INTRODUCTION

Drug discovery is identification of novel active constituents. One of the major tools in the drug discovery is the Pharmacophore approaches. Screening of compounds for biological activity and structure elucidation of chemical compounds by Mass, IR and NMR spectroscopy are the two approaches which help in finding of new chemical compounds from the natural sources. Medicinal plants are also rich in the secondary metabolites which have a potent physiological effect on the living system. These are called as active plant principles.

LITERATURE REVIEW

Detailed literature survey of the plant kyllinga triceps rottb suggest that not much work is reported on the plant except few ethnomedicinal studies, while plant is reported in ayurvedic literatures as medicine for several diseases.

AIM AND SCOPE OF THE STUDY

*Kyllinga triceps* rottb is distributed throughout India. Except few ethnomedicinal studies not much work has been reported on this plant. Hence we are interested to submit this plant for detailed pharmacognostical, preliminary phytochemical and pharmacological investigations which may help in future research in field of hepatoprotective, Antioxidant and diuretic medication,

MATERIAL AND METHODS

Collection of Specimen.

The species for the proposed study that is *Kyllinga triceps* rottb were collected from Bhoora Khon area of Shivpuri District of Gwalior Division (M.P.) with the help of Mr. N.K. Pandey (R.O.) National Research institute for ayurvedic-siddha (CCRAS) Amkho, Gwalior.
Taxonomical Identification.

The species for the proposed study was identified as *Kyllinga triceps* by Dr. (Smt.) M.D. Gupta (Asst Director) and Mr. N.K. Pandey (R.O.) National Reseach Institute for Ayurveda and siddha (C.C.R.A.S.) under Ministry for Health and Family Welfare, Govt. of India, Amkho Gwalior (M.P.)

PHARMACOGNOSTICAL STUDIES

Morphological and microscopical studies were performed.

PHYTOCHEMICAL STUDIES

Extraction, separation of phytoconstituent by TLC column, reverse TLC, mass $^{13}$CNMR $^{1}$HNMR spectral studies was performed with structure elucidation of isolated component.finally GCMS analysis was performed for identification of constituents of essential oil.

PHARMACOLOGICAL STUDIES

Acute oral toxicity study

Study was performed by using OECD guidelines -423 (Organization of Economic Co-Operation Development)-Fixed dose procedure (FDP).

HEPATOPROTECTIVE AND ANTIOXIDANT STUDIES

Assessment of Hepatoprotective activity.

Ethical Aspects

The study was approved by the institutional ethical committee (protocol No. 891/Po/ac/05/CPCSEA).

Induction of Hepatotoxicity using CCl4 and Grouping of Animal.

The rats were randomly divided into seven groups, comprising of six animals in each group.
Collection of blood.

The blood samples were collected from the retro orbital plexus, Later serum was separated by centrifugation of blood at a speed of 2000 rpm for 10 minutes. The serum was collected and quantitatively analysed for SGOT, SGPT and ALP, direct bilirubin, total cholesterol and triglycerides etc.

Biochemical parameter estimation.

- **Determination of Glutamate Pyruvate Transaminase (SGPT)**
  Serum GPT assayed by using SGPT kit obtained from, Ranbaxy Diagnostic ltd, Baddi, H.P.

- **Determination of Glutamate Pyruvate Transaminase (SGOT)**
  Serum GPT assayed by using SGOT kit obtained from, Ranbaxy Diagnostic ltd, Baddi, H.P.

- **Determination of Alkaline Phosphatase (ALP)**
  Serum ALP assayed by using ALP kit obtained from Roche Diagnostics India Pvt. Ltd. Mumbai, India.

- **Determination of Acid Phosphatase**
  Serum ACP assayed by using ACP kit obtained from Roche Diagnostics India Pvt. Ltd. Mumbai, India.

- **Determination of direct bilirubin**
  Bilirubin test kit Jendrassik and Grof, Span Diagnostic Ltd. (Liquid Gold), Surat.

- **Determination of Total Cholesterol**
  Plasma total cholesterol estimated by using kit obtained from M/s Excel Diagnostics Pvt. Ltd, Hyderabad.
• **Determination of Triglycerides**
  Plasma triglycerides estimated by using kit obtained from M/s Excel Diagnostics Pvt. Ltd, Hyderabad.

• **Determination of Total Proteins**
  Plasma total protein estimated by using kit obtained from Span Diagnostics Ltd, Surat.

• **Determination of Albumin**
  Plasma albumin estimated by using kit obtained from Span Diagnostics Ltd, Surat.

• **Determination of globulin**
  Plasma globulin estimated by using kit obtained from Span Diagnostics Ltd, Surat.

• **Determination of Urea**
  Plasma urea estimated by using kit obtained from M/s Excel Diagnostics Pvt. Ltd, Hyderabad.

• **Determination of Creatinine**
  Plasma creatinine estimated by using kit obtained from M/s Excel Diagnostics Pvt. Ltd, Hyderabad.

**Body weight.**

Mean body weights of 7 experimental groups at 0 days (initial) and 60 days (final) were noted

**Liver weight.**

Relative tissue weights per 100 gms body weight of liver were measured in all groups.
Histopathological Studies of the Liver.

Assessment of Antioxidant activity.

Studies on Antioxidant and Oxidative stress

Immediately after separation of liver, 10% tissue homogenate was prepared in 0.15 M potassium chloride using homogenizer at 0° C. The whole homogenate was used for estimation of glutathione and lipid peroxidation.

Diuretic Activity.

Experimental animals.

Albino rats weighing between 175-200 g of either sex were used in the study. The experimental protocol was approved by the Institutional Animal Ethical Committee, and these animals were used to evaluate the diuretic activity of Ethanolic and petroleum Ether Extracts of the plant *kyllinga triceps* rottb.

Drug used.

Furosemide 10 mg/ml.

Experimental model

Lipschitz test

The method of lipschitz et.al was employed for the assessment of diuretic activity.

Estimation of urinary electrolytes.

Sodium, potassium and chloride levels of urine and the plant extract were analyzed.
Statistical analysis.

The experimental results were expressed as the mean ± standard error of mean and the statistical significance was evaluated by using students’ t-test.

RESULT AND DISCUSSION

PHARMACOGNOSTICAL STUDIES

Microscopy of Leaf.

presence of anomocytic stomata is the characteristic feature of T.S. of *Kyllinga triceps* rottb. Stamatal No. is 14 and index in 19.44.

Microscopy of culm (stem).

The aerial stem or the culm is triangular in cross sectional view with three prominent wings the wings are semicircular. The epidermis of the culm has fairly thick epidermal layer made up of rectangular or squarish cells. The vascular system of the culm consists of outer vascular bundles and inner vascular strands. The outer vascular strands are smaller circular and collateral.

Microscopy of Root.

The root is thin and fibrous it has crushed epidermal layer.

Microscopy of Rhizome.

There are smaller vascular bundles situated along the outer epidermis.

Microscopy of Inflorescence.

In sectional view, the inflorescence axis has many ridges and furrows. The florets are attached to the axis in the furrows with a short stalk. The floret have thin, membranous perianth members.
**Powder Microscopy.**

Vessel elements: The vessel elements are long, narrow and cylindrical. They are 90 \( \mu m \) and 20 \( \mu m \) wide.

**PHYTOCHEMICAL STUDIES**

Kyllinga triceps rottb rhizome powder contains Ash content, it is due to the presence of high inorganic contents, how ever the Ash content is possibly due to the \( \text{Na}^+ \) and \( \text{Ca}^{++} \) salts which are not harmful. As a part of phytochemical study plant extractive values were analysed to estimate the percentage yield of individual extract and found that the yeild was abundant in both extracts may be due to the presence of phytoconstituents having solubility and affinity in both solvents. Preliminary phytochemical studies of KTR extracts confirmed the presence of saponins, carbohydrate ,tannins, phenolic compounds, flavonoids, steroids, terpenes and tri terpenoids.

TLC profiles of ethanolic and petroleum ether extracts of KTR showed distinct characteristics. \( R_f \) values of spots obtained from ethanolic extract with solvent system chloroform : ethyl-acetate (60:40) was 0.52, 0.45 and 0.50 similarly 3 spots obtained from petroleum ether extract with \( R_f \) values 0.60,0.48 and 0.40. Petroleum ether extract was subjected to column chromatography because it showed three coloured highly resolute spots and also in our previous phytochemical studies it showed positive results. Fraction 21-24 obtained from the column were taken as fraction I similarly fraction 25-26 were taken as fraction II because they not only showed the homogeneity in \( R_f \) values (0.60,0.48) of TLC but also the spots obtained were nearly same in colour (blue,dark blue), which might be due to the same chemical constituents and its \( R_f \) values was also near to \( R_f \) values (0.60,0.48,0.40) shown by TLC of petroleum ether extract. Thus two compounds were isolated from two fractions, were subjected to spectral studies for identification. The structure of isolated...
The compound was analysed by $^1$HNMR, $^{13}$CNMR and mass spectroscopy. Both isolated compounds are colourless liquid. Constituent separated from fraction I of petroleum ether extract of rhizomes of *kyllinga triceps* rottb. Has the molecular formula C$_{20}$H$_{30}$O, was established by its mass spectrum data as its showed molecular ion-peak at m/z (%) 286 (35) where the base peak is at 271. The fragment ion peaks are at 253(12), 187(78), 145(30), 117(14) and 91(9). The $^1$HNMR spectral data confirm the structure of the compound the presence of phenolic OH which was observed at δ 4.62 ppm. The aromatic protons were reported at δ 6.79 and 6.63 ppm. An angular methyl of trans configuration were recorded at δ 1.06 ppm. The geminal methyls of isopropyl moiety were observed at δ 1.12 ppm. The structure was finally confirmed by its $^{13}$CNMR spectra. A shift at δ 151.0 ppm revealed the presence of a phenolic OH at C-13. The aromatic carbons were found at δ 132.0, 127.3, 125.7, and 110.7 ppm. The geminal dimethyl carbons of isopropyl moiety were at δ 22.5 ppm. The trans configuration of the compound was finally confirmed by the comparison of $^{13}$CNMR data with its cis-isomers, where in the ring carbon C-3 and C-2 of cis-isomer were reported at δ 37.6 and 50.1 ppm, which are more shielded than the ring carbons of trans-isomer, Present at δ 40.7 and 54.2 ppm of carbons C-1 and C-2. Similarly two carbons being shared by the cyclohexane and aromatic moiety of both the isomers possess different values. The carbons of cis-isomers at C-7 and C-9 are found at δ 126.0 and 145.3 ppm, whereas in trans-isomer they were found at δ 127.0 and 146.4 ppm.

Constituent separated from fraction II of petroleum ether extract of rhizomes of *kyllinga triceps* rottb. The molecular formula C$_{12}$H$_{14}$O was established by its mass spectral data and further confirmed by $^1$HNMR and $^{13}$CNMR spectral studies. $^1$HNMR data confirms the presence of aromatic protons as it is absorbed at δ 7.08 (1H) 7.8 (1H) and 7.58 (1H) ppm these observation were confirmed by $^{13}$CNMR data at δ 124.3, 125.6, 127.3, 135, 136 and 144.7 ppm. Presence of c=0 linkage
was confirmed by $^{13}$CNMR data which showed a down field shift at $\delta$ 199.3 ppm, CH$_2$ carbons of C-8 and C-6 were reported at $\delta$ 36.1 and 30.6 ppm. Two methyl carbons at $\delta$ 20.5 and 20.2 ppm were reported as C-4 and C-7. These spectral data confirms the structure 4,7-dimethyl-1-tetralone for compound b, having molecular formulae C$_{12}$H$_{14}$O.

**PHARMACOLOGICAL STUDIES**

Acute toxicity study was carried out to determine the safe dose for pharmacological studies. The alcoholic & Petroleum Ether extract of Kyllinga triceps rottb at dose of 50, 100, 200, 500, 1000 mg /kg body weight did not produce any toxicity in tested animals. The alcoholic & Petroleum Ether extract of Kyllinga triceps rottb falls under class 5 for (LD$_{50}>2000$ mg/kg). Oral administration at this dose level did not show any sign of behavioural toxicity and neurological toxicity during the experimental period according to the OECD Guidelines at different doses of 50, 100, 200, 500, 1000 mg /kg. No sign of mortality was seen hence, it could be concluded that the estimated LD$_{50}$ of alcoholic & Petroleum Ether extract of Kyllinga triceps rottb is above 2000 mg/kg bodyweight. Hence, 1/10th and 1/20th dose of the maximum tolerated dose was selected for the efficacy studies. Based on results obtained, the 100 and 200 mg/kg body weight was considered as safe dose for in-vivo pharmacological studies.

The CCl$_4$ is a well-known hepatotoxic agent and most commonly used model system for the screening of hepatoprotective activity of plant extracts/drugs. In general, protective action of the drugs against the liver damage induced by CCl$_4$ has been taken into consideration as an indicator of liver protective activity. The changes associated with CCl$_4$-induced liver damage are comparable to that of acute viral hepatitis. The mechanism by which CCl$_4$ causes hepatic injury involves two phases. In the first phase CCl$_4$ is
metabolized to toxic trichloromethyl radical (CCl₃ •) by cytochrome P450 2E1. This is further metabolized to trichloromethyl peroxy radical (CCl₃OO•), which causes initiation of lipid peroxidation and causes peroxidative degradation of cellular membrane leading to the necrosis of hepatocytes. In the second phase, the activation of kupffer cells by free radicals leads to release of pro-inflammatory mediators like TNF-α (induces cytotoxicity and apoptosis) and nitric oxide (NO; inhibits mitochondrial respiration and DNA synthesis). Therefore, the model was thought to be suitable for confirmation of hepatoprotective activity of selected medicinal plants. The results observed from serum biochemical parameters in pre-treatment of KTR extracts with respect to induction of hepatotoxicity using CCl₄ are provided in Marked increase in the levels of SGOT, SGPT, ALP, ACP and DB (P < 0.01) in the group treated with CCl₄, when compared with normal control was observed. The marked elevation in serum hepatic biochemical markers in rats treated with CCl₄ (hepatotoxicant) was an indication of hepatotoxicity. The groups received the pre-treatment of KTR extracts at dose levels of 100 and 200 mg/kg body weight significantly controlled the change in the biochemical parameters in dose dependant manner. All the extracts at dose levels of 200 mg/kg exhibited significant activity compared to lower doses. Among all the extracts of KTR, the AE-KTR extract significantly decreased (P < 0.01) the SGOT, SGPT, ACP, ALP and TB. However, PE-KTR extract (200 mg/kg) showed highest hepatoprotective activity almost comparable to the Silymarin treated group. Plant extracts exhibits good antioxidant and diuretic activities.

CONCLUSION

The liver is a vital organ present in vertebrates and some other animals. It has a wide range of functions, including detoxification, protein synthesis, and production of biochemicals necessary for digestion. 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. Thus, to maintain a healthy liver is a crucial factor for overall health and well being. But it is continuously and variedly exposed to environmental toxins, and abused by poor drug habits, and alcohol and prescribed & over-the-counter drug which can eventually lead to various liver ailments like hepatitis, cirrhosis and alcoholic liver disease. Today, human beings are exposed on a daily basis to certain environmental pollutants and foreign chemicals collectively referred to as xenobiotics which are causing serious health problems. Liver diseases have become a global problem and about 20,000 deaths occur every year due to liver disorders. Approximately two million people die annually from hepatic related disorders in the world. Liver diseases are the 10th leading cause of death and account for significant morbidity across the entire age and gender spectrum of the US population. Chronic liver diseases are common worldwide and are characterized by a progressive evolution from steatosis to chronic hepatitis, fibrosis, cirrhosis, and hepatocellular carcinoma.

Various xenobiotics are known to cause hepatotoxicity, one among them is carbon tetrachloride (CCL₄) that may cause lipid peroxidation. It was the first toxin for which was shown that the injury it produces is largely or entirely mediated by a free radical mechanism. Toxic levels administered to animals produce fatty accumulation in the liver due to blockage in the synthesis of lipoproteins that carry triglycerides away from this organ. It is believed that CCL₄ is metabolised by the P450 system to give the trichloromethyl radical. Several P₄₅₀ are involved including CYP2E1, the ‘ethanol-inducible’ cytochrome P₄₅₀. Hence, CCL₄ induced hepatotoxicity serves as an excellent model to study the molecular, cellular and morphological changes in the liver. There are increasing evidences that free radicals and reactive oxygen species (ROS) play a crucial role in the various steps that initiate and regulate the
progression of liver diseases independently of the agent in its origin. Oxidative stress is also involved in liver damage induced by alcohol abuse, viral infection, alteration of lipid and carbohydrate metabolism and xenobiotics. Steroids, vaccines, and antiviral drugs, have been used as therapies for liver pathologies, have potential adverse side-effects, especially if administered chronically or sub-chronically. Therefore, herbal products and traditional medicines with better effectiveness and safe profiles are needed as a substitute for chemical therapeutics. As oxidative stress plays a central role in liver pathologies and their progression, the use of antioxidants have been proposed as therapeutic agents, as well as drug co-adjuvants, to counteract liver damage. There are no detailed reports on the antioxidant defense of Kyllinga triceps rottb in CCl₄ induced hepatotoxicity. Hence we considered it worthwhile and carried out this investigation to assess the effect of Kyllinga triceps rottb on marker enzymes, enzymatic antioxidants in CCl₄ induced hepatotoxicity in rats. CCl₄ induced rats showed decreased body weight, increase in liver tissue weights, elevated levels in serum marker enzymes, antioxidant enzyme levels (CAT, SOD, GPx, GST, GR). Whereas, clinical symptoms (above mentioned) of CCl₄ intoxication were rectified with Kyllinga triceps rottb treatment. In contrary Kyllinga triceps rottb (PE-KTR 200mg/kg) is effective treatment and it showed almost near result with standard drug treatment (silymarin). Thus our results strongly support the notion that treatment with Kyllinga triceps rottb to CCl₄ induced cirrhotic subjects would help in achieving hepatoprotection. Due to antioxidant potential it could be beneficial for protection and alleviation of the liver fibrosis complications.

Diuretic activity may be very useful in a number of conditions like hypertension, hypercalciuria, and cirrhosis of liver. Since diuretics are employed clinically in the treatment of edema, it would be highly important to demonstrate effectiveness in the presence of electrolyte and water. Thus, it is presumed to be advantageous to ‘pre-treat’ or ‘prime’ the test animals with
various fluids in screening agents for potential diuretic activity. The petroleum ether and alcoholic extracts showed an increase in urine volume that appeared to vary with dose and time as well as the nature of the extract. Compared to the alcoholic extract, the petroleum ether extract produced a better diuretic effect. The lower doses of both extracts did not produce an appreciable effect, but, whilst the high dose of the petroleum ether extract was able to produce significant effect beginning from the fourth hour, the same dose of the alcoholic extract was devoid of any effect until the end of the observation period. This could probably suggest that the lower doses might represent sub threshold doses.

Phytochemical studies confirms the presence of terpenes and terpenoids as 1,8-cineole, cyperene, valencene, ferruginol etc. thus the present study suggests that pharmacological actions of monocot grass Kyllinga triceps rottb. Is due to presence of these terpenes and terpenoids. Main active constituents of the plant rhizome may be Ferruginol and 4,7-dimethyl-1-tetralone.