HEPATOPROTECTIVE ACTIVITY OF AQUEOUS EXTRACT OF
HIBISCUS SABDARIFFA ON ALCOHOL INDUCED HEPATOTOXITY IN RATS

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ABSTRACT
The aim of present study was to investigate the Hepatoprotective activity of aqueous extract of Hibiscus Sabdariffa leaves in female albino rats on alcohol induced hepatotoxic activity. The efficacy of any hepatoprotective drug is essentially dependent on its capability of either reducing the harmful effects or in maintaining the normal hepatic physiological mechanism. Orally administered doses of 200 and 400 mg/kg of aqueous extract of leaves of Hibiscus Sabdariffa produced significant decrease in AST, ALP, ALT levels and bilirubin. The activity of the extract is found to be dose dependent. The hepatoprotective effect of the drug was further supported by the histopathological examinations of the liver sections which revealed that the normal liver shapes was distributed by hepatotoxic intoxication. In the liver sections of the rats treated with Hibiscus Sabdariffa extract and intoxicated with Alcohol the normal cellular shape was retained as compared to silymarin, thereby confirming the protective effect of the extracts of Hibiscus Sabdariffa. The hepatoprotective activity of Hibiscus sabdariffa could be due to the presence of bioflavonoids which have hepatoprotective properties. The result of this investigation indicated that the aqueous extract of Hibiscus Sabdariffa leaf possess hepatoprotective activity against alcohol induced liver damage in rats.

KEYWORDS: Hepatoprotective activity, Alcohol Induced Hepatotoxic activity, Hibiscus Sabdariffa, ANOVA, ALT, AST, AEHS, SGPT, SGOT.
1. INTRODUCTION

Herbal medicine ("herbalism") is the study and use of medicinal properties of plants. The scope of herbal medicine is sometimes extended to include fungal and bee products as well as minerals, shells and certain animal parts. Pharmacognosy is the study of all medicines that are derived from natural sources. The bark of willow trees contains large amounts of salicylic acid, which is the active metabolic of aspirin. Willow bark has been used for millennia as an effective pain reliever and fever reducer. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and to defend against from predators such as insects, fungi and herbivorous mammals.\textsuperscript{[1,2]} Many of these phytochemicals have beneficial effects on long-term health when consumed by human disease. Thus herbal medicines to be as effective as conventional medicines, but also gives them the same potential to cause harmful side effects. The use of plants as medicines predate written human history. Ethnobotany (the study of traditional human use of plants) is recognized as an effective way to discover future medicines. Many of the pharmaceuticals currently available to physicians have a long history of use as herbal remedies, including aspirin, digitalis, quinine, and opium. The use of herbs to treat disease is almost universal among non-industrialized societies and is often more affordable than purchasing expensive modern pharmaceuticals.\textsuperscript{[3,4]} The world health organization (WHO) estimates that 80 percent of the population of depends on herbal medicine. Asian and African countries presently use herbal medicine for some aspects of primary health care. Natural products have played an important role in drug discovery. These products as drugs play a significant role in pharmaceutical care. The terrestrial plants have played a dominant role in treatment of human ailments from time immemorial. Plants remain the basis for a large proportion of the medications used today for the treatment of variety of diseases. A number of researches have documented the use of traditional medicinal plants in India. From ancient times, plants are the main sources of the treatment and nowadays they are the hub of medical source not only in developing countries, but also in developed countries where modern medicines are predominantly used. In the Indian system of medicines, most practitioners formulate and dispense their own recipes; hence this requires documentation and maintenance. In western countries use of herbal medicines is steadily growing. To be accepted as viable alternative to modern medicine, the same vigorous method of scientific and clinical validation must be applied to prove the safety and effectiveness of a therapeutic product.\textsuperscript{[5-8]}
Hibiscus Sabdariffa is the plant belonging to Malvaeceae family having very good medicinal value. It is properly known as Konda Gongura is one of the essential food constituents. It is widely used to treat hepatotoxicity in traditional medicines. However there is passivity of scientific data to support this activity, the present study was designed to evaluate the hepatoprotective activity of aqueous extract of Hibiscus Sabdariffa alcohol intoxicated rats.

2. PLANT PROFILE

2.1. Botanical Description
Hibiscus sabdariffa L. locally known as Assam kumbang, asamsusur, and asampaya is belonging to the large “family” of “Malvaceae”. It is also commonly known as rosella (English), l’Oiselle (French), Spanish (Jamaica), Kerkrade (Arabic), and Krachiapdaeng (Thailand).

2.2. Botanical Classification
Kingdom: Plantae (Plants)
Subkingdom: Tracheobionta (Vascular plants)
Superdivision: Spermatophyte (Seed plants)
Division: Magnoliophyta (Flowering plants)
Class: Magnoliopsida (diCotyledons)
Subclass: Dilleniidae
Order: Malvales
Family: Malvaceae (Mallow family)
Genus: Hibiscus L. (Rose mallow)
Species: Hibiscus sabdariffa L

Fig-1- Leaves of Hibiscus sabdariffa
2.3. Medicinal Value\textsuperscript{[15]}

*Hibiscus sabdariffa* is used in many folk medicines. It is claimed as a Thai traditional medicine for kidney stones\textsuperscript{[35]} and urinary bladder stones. *Hibiscus sabdariffa* also is said to have diuretic effects, used effectively in folk medicines for treatment of inflammatory diseases and cancer. The positive effect of *Hibiscus sabdariffa* extract consumption to decrease blood pressure has been proved in study on both man and rats. More recently, the antihypertensive action of *Hibiscus sabdariffa* has been confirmed with experimental hypertension. In addition, studies on humans also proved the anti-inflammatory effect of *Hibiscus sabdariffa* consumption. *Hibiscus sabdariffa* extract is also reported used as an antibacterial, antifungal, diuretic, uricosuria, and mild laxative substance. In addition, the components of *Hibiscus sabdariffa* extract exhibit anti-tumour characteristics, immune-modulating and anti-leukemic effects.\textsuperscript{[16]}

Table: 1 Physicochemical constituents of the fresh calyces and leaves of *H.sabdariffa*

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Calyces (fresh)</th>
<th>Leaves (fresh)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>9.2g</td>
<td>86.2%</td>
</tr>
<tr>
<td>Protein</td>
<td>1.145g</td>
<td>1.7-3.2%</td>
</tr>
<tr>
<td>Fat</td>
<td>2.61g</td>
<td>1.1%</td>
</tr>
<tr>
<td>Fibre</td>
<td>12.0g</td>
<td>10 %</td>
</tr>
<tr>
<td>Ash</td>
<td>6.90g</td>
<td>1 %</td>
</tr>
<tr>
<td>Calcium</td>
<td>12.63mg</td>
<td>0.18 %</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>273.2mg</td>
<td>0.04 %</td>
</tr>
<tr>
<td>Iron</td>
<td>8.98mg</td>
<td>0.0054 %</td>
</tr>
<tr>
<td>Carotene</td>
<td>0.029mg</td>
<td>—</td>
</tr>
<tr>
<td>Thiamine</td>
<td>0.117mg</td>
<td>—</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>0.277mg</td>
<td>—</td>
</tr>
<tr>
<td>Niacin</td>
<td>3.765mg</td>
<td>—</td>
</tr>
<tr>
<td>Ascorbic Acid</td>
<td>6.7mg</td>
<td>—</td>
</tr>
</tbody>
</table>

3. MATERIALS AND METHODS

3.1. Drugs/Chemicals/Reagents

Ethyl Alcohol, Alpha Naphtol, Conc. Sulphuric acid, Ether, Ammonia, Caustic Soda, Pyridine, Sodium Nitroprusside, Sudan Red 3, Picric Acid, Ferric Chloride, Total Bilirubin Kit, ALT, AST estimation kit, Silymarin.

3.2. Plant Material

The plant was collected from fields in Guntur.
3.3. Experimental Animals
Female albino rats weighing (B.W 180-225 gm) were obtained from Animal House of Hindu College of Pharmacy. They were housed in standard cages at room temperature (25+_2C) with relative humidity (55+_5%) and 12/12 h light/dark cycle. The animals were provided with standard pellet diet and water. All animals were acclimatized for 10 days.

3.4. Preparation of Aqueous Extract
3.4.1. Maceration Process\cite{17, 18}
This simple, but still widely procedure used which involves leaving the pulverized plant to soak in suitable solvent in a closed container at room temperature. The method is suitable for both initial and bulk extraction. Occasional or constant stirring of the preparation can increase the speed of extraction. The extraction ultimately stops when equilibrium is attained between the concentration of metabolites in the extract and that in the plant material. After extraction, the residual plant material (marc) has been separated from the solvent. This involved a rough clarification by decanting, which is usually followed by a filtration step. Centrifugation may be necessary if the powder is too fine to be filtered.

To ensure exhaustive extraction, it is common to carry out an initial maceration, followed clarification, and an addition of fresh solvent to the mark. This can be performed periodically with all filtrates pooled together. The main disadvantage of maceration is that the process can be quiet time consuming, taking few hours up to several weeks. Exhaustive maceration can also consume large volumes of solvent and can lead to potential loss of metabolites and some compounds may not be extracted efficiently if they are poorly soluble at room temperature. On the other hand, as the extraction is performed at room temperature, maceration is less likely to lead to degradation of thermo labile compounds.

3.5. Preliminary Phytochemical Analysis\cite{18-20}
The extracts obtained were subjected to various chemical tests to detect the chemical constituents present in them.
4. RESULTS

Table: 2 Preliminary phytochemical screening

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Phytochemical Tests</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Test for Carbohydrates</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Test for Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Test for Tannins</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Test for Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Test for Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Test for Phenols</td>
<td>+</td>
</tr>
</tbody>
</table>

Table: 3 Effect of pre-treatment with *H.Sabdariffa* aqueous leaf extract on serum level changes in chronic alcohol Intoxicated Rats

<table>
<thead>
<tr>
<th>Serum levels</th>
<th>Normal</th>
<th>Toxicant</th>
<th>Standard</th>
<th>200mg/kg+Alc</th>
<th>400mgS/kg+Alc</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST</td>
<td>83.27±0.25</td>
<td>337.53±0.4***</td>
<td>87.73±0.52***</td>
<td>249.20±0.63**</td>
<td>92.91±0.72***</td>
</tr>
<tr>
<td>ALT</td>
<td>37.22±0.30</td>
<td>257.43±0.5***</td>
<td>47.27±0.56***</td>
<td>186.54±0.77**</td>
<td>52.23±4.26 ***</td>
</tr>
<tr>
<td>ALP</td>
<td>0.12±0.20</td>
<td>391.42±0.3***</td>
<td>132.82±0.62***</td>
<td>253.24±0.66**</td>
<td>139.65±0.77***</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>0.58±0.24</td>
<td>3.48±1.20***</td>
<td>0.98±15***</td>
<td>2±1.55 **</td>
<td>0.97±1.21***</td>
</tr>
</tbody>
</table>

Results are Mean ± S.D (n = 5).

A Significantly lower than control group 4(p<0.05).

B Significantly lower compared to normal 5 (p<0.05).

C Significantly lower compared to normal rats at week 6 (p<0.05).

Fig-2- Effect of pre-treatment with *Hibiscus Sabdariffa* aqueous leaf extract on serum level changes in chronic alcohol Intoxicated Rats
Fig-3-: A section from the liver of group A, showing central vein (C) lined by endothelial cells (Ec) surrounded by radiating cords of hepatocytes (H) enclosing hepatic sinusoids (S) that was lined with Kupffer cells (K).

Fig-4- A section from the liver of group A, showing portal area comprising portal vein (V), hepatic artery (A) and bile duct (B). The surrounding cells contain central nuclei (N) with one or two nucleoli (Nu).

Fig-5-: A section from the liver of group B, showing central vein (C), continuing with hepatic Sinusoids (S); surrounded by cords of hepatocytes (H) that contain cytoplasmic vacuoles (V).
Fig 6: The portal area showed lymphocytes infiltration; the portal vein and hepatic artery contained erythrocytes and bile duct was normal in appearance.

Fig 7: A section from the liver of group C, showing central vein (C) lined by flattened endothelial cells (Ec) surrounded by radiating cords of hepatocytes (H) enclosing sinusoids (S) that contain Kupffer cells (K); pyknotic nuclei (Pn) are also observed.

Fig 8: A section from the liver of group showing portal area comprising portal vein (V), hepatic artery (A) and bile duct (B); the surrounding cells contain glycogen granules (G), cytoplasmic vacuoles (Vc) and nucleus (N) with one or two nucleoli (Nu); there is lymphocyte infiltration (L) in portal area, pyknotic nuclei (Pn) are also seen.
Table: 4 Effect of *Hibiscus Sabdariffa* on Bilirubin in Alcohol induced hepatotoxicated Rats.

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>BILURUBIN</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rat1</td>
<td>Rat2</td>
<td>Rat3</td>
<td>Rat4</td>
<td>Rat5</td>
<td>Rat6</td>
<td>Mean±SEM</td>
</tr>
<tr>
<td>NORMAL</td>
<td>0.4</td>
<td>0.49</td>
<td>0.51</td>
<td>0.6</td>
<td>0.7</td>
<td>0.82</td>
<td>0.58±0.2</td>
</tr>
<tr>
<td>TOXICANT</td>
<td>3.1</td>
<td>3.22</td>
<td>3.42</td>
<td>3.6</td>
<td>3.72</td>
<td>3.84</td>
<td>3.48±1.2</td>
</tr>
<tr>
<td>STANDARD</td>
<td>0.91</td>
<td>0.92</td>
<td>0.94</td>
<td>0.95</td>
<td>1</td>
<td>1.2</td>
<td>0.98±1</td>
</tr>
<tr>
<td>200Mg/kg AEHS</td>
<td>1.91</td>
<td>1.93</td>
<td>1.99</td>
<td>2</td>
<td>2.1</td>
<td>2.3</td>
<td>2±1.5</td>
</tr>
<tr>
<td>400mg/kg AEHS</td>
<td>0.91</td>
<td>0.92</td>
<td>0.94</td>
<td>0.96</td>
<td>1</td>
<td>1.1</td>
<td>0.97±1.2</td>
</tr>
</tbody>
</table>

EVALUATION OF EXPERIMENTAL DATA ANALYSIS OF HEPATOPROTCTIVE ACTIVITY

The results of serum parameters revealed that ALT, ALP, AST are significantly increased several times in case of disease control group compared to normal control group. The plant extract at a dose of 400 mg/kg showed significant hepatoprotective activity compared to the disease control group. This is manifested by decreased levels of AST, ALP and ALT etc. The biochemical parameters of experimental animals are restores towards its normal value by higher dose of extract. Standard drug Silymarin treated group animals exhibited its usual significant hepatoprotective activity compared to disease control group along with serum parameters.

5. DISCUSSION

Chronic consumption of alcohol, which is rich in energy (7.1cal/g), does not produce any gain in body weight. Substantial use of alcohol has profound effect on nutritional status which may cause primary malnutrition by displacing other nutrients in the diet of high energy content or because of associated medical disorders. Secondary malnutrition results due to maldigestion or malabsorption of nutrients caused by gastrointestinal complications. Pre-treatment of rats with aqueous extract of *Hibiscus Sabdariffa* leaf prior to alcohol feeding significantly increased their body weight gain compared to rats administered alcohol only. Pre-treatment with 400 mg/kg and aqueous extract of *Hibiscus Sabdariffa* leaf prevented decrease in body weight gain similar to the group pre-treated with silymarin. Other researchers earlier reported significantly decreased body weight gain due to alcohol compared to control rats.

In the current investigations we observed that there was statistically significant increase in size of hepatocytes, number of micro and macro cytoplasmic vacuoles in group B when compared to those of group A (p value being <0.05). This was presumably due to
accumulation of fats, bilirubin and water; these results were comparable with the observations of Ronin, who reported that ingestion of ethanol along with low carbohydrate diets in rats produced fat vacuoles and inflammatory changes in the liver. In the other hand in group C, there was statistically significant decrease in mean size of hepatocytes, numbers of micro and macro vacuoles as compared to group B (p value <0.005); hepatocytes were, however, comparable to those of group A, as far as their size and number of micro and macro vacuoles were concerned; it is implied from this finding that silymarin effectively protected the liver against ethanol inducer.

In groups B and C, the nuclei of hepatocytes were larger and vesicular with dispersed materials; there was, however, no statistically significant change in their size as compared to those of group A (p value being >0.05). There was no statistically significant change in the diameter of central vein in groups B and C as compared to group A (p value being >0.05).

Therefore ethanol treatment to albino rats for 8 weeks induced a fair degree of de-arrangement of liver structure and function. There was a significant elevation in the levels of serum marker enzymes like AST, ALT & ALP etc. content of ethanol in toxicated animals. In contrast, pre-treatment with AELHS (200,400 mg/kg) and silymarin\textsuperscript{49} (100 mg/kg p.o) exhibited an ability to counteract the hepatotoxicity by decreased serum marker enzymes.

In the ethanol treated groups, there was a significant increase in total bilirubin content. Whereas, pre-treatment with AELHS can significantly reduced the increased total bilirubin against alcohol in toxicated rats.

6. CONCLUSION

The efficacy of any hepatoprotective drug is essentially dependent on its capability of either reducing the harmful effects or in maintaining the normal hepatic physiological mechanism, which have been imbalanced by a hepatotoxin\textsuperscript{56}. Orally administered doses of 200 and 400 mg/kg of aqueous extract of leaves of \textit{Hibiscus Sabdariffa} produced significant decrease in AST, ALP, ALT levels and bilirubin. The activity of the extract is found to be dose dependent. The hepatoprotective effect of the drug was further supported by the histopathological examinations of the liver sections which revealed that the normal liver shapes was distributed by hepatotoxic intoxication. In the liver sections of the rats treated with \textit{Hibiscus Sabdariffa} extract and intoxicated with Alcohol the normal cellular shape was retained as compared to silymarin, thereby confirming the protective effect of the extracts of \textit{Hibiscus Sabdariffa}. The hepatoprotective activity of \textit{Hibiscus Sabdariffa} could be due to the
presence of bioflavonoids which have hepatoprotective properties. The result of this investigation indicated that the aqueous extract of leaf possess hepatoprotective activity against alcohol induced liver damage in rats. Attempts are being made to isolate and characterize the active principle to which the hepatoprotective activity can attribute. The use of herbal drugs may be beneficial and safe when compared to the synthetic medicines which possess several side effects\cite{59} in addition to the therapeutic use. Hence further research was suggested to explore the exact pharmacology of the drug and the study was closed with delight.

7. REFERENCES


