Plant is also used in stiffness of limbs, enlargement of scrotum and sciatica [15-17].

Cleome gynadra commonly known as spiderwisp in English. Cleome gynandra is used as a medicinal plant and can be found in all over world. It grows as a weed in paddy fields and also in road sides and in open grass lands. In India it is never cultivated but grows spontaneously everywhere. Different species of Cleome can be found in all states of India. In Ayurvedic medicine it is a chief constituent in *Narayana Churna.* In Ayurveda it is used as an Anthelmentic, in ear diseases, pruritis and several other diseases like gastro intestinal disorders and gastrointestinal infections etc [20].

Materials and Methods

Sources of drugs and chemicals

All reagents, solutions, chemicals and solvents used in present study were obtained as analytical grades. Centrifuge (Mini centrifuge, Spinnwell), Micropipette (100–1000 µl, 10–100 µl, Superfit), Digital balance (Shimadzu AUX220 Unibloc), UV-spectrophotometer (PharmaSpec UV- 1700 Shimadzu, Japan) and Refrigerator from Intello cool LG.

Collection and authentication of plant material

Fresh leaves of *Cleome gynadra* and *Barleria gibsonii* were collected from Kadapa district of Andhra Pradesh, India, in the month of October and authentified by Prof. Dr. K. Madhava Chetty, Taxonomist, S.V. University, Tirupati. A.P. Specimen vouchers were deposited at Department of Pharmacognosy for further reference.

Extract Preparation

The extract procedure used in this study is soxhlation using ethanol as solvent. An amount of five hundred grams was taken and the soxhlation was proceeded till the infusion get colorless. The acquired infusion was evaporated and dried out, kept under dessicator and used for research.

Experimental animals

Experimental Protocol

The animal selected for this study is male wistar albino rat of body weight varying 200 to 250 gm. These animals are set in polypropylene cages in temperature of 22 degrees centigrade at half-day light and half-day darkness. Rats were offered standard pellet food and water ad libitum. The investigation procedures have been stringently implemented according to the recommendations of CPCSEA and legislation of the institute. Single dose of thioacetamide induces hepatotoxicity. In the current research rats were divided in to 9 groups of six animals in each and Hepatoprotective activity of EECG and EEBG was screened at 200, 400 and 500 mg dosages.

Evaluation of liver function test

The serum AST (modified UV kinetic assay), ALT (modified UV kinetic assay), ALP (modified UV kinetic assay), TC (CHOD-PAP assay), ALB (modified UV kinetic assay), SBL (Jendrassik-Grof assay), TP (modified UV kinetic assay) and TG (modified UV kinetic assay) were resoluted and evaluated by using regular kit sets from the manufacturer Span Diagnostic Ltd, India. LDH (Infinite LDH UV kinetic assay) was evaluated by using regular kits from the manufacturer Accurex Biochemical Private Limited, India. UV-spectrophotometer (Shimatzu) was applied for all the enzymatic evaluations as per regular kit set methods. The detailed standard procedures for such estimations were obtained from the leaflets provided in the commercial kits and also the same kit-leaflets methodology mentioned in the reference [21]

Assessment of histopathology

Before histopathological examination and for the preservation of isolated livers of rats of different groups, 10% formalin solution was used. The liver was mounted by embedding it in paraffin; it was cut into size of 6 mm sections, put on slide and stained consequently with the dyes eosin and hematoxylin and observed in the light microscope intended for TTA induced Liver damage or hepatoprotection. They were observed for lymphocyte infiltration, centrilobular necrosis, fatty infiltration and fibrosis [21]

Statistical analysis

The biochemical data have been expressed as mean ± SEM (standard error of mean). All the values were futher subjected to ANOVA using Graphpad Prism version 9.0. Variance was estimated by Dunnettes multiple comparison test. The $P < 0.05$ value was measured as significant value.

Results:

Evaluation of liver function test

When compared to the normal group, rats treated with thioacetamide showed severe liver damage as indicated by raised serum levels of hepatospecific enzymes such SGPT, SGOT, and ALP. Both plant extracts demonstrated effective protection against thioacetamide-induced liver damage following treatment with silymarin and ethanolic extracts. When compared to toxic control rats, extract-treated animals showed a substantial decrease in elevated serum enzyme levels according to Dunnet's 't' test. In comparison to the toxic control, which was a 100% rise, the hepatoprotective efficacy was represented as a percentage reduction in biochemical markers.

Other biochemical parameters

Effect on Serum direct bilirubin and total bilirubin

After thioacetamide administration, elevated levels of direct and total bilirubin show that the drug is toxic to the liver. When compared to the toxic control group, silymarin, and plant extract treatment dramatically decreased levels of direct and total bilirubin, demonstrating the hepatoprotective action of *cleome gynandra and barleria gibsoni* leaf extracts.

Effect on Serum total protein and albumin levels

Albumin and total protein levels in serum have significantly decreased after treatment with thioacetamide. In comparison to the toxicant group, pretreatment with *cleome gynandra* and *barleria gibsoni* leaf extracts in silymarin, ethanolic extracts resulted in significantly higher levels of total protein and albumin.

Effect on Serum cholesterol and Triglycerides

From the results it was found that rats treated with thioacetamide have showed a marked increase in cholesterol and triglycerides levels when compared to normal control group. The rats pretreated with silymarin and ethanolic leaf extracts of *cleome gynadra* and *barleria gibsoni*, the serum cholesterol and triglycerides levels had significantly decreased when compared to toxicant group.

Histopathology

The normal control group's liver sections underwent histopathological investigation using silymarin as the reference medication and revealed normal cellular architecture with separate hepatic cells. However, with the treatment of thioacetamide, significant hepatic necrosis was seen along with hepatic cell death. Treatments with EECG and EECG for such thioacetamide -intoxicated rats demonstrated hepatocyte recovery from necrosis. This shows that the plant extract has a great ability to restore the abnormal alterations brought on by thioacetamide hepatotoxicity.

Wet liver weight and Wet liver volume

Rats treated with thioacetamide had enlarged livers, as shown by an increase in wet liver weight and volume. Wet liver weight and volume were significantly restored in the groups treated with silymarin, and alcoholic leaf extracts of *cleome gynandra* and *Barleria gibsoni*.

Discussion

The liver has an indispensable role in life via its endowed metabolic and detoxification capability. As it is exposed to several endogenous and xenobiotic agents, a myriad of intermediate and end products is produced and can cause hepatocellular death and constitute the principal causes of liver disease [22]. TAA at 300 mg kg^{-1} body weight as a single dose has been reported to cause oxidative stress.19 The aim of this study was TAA acts as an electrophilic agent and leads to formation of s-oxide that can covalently bind to the lysine residues forming adducts with sulfhydryl groups hence lowering the protein levels and causing significant damage. Protein adducts with TAA were also reported in previous studies [23]. Results from present study evidently reflect hepatocellular damage in TAA in animal model and EECG and EEBG at the doses of 200, 400 and 500 mg/kg significantly lowered the SGPT, SGOT, ALP, total cholesterol and triglycerides in the thioacetamide intoxicated rats of groups. Toxic metabolite in turn causes liver damage which disturbs transport function of the hepatocytes resulting in the leakage of plasma membrane and thereby causing an altered level of enzyme in the serum [24]. Flavonoids and phenolics are reported bioactives which decrease different destructive processes in human being due to their ability to scavenge free radicals and reduce its formation. Hepatoprotection by plant extracts might be caused by the anti-oxidant activity of phyto-constituents available in plant extracts which reduced an oxidative stress incorporated by thioacetamide and another similar analgesic and anti-inflammatory qualities preventing the inflammatory liver damage [25].

Conclusion

Present study has demonstrated that ethanolic extract of two plant leaves possesses a potent hepatoprotective effect against thioacetamide-induced liver damage. Thus, both plant extracts may provide a novel potent hepatoprotective agent for liver damage, which could be considered for further clinical studies for future drug development.

Declaration of Competing Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article.

Table 1: Treatment schedule for thioacetamide Induced hepatotoxic Method

Groups	Treatment
I	Normal control 10 ml/kg p.o
II	Toxicant control (Thioacetamide 100mg/kg, s/c)
III	Silymarin (100mg/kg, p.o) +Thioacetamide
IV	EECG (200mg/kg, p.o) + Thioacetamide
V	EECG (400mg/kg, p.o) + Thioacetamide
VI	EEBG (200mg/kg, p.o) + Thioacetamide
VII	EEBG (400mg/kg, p.o) + Thioacetamide
VIII	EECG+EEBG (200mg/kg, p.o) + Thioacetamide
IX	EECG+EEBG (400mg/kg, p.o) + Thioacetamide

Table 2: Effect of Effect of EEBG & EECG on Lipid levels on different bio chemical parameters

Groups	Treatment	SGPT	SGOT	ALP	Total bilirubin	Direct bilirubin	Albumin	Total Protein
I	Normal control 10 ml/kg p.o	40.18 ± 4.12	107.23 ± 15.354	107.22 ± 7.397	0.296 ± 0.02076	0.2067 ± 0.02076	4.408 ± 0.1623	13.568 ± 0.5712
II	Toxicant control (Thioacetamide 100mg/kg, s/c)	120.99$^{###}$ ± 1.56	229.23$^{###}$ ± 3.444	210.23$^{###}$ ± 1.200	2.758$^{###}$ ± 0.06498	2.297 $^{###}$± 0.07522	3.355$^{###}$ ± 0.1150	6.517$^{###}$ ± 0.3355
III	Silymarin (100mg/kg, p.o) +Thioacetamide	42.42*** ± 1.21	112.50*** ± 1.408	116.02*** ± 0.7483	0.3083*** ± 0.009	0.2267***± 0.008	4.198*** ± 0.03877	11.958** ± 0.1510
IV	EECG (200mg/kg, p.o) + Thioacetamide	114.65^{ns} ± 2.11	205.58^{ns} ± 1.511	181.70** ± 1.761	2.340** ± 0.0391	2.028** ± 0.0249	3.447^{ns} ± 0.02246	7.675^{ns} ± 0.0864
V	EECG (400mg/kg, p.o) + Thioacetamide	87.25** ± 1.65	155.59** ± 7.636	156.47** ± 2.800	1.760** ± 0.02817	1.222** ± 0.0257	3.673** ± 0.03363	8.448** ± 0.0709
VI	EEBG (200mg/kg, p.o) + Thioacetamide	71.40*** ± 1.05	145.22*** ± 2.142	137.80*** ± 1.748	1.053*** ± 0.0328	0.8367*** ± 0.013	4.008***± 0.0294	11.242*** ± 0.5170
VII	EEBG	110.86* ± 1.97	210.64^{ns} ± 6.506	195.69* ± 2.589	2.253** ± 0.0253	1.825** ± 0.041	3.52^{ns} ± 0.03109	7.588^{ns} ± 0.1060

	(400mg/kg, p.o) + Thioacetamide							
VII	EECG+EEBG (200mg/kg, p.o) + Thioacetamide	90.09** ± 2.84	184.95** ± 3.956	171.28* ± 1.108	1.585** ± 0.0189	1.228** ± 0.0164	3.840** ± 0.01528	9.868** ± 0.1710
IX	EECG+EEBG (400mg/kg, p.o) + Thioacetamide	68.32*** ± 1.00	146.29*** ± 3.065	142.37** ± 2.757	0.9967*** ± 0.0128	0.7183*** ± 0.010	4.075***± 0.0455	11.812*** ± 0.472

All values are shown as mean ± SEM and n=6. ### indicate $p<0.001$ when compared to normal control group. * indicate $p<0.05$, ** indicate $p<0.01$, *** indicate $p<0.001$ when compared to toxicant control group. ns= nonsignificant

Table 3: Effect of EEBG & EECG on Lipid levels

Groups	Treatment	Cholesterol levels (U/L)	Triglycerides (U/L)
I	Normal control 10 ml/kg p.o	133.69 ± 2.367	30.538 ± 1.451
II	Toxicant control (Thioacetamide 100mg/kg, s/c)	210.28### ± 2.494	117.91### ± 1.875
III	Silymarin (100mg/kg, p.o) +Thioacetamide	147.57*** ± 1.451	41.895*** ± 1.47
IV	EECG (200mg/kg, p.o) + Thioacetamide	200.71* ± 1.431	102.38** ± 102.38
V	EECG (400mg/kg, p.o) + Thioacetamide	180.38** ± 3.670	73.788** ± 1.752
VI	EECG (200mg/kg, p.o) + Thioacetamide	153.90*** ± 1.625	63.143*** ± 2.385
VII	EECG (400mg/kg, p.o) + Thioacetamide	202.94ns ± 3.231	111.21ns ± 3.050
VII	EECG+EECG (200mg/kg, p.o) + Thioacetamide	186.81** ± 2.940	89.450** ± 3.414
IX	EECG+EECG (400mg/kg, p.o) + Thioacetamide	156.21*** ± 1.449	59.387*** ± 2.987

All values are shown as mean ± SEM and n=6. ### indicate $p<0.001$ when compared to normal control group. * indicate $p<0.05$, ** indicate $p<0.01$, *** indicate $p<0.001$ when compared to toxicant control group. ns= nonsignificant

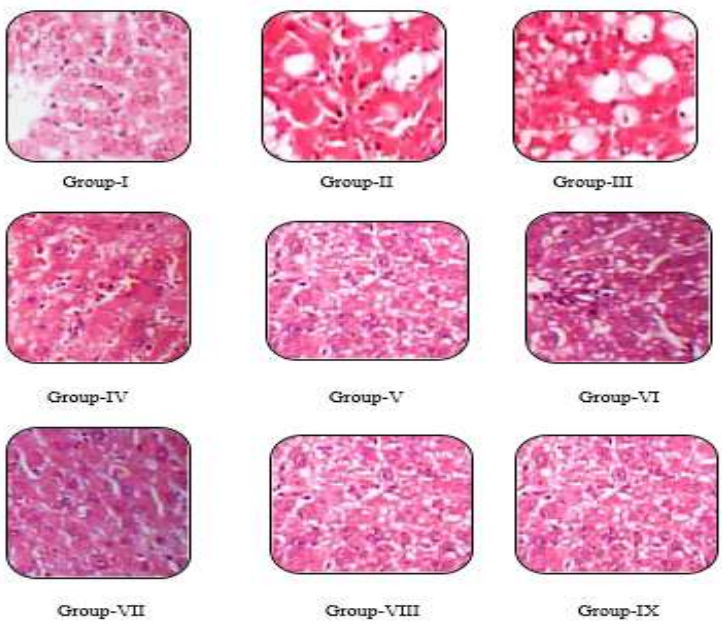

Figure 1: Histological changes showing efficacy by EECG & EEBG on thioacetamide intoxicated rats

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