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EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF ETHANOLIC LEAVES EXTRACTS OF *HIBISCUS PLATANIFOLIUS*

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ABSTRACT

Aim of the study is to evaluate the Hepatoprotective activity of ethanolic leaves extracts of *Hibiscus platanifolius.* In present investigations leaf of *Hibiscus platanifolius* was subjected to proximate analysis and showed significant values. In that the ethanolic extract (6.7w/w) shows more extractive value compared to water extractive value (5.8w/w). The assessment of hepatoprotective activity was carried out by estimation of various biochemical parameters i.e. Glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), alkaline phosphatase (ALKP), total bilirubin (TBL), total cholesterol (CHL), lactate dehydrogenase (LDH), total protein (TPTN), and albumin (ALB) in serum. The biochemical observations were supported by histological examination of liver sections of rats. Results showed significant Hepatoprotective activity of ethanolic leaves extracts on tested dose 200mg/kg b wt and 400mg/kg b wt.

Key Words: *Hibiscus platanifolius,* Hepatoprotective activity, ethanolic leaves extracts

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INTRODUCTION

Herbal medicine continue to grow in popularity as consumers adopt more natural approaches for staying healthy, and these have been used since ancient times. In fact, every major culture has used herbalism as the method of healing at some time or the other. Since past two decades World Health Organization is encouraging the use of principal indigenous medicinal plants in developing countries. World Health Organization estimates that about 80% of population living in developing countries relies almost exclusively on traditional medicines for their primary health care needs. Since the medicinal plants are the backbone of traditional medicine, this mean that, 3300 million people in the under developed countries utilize medicinal plants on a regular basis. This assumption does not include the developed countries where there has been a great fascination for the herbal medicines and dietary food supplements in the last decade. Although worldwide growth in usage of traditional systems of health care has been seen in recent years, countries like India, China, Tibet and Brazil, the health care scenario has always been associated with these traditional systems of medicines (TSM). These countries are still having very rich biological as well as cultural diversity and the traditional health care systems have a deep influence on the current healthcare means in these nations. Today these

traditional systems are not only flourishing in their respective countries but are also becoming immensely popular among other nations including the western world. In India a number of traditional health care systems have been practiced for many centuries, namely Ayurveda, dating back to more than 4000 years, siddha, Unani and more recently homeopathy. Apart from these systems, there has been a rich heritage of ethno botanical usage of herbs by various tribal communities in the country. All these systems have been codified and documented in oriental languages. the fact that many important drugs in use today in modern medicine were derived from plants or from starting molecules of plant origin. Digoxin/digitoxin, the vinca alkaloids, reserpine and tubocurarine are some important examples. Plants have also yielded molecules which are extremely valuable tools in the characterization of enzymes and the classification of receptor systems: morphine, physostigmine, muscarine, atropine, nicotine and tubocurarine, are the examples. Some scientists thus expect that the plant kingdom holds the key to the understanding of complex human biochemistry/pathology and the cure of man's perplexing diseases. The initial optimism, engendered by the idea that a sophisticated understanding of receptor systems and of the biochemistry of disease would pave the way to predictable drug development, has not been released., therefore, laboratories around the world are engaged in the screening of plants for biological activity with therapeutic potential. One major criterion for the selection of a plant for such study is to ascertain traditional healer's claims for its therapeutic usefulness. It is thus worth reflecting on the cultural environment in which traditional healers use plant remedies, as well as the materials of plant use, in order to strengthen the research design.

The modern system of medicine still lack in providing suitable medicament for a large number of adverse conditions, in spite of tremendous advances made in discovery of new compounds. A few of these diseases can be mentioned like, hepatic disorders, viral infections, AIDS, rheumatic diseases etc. The available therapeutic agents only bring about symptomatic relief without any influence on the curative process, thus, causing the risks of relapses and danger of untoward effects. A large number of populations suffer, due to various reasons, from hepatic diseases and also inflammatory conditions of known and unknown origin. The development of antihepatotoxic drugs being a major thrust area has drawn attention of majority of workers in the field of natural product research. Liver, the largest and the most versatile by organ for metabolism and excretion, plays an important role in the maintenance of body's internal environment through its multiple and diverse functions. It is continually exposed to a variety of xenobiotics and therapeutic agents due to inadequately controlled environmental pollution and expanding therapeutic uses of potent drugs. Thus, the disorders associated with this organ are numerous and varied. Although a strict delineation of various hepatic disorders is not yet possible, from didactic point of view, these may be classified as acute or chronic hepatitis (inflammatory diseases), hepatosis (non-inflammatory disorders) and liver cirrhosis (degenerative disorder resulting in fibrosis) (1-5)

Aim of the study is to evaluate the Hepatoprotective activity of ethanolic leaves extracts of *Hibiscus platanifolius.*

MATERIALS AND METHOD

Plant collection

Fresh leaves of *Hibiscus plantanifolius* were collected from the Tirumala hills of Chittor district. These were coarsely powdered & extracted using 90% methanol for *Hibiscus platanifolius* on a reflux water bath for 3 hours. The cycle was repeated for three times. The extract was concentrated on rotary flash evaporator to semi solid consistency .To it 1-2 drops of chloroform was added and stored at 8º C in screwed glass vials.

Determination of Loss on drying

About 5 g of the leaves of *H. plantanifolium* was weighed into a previously dried and tare flat weighing bottle and dried in an oven at 105°C. Drying was continued until two consecutive weighing do not differ by more than 5 mg. The difference in the weight of drug before and after drying was noted and loss on drying was calculated with reference to the air-dried material.

Determination of Extractive values

Alcohol soluble extractive

This method determines the amount of active constituents extracted with solvents from a given amount of the powder material. 5 g of *H. plantanifolium* was macerated with 100 ml of 95% ethanol in a glass stoppered conical flask for 24 h shaking frequently during first 6 h and the allowing to stand for 18 h. The solution was filtered rapidly taking care not to lose any solvent and evaporated 25 ml of the solvent in a tared flat bottomed dish on water bath. The residue was dried at 105°C and cooled in desiccators for 30 min and weighed. The content of extractable matter was calculated on the basis of air dried material (WHO, 1998).

Water soluble extractive value

5 g of *H. plantanifolium* was macerated with 100 ml of chloroform water in a glass stoppered conical flask for 24 h shaking frequently during first 6 h and the allowing to stand for in a 18 h. The solution was filtered rapidly taking care not to lose any solvent and evaporated 25 ml of the solvent in a tared flat bottomed dish on water bath. The residue was dried at 105°C , cooled in a desiccators for 30 min and weighed. The content of extractable matter was calculated on the basis of air dried material.

Successive solvent extraction

The presence of different chemical constituents in *H. plantanifolium* can be detected by subjecting them to successive extraction using solvents in the order of increasing polarity and subjecting the extracts so obtained to qualitative tests for various chemical constituents. The selected drug samples in the present study were therefore, subjected to successive extraction followed by qualitative chemical tests in order to know the phytochemical profiles on a preliminary basis. *H. plantanifolium* powder weighing about 100 g each was taken and extracted successively in soxhlet apparatus using solvents was Ethanol. Then physical characters and percentage yield of extracts were recorded.

Experimental animals (6-8)

Male Wister albino rats weighing 150-200 g were used in the present study. They were housed in individual polypropylene cages under standard laboratory conditions of light, temperature and relative humidity. Animals were given standard rat pellets (Pranav Argo's ltd.) and drinking water *ad libitum*.

Each set of experiment was divided into groups consisting of control, toxicant, standard, and test. The rats of control group received a single daily dose of 5% acacia mucilage (1 ml/kg, p.o.). The rats of toxicant group received a single daily dose of vehicle (1 ml/kg, p.o.) for three days and a single intraperitoneal injection of thioacetamide (100 mg/kg) in water for injection, 30 min after the administration of the vehicle on the second day of treatment. The rats of silymarin group received silymarin (100 mg/kg p.o.) three times at 24 h intervals. Thioacetamide was administered 30 min after the second dose of silymarin while test groups were given orally a single daily dose of extracts in vehicle for three days and a single dose of thioacetamide (100 mg/kg i.p.) on the second day 30 min after the administration of respective test suspensions. After 48 h of thioacetamide administration i.e. 4^{th} day of the experiment the blood was collected and serum was used for determination of biochemical parameters.

Assessment of liver function

Blood was collected from all the groups by puncturing the retro-orbital plexus and was allowed to clot at room temperature and serum was separated by centrifuging at 2500 rpm for 10 min. the serum was used for estimation of biochemical parameters to determine the functional of the liver. Glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT) were estimated by a UV-Kinetic method based on the reference method of International Federation of Clinical Chemistry (Bergmeyer et al., 1985). Alkaline phosphatase (ALKP) was estimated method by PNPP method (Mac Comb and Bowers, 1972), while total bilirubin (TBL) by modified DMSO method (Walter & Gerard, 1980), total cholesterol (CHL) by CHOD-PAP method (Richmond, 1973), total protein (TPTN) by colour complexation with copper ions in an alkali solution (Peters, 1968). Albumin was estimated by bromo cresol green method (Webster, 1974).Lactate dehydrogenase (LDH) was estimated by SCE (Scandinavin committee on enzymes) recommended method (Wei bahaar, 1975). All the estimations were carried out using standard kits on Semi auto analyzer of Agappe make (Misapa Exel).

RESULTS AND DISCUSSION

Microscopic Characterstics

Transverse section of midrib of leaf showed chained, small and numerous epidermal cells. The mesophyll layer is irregular and comprised of 5-6 layers. Cells of parenchyma varied greatly in shape and size and were sometimes, elongated or lobed. The xylem vessels were numerous, very big in size and circular in shape. Phloem vessels were small in size, numerous and circular in shape. Calcium oxalate crystals were dark stained and numerous in mesophyll parenchyma. Trichomes were present on both upper and lower surface. Transverse section of lamina showed cuticle and thick walled cells in upper and lower epidermis. Epidermal cells were large in size, elongated and compact. Palisade parenchyma showed 3 layers of large, compact and dark cells. Dark stained crystals were present in mesophyll layer. The spongy mesophyll was wider comprising of 6-7 layers of lobed tightly interconnected cells. Vascular bundles had compact parallel rows of xylem vessels and fibres (Fig-1).

Fig-1 TS of *Hibiscus platanifolius* Leaf

Proximate Analysis

Proximate analysis helps to set up certain standards for the formulation in order to avoid batch to batch variations and also to judge their quality and purity. The results of proximate analysis of *Hibiscus platanifolius* were recorded in Table-1.

Table-1 Proximate analysis of *Hibiscus platanifolius*

Parameter	*Hibiscus platanifolius*
Alcohol soluble extractive	10.4 % w/w
Water soluble extractive	15.2 % w/w
Loss on drying	6.8% w/w
Bulk density	0.59 gm/cc
Angle of repose	44.305º
Total ash value	10.5%

Based on extractive value qualitative phytochemical screening was done to different extracts by using various qualitative tests. The above results shows presence of various phytoconstituents in different extracts.Here the ethanolic extract containing more number of constituents when compared to other extracts.

Acute toxicity studies

The ethanolic extract (EE) of the *Hibiscus platanifolius* was subjected to acute toxicity determinations as per OECD guidelines. None of these showed mortality even at the dose level of 2000 mg/kg and therefore considered safe.

Hepatoprotective activity

The ethanolic extract (EE) of the *Hibiscus platanifolius* selected was subjected to hepatoprotective activities in using thioacetamide model. The ethanolic extract was administered at dose levels of 200 and 400 mg/kg, Silymarin being positive control was administered at dose level of 100 mg/kg.

Thioacetamide-induced hepatotoxicity

Administration of thioacetamide (100 mg/kg i.p.) induced a marked increase in the serum levels of GOT, GPT, ALKP, TBL, LDH and CHL; and decrease in the levels of TPTN and ALB, indicating parenchymal cell necrosis. Significant decrease ($p<0.05$) in all the elevated levels of biochemical parameters and significant ($p<0.05$) increase in depleted TPTN and ALB levels was observed with the groups of rats which received EE at dose levels of both 200 mg/kg and 400 mg/kg as observed in case of silymarin treated group. Results of thioacetamide-induced hepatotoxicity are shown in Table-2- 4 and Fig-2-4.

Table-2 Effect of Ethanolic extract of *Hibiscus Platanifolius* on GOT level

S.NO.	GROUP	GOT (IU/L)
1	Control	56.84 ± 3.46
2	Thio-Acetamide	376.48 ± 37.21
3	Silymarin	101.34 ± 54.32*
4	T 1 200mg/kg	301.58 ± 63.53*$
5	T 2 400mg/kg	175.18 ± 42.73*#

Table-3 Effect of Ethanolic extract of *Hibiscus Platanifolius* on GPT levels

S.NO.	GROUP	GPT (IU/L)
1	Control	78.92 ± 6.65
2	Thio-Acetamide	536.13 ± 69.96
3	Silymarin	79.99 ± 47.53*
4	T 1 200mg/kg	383.08 ± 69.45*#
5	T 2 400mg/kg	216.91 ± 53.94*#

Table-4 Effect of Ethanolic extract of *Hibiscus Platanifolius* on ALKP levels

S.NO.	GROUP	ALKP (IU/L)
1	Control	53.69 ± 13.84
2	Thio-Acetamide	889.65 ± 99.23
3	Silymarin	431.56± 75.74*
4	T 1 200mg/kg	654.93 ± 73.14*#
5	T 2 400mg/kg	489.54 ± 56.35*#

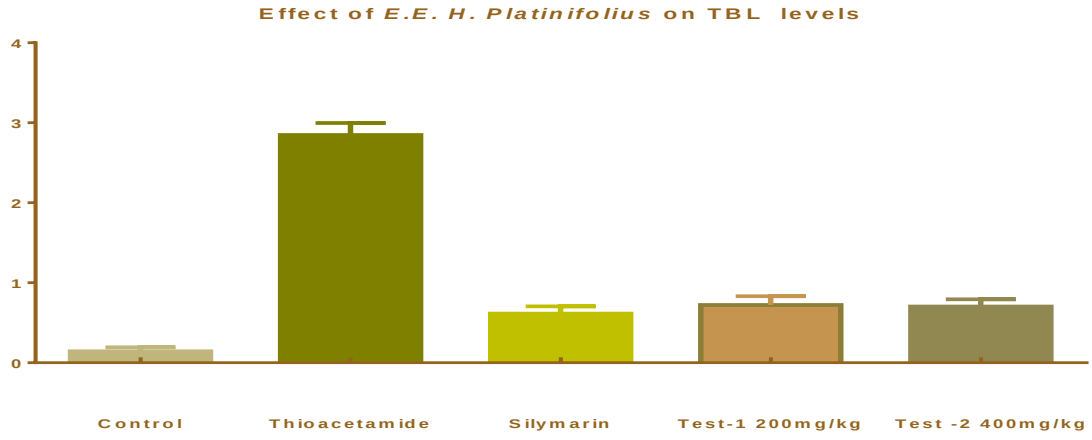

Fig-2 Effect of Ethanolic extract of *Hibiscus Platanifolius* on TBL levels

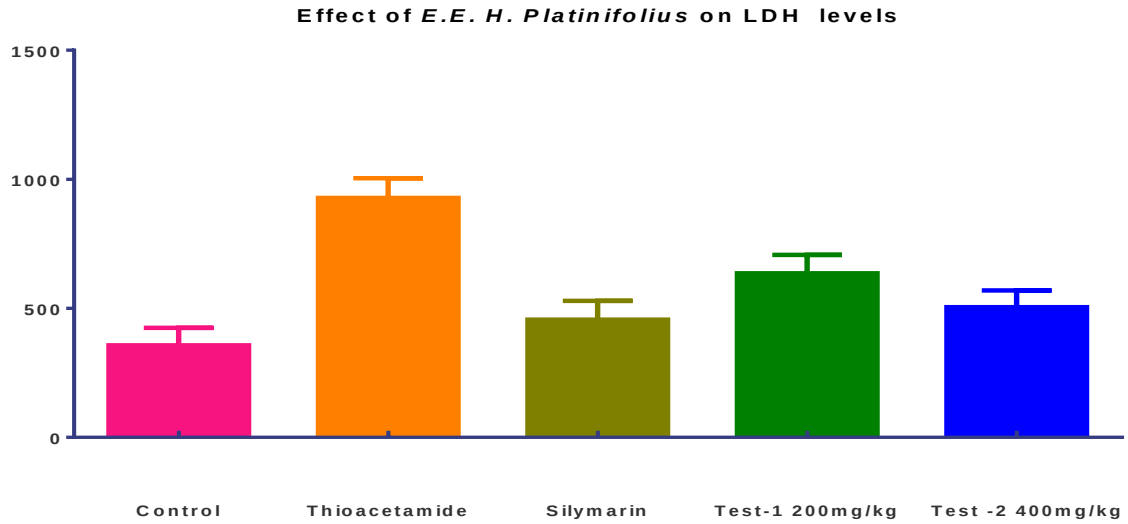

Fig-3 Effect of Ethanolic extract of *Hibiscus Platanifolius* on LDH levels

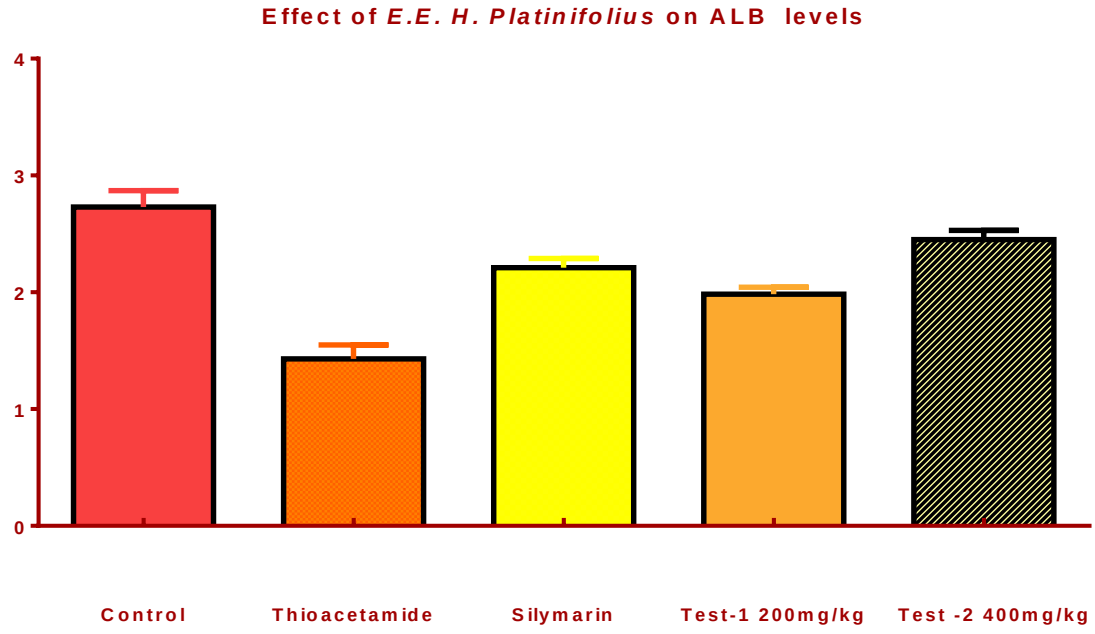

Fig-4 Effect of Ethanolic extract of *Hibiscus Platanifolius* on ALB levels

Administration of a single dose of thioacetamide in rats produces centrilobular hepatic necrosis. It get metabolises to a toxic metabolite thioacetamide-S-oxide, a direct hepatotoxins, which is further metabolised, at least in part, by cytochrome P_{450} monooxygenases. The subsequent product formed exerts hepatotoxicity by binding to hepatocyte macromolecules and causes centrilobular necrosis by generation of reactive oxygen species. The chronic thioacetamide exposure leads to cirrhosis in rats. It induces toxicity by altering semi permeable character of the cell membrane of hepatocytes resulting in an increased influx of calcium ions and leading to necrosis and finally death. Therefore extracts under study antagonise the effect of thioacetamide by inhibiting cytochrome P_{450} or by acting either as membrane stabiliser, thereby preventing the distortion of the cellular ionic environment associated with

thioacetamide intoxication, or by preventing interaction of thioacetamide with the transcriptional machinery of the cells resulting in regeneration of hepatic cells. Thus the hepatoprotective activity of these extracts and fractions may be due to their ability to affect the cytochrome P_{450} mediated functions or stabilisation of endoplasmic reticulum resulting in hepatic regeneration.

CONCLUSION

In present investigations leaf of *Hibiscus platanifolius* was subjected to proximate analysis and showed significant values. In that the ethanolic extract (6.7w/w) shows more extractive value compared to water extractive value (5.8w/w). The successive solvent extraction was done by using selective solvents. Here the extractive value of ethanolic extract (6.7w/w) was more when compared to other solvents like benzene, petroleum ether (2.04w/w), water (5.8w/w). Based on above result further subjected to qualitative phytochemical screening to detected various phytoconstituents like flavanoids, alkaloids, phenols and steroids and conformed by using thin layer chromatography. Further study was done by using ethanolic extract and the doses 200 mg/kg b.wg 400 mg/kg b.wg were confirmed by performing acute toxicity studies. Then the hepatoprotective study of *H. plantifolius* against thioacetamide induced toxicity was done by performing biochemical parameters.

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