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EVALUATION OF ANTIOXIDANT AND HEPATOPROTECTIVE ACTIVITIES OF *KYLLINGA TRICEPS* ROTTB. RHIZOMES IN CARBON TETRA CHLORIDE-INTOXICATED RATS

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ABSTRACT

The anti oxidant and hepato protective activities of the extract of *kyllinga triceps* rottb. Rhizome were investigated against ccl4-induced hapatotoxicity in rats. Hepatotoxic rats were treated with eathanol and pet. Ether extracts of rhizome of *kyllinga triceps* with two dose levels 100 mg/kg and 200 mg/kg body weight/day, orally. The activities were studied by assaying the serum marker enzymes like SGOT, SGPT, ALP and ACP as well as total Bilirubin (mg/100 ml) of blood, was also estimated. All the biochemical Investigations were confirmed by the histopatholoigical observations and compared with the standard drug sillymarin. Antioxidant activity is evaluated by estimations of SOD, catalase, glutathione peroxidase TBRARS, result suggest significant hepatoprotective and antioxidant effects of the plant rhizomes which might be due to the presence of terpenes and terpenoides. As diterpene is isolated from the ethanolic extract of *kyllinga triceps* rhizomes.

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INTRODUCTION

Kyllinga triceps Rottb. Is a monocot grass of the cyperaceae family, known as nirvishi in hindhi and musta in ayurvedic literatures. It is considered as valuable medicinal plant in ayurveda. It is found as a weed in fields and forests of Gwalior-chambal region. Plant is reported to contains volatile oil as main active constituent. It is reported that plant is used in liver ailments by tribals of various places in India. Present study confirms its hepatoprotective potential.

MATERIALS AND METHODS

Plant Material-fresh rhizomes of *kyllinga Triceps* Rottb. Were collected from Bhoora Khon area of Shivpuri District of Gwalior region, authenticated by Dr. (Smt) M.D. Gupta (Asst. Director) and Mr. N.K. Pandey (R.O.) National Research institute of Ayurveda and Siddha (CCRAS), Ministry for health and family welfare, Govt. of India, Amkho, Gwalior (M.P.)

Extraction and Isolation-

Freshly Collected Rhizomes were shade dried and powdered, the powder was subjected to cold percolation using eathnol and petroleum ether as a solvent, the extract-was concentrated under vacuum, yields of ethanolic and petroleum. Ether extracts were 6.6 and 5.4 respectively half of the extract was suspended in an appropriated volume of olive oil to prepare desire concentration for oral administration to rats during experiments. The rest of the extract was subjected to traditional column chromatography by fractionation with different solvents. The purity of fractions was checked by thin layer chromatography. After ascertaining the purity of compound, it was subjected to spectral analysis (Mass,^{1}HNMR, ^{13}CNMR) to establish the structure, as a result, compound isolated was characterized as monoterpene.

Characterization of compound:-

Present study confirms the presence of Saponins, carbohydrates, tannins, flavonoids and terpenoids. Both the extracts were subjected to thin layer chromatography for the separation of phytoconstituents, ethanolic extract show three sport with RF value 0.60, 0.48, 0.40 respectively and petroleum ether extract-showed three spots with Rf value 0.52, 0.45 and 0.76 respectively after that column chromatography was performed for ethanolic extract because it showed three coloured highly 585unes585al spots and also in our previous Phytochemical studies, it has given positive result fraction 21-24 obtained from the column were selected because it not only showed homogenicity in Rf values of TLC but also the spots obtained were nearly same in colour which might be due to same chemical constituents and its Rf value was also to the Rf value shown by the TLC of ethanolic extract.

Isolated component is subjected to mass spectroscopy, molecular formula is established as C_{20} H_{30} 0, mass spectrum data showed molecular ion peak at m/2 (%) 286 (35) where the base peak in at 271, the fragment ion peaks at 253 (12), 187 (78) 145 (30), 117 (14) and 91 (9).

The ^{1}H NMR spectral study in used for establishment of the structure of the isolated compound. The presence of phenolic 0H was observe at δ4.62 ppm. The aromatic protons were reported δ 6.79 and 6.63 ppm. The germinal methyls of iso propyle moiety were observed at δ 1.12 ppm, angular methyl of trans configuration were recorded at δ 1.06 ppm.

The structure was also supported by ^{13}CNM R spectral data. Aromatic carbons were observed at δ 132, 125.7 127.3 and 110.7 ppm. A shift at δ 151.0 ppm indicate the presence of a phenolic-0H at C-13, 585unes585al dimethyl carbons of isopropyl moiety were recorded at δ 22.5 ppm.

Hepatoprotective Activity:

Silymarin:

Silymarin is natural product obtained from seeds and fruits of silybum marianum (milk thistle). Silymarin for present study was obtained as gift from unicure, noida, India, it was dissolved in olive ail for oral administration to rats during experiment at the dose level-25mg/kg body weight/day.

Chemicals:

All Chemical were analyrical grade and chemical require for biochemical assay were obtained from span diagnostics, surat, India.

Animal Model-

Colony Bred healthy, adult male wistar albino rats (rattus norvegicus) weighing 175-200gm were used in the present study. The rats were housed in polypropylene cages under controlled conditions temperature (23-26^{0}c), humidity (60-70%) and light (12 hrs light/dark cycle). They were provided with nutritionally adequate standard laboratory diet (lipton, india ltd. Banglore). And tap water ad libitum.

Ethical Aspects:-

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

Acute oral toxicy:

Acute oral toxicity was performed by using OECD guide lines-423, fixed dose procedure (FDP). Five wistar albino rats of either sex having weight 175-200gm were used for the study. Fixed dose levels of 50, 100, 200, 500, 1000 mg/kg were given initially to allow identification of a dose producing evident toxicity for the ethanolic and petroleum ether extracts of *kylling tricps* rottb. LD 50 of the alcoholic and pet. Ether extract of *kyllinga triceps* rottb. Was done as per OECD guideline. The alcoholic and pet. Ether extracts falls under class 4 (LD50>2000mg/kg). the animals did not show any signs of toxicity and behavioral changes.

Experimental Animals:

Male wistar albino rats 4-6 weeks, 175-200 gm were used for the pharmacological stidies. The animals were maintained in well ventilated room temperature (23-26^{0}c) with natural day-night (12 hrs light/dark) cycle in the polypropylene cages. They were fed with balanced rodent pellet diet and tap water, throughout the experimental period. The animals were housed for one week, prior to the experiments to acclimatize the laboratory environment.

Study Protocol:

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

Group-I

Received normal saline (10ml/kg) for 60 days. This group served as a normal control.

Group-II

Rats were intoxicated with, CCL_4 at the dose level of 0.3 ml/kg body weight/twice a week, I.P. with olive oil (50% v/v)

Group-III

Received ethanolic extract of rhizome of *kyllinga triceps* rottb. 100 mg/kg orally daily once for 60 days and CCl_4 as group-II for 60 days.

Group-IV

Received ethanolic extract of rhizome of *Kyllinga triceps* rottb 200mg/kg orally daily once for 60 days and CCl_4 as group-II for 60 days.

Group-V

Received petroleum Ether extract of rhizome of *kyllinga triceps* rottb. 100mg/kg orally daily once for 60 days and CCl_4 as group-II for 60 days.

Group-VI

Received petroleum ether extract of rhizome of *kyllinga triceps* rottb. 200 mg/kg orally daily once for 60 days and CCl_4 as group-II for 60 days.

Group-VII

Received silymarin (25 mg/kg orally) daily once for 60 days and CCl_4 as group-II for 60 days.

Autopsy schedule:

After the last dose delivery rats of each group were kept on starvation for 24 hrs and after that anaesthetized under mild ether anesthesia. Blood samples were collected by cardiac puncture. The blood samples of each animal were taken and allowed to clot at 37^{0}C and the serum was sperates by antrifugation then stored at 4^{0}C until assayed.

After the collection of blood the liver was immediately excised, washed with cold normal saline. Half of the liver was fixed in bouin's fixative for histological studies and the remaining half was immediately frozen at 7^{0}C for biochemical assays.

Analysis and processing of the sample:

The biochemical analysis in the serum sample viz. Serum glutamic oxalo acetic transaminase (SGOT), serum glutamic, pyruvic transaminase (SGPT), Alkaline phosphatase, (ALP). Acid phosphatase (ACP) and total bilirubin were performed using kit methods. SGOT SGPT, kits were purchased from Accurex biomedical Pvt. Ltd. Mubai India. Kits for ALP and ACP, total bilirubin purchased from span diagnostics ltd. Surat India.

In tissue samples, a part of liver tissue was minced and homogenized for 1 min. to make 10% w/v liver homogehate, with 1.15 % W/V KCl. The quantitative estimation of hepatic antioxidant enzymes such as SOD, Catalase glutathione peroxidase thiobarbituric acid reactive substance were performed in liver homogenate.

Histopathology:-

Liver was fixed in bouin's fixative for 24 hrs and after that dehydrated in ethanol series (50%-100%) cleared in xylene, embedded in paraffin using the standard microtechniquas, section of the liver (5μm) were stained with alum harmatoxylin and eosin for histopathological changes.

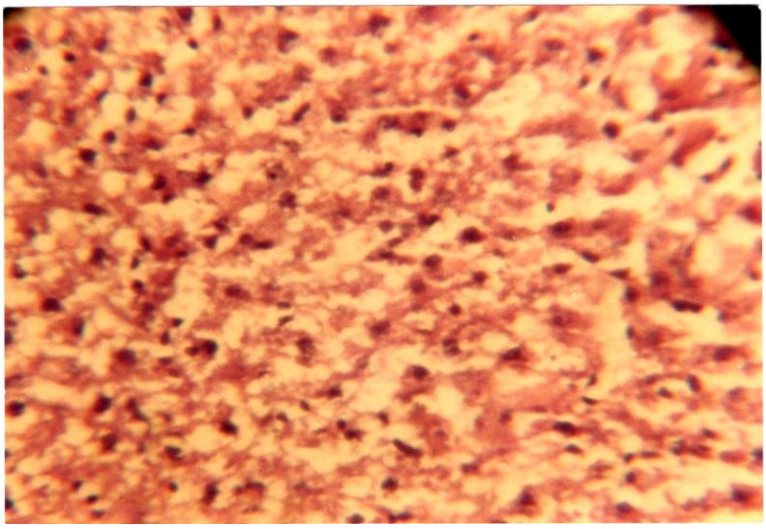

Fig-I, Group-I (Normal Control).

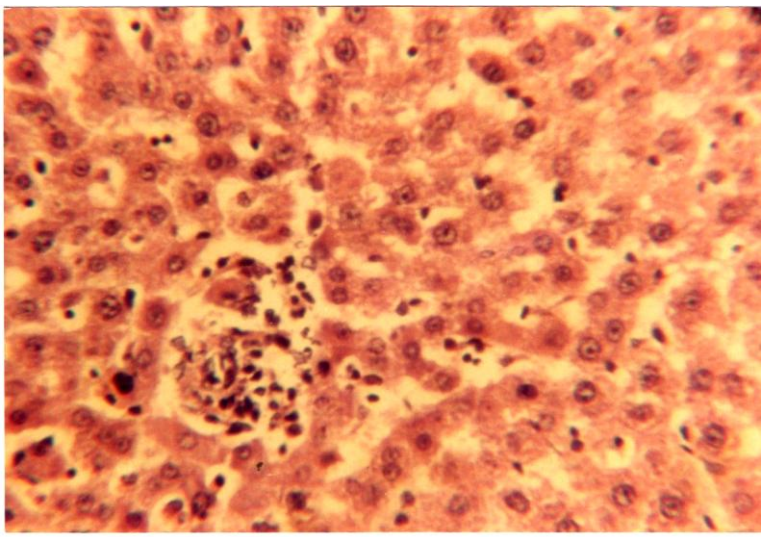

Fig-II, Group-II (CCl 4 Challenged).

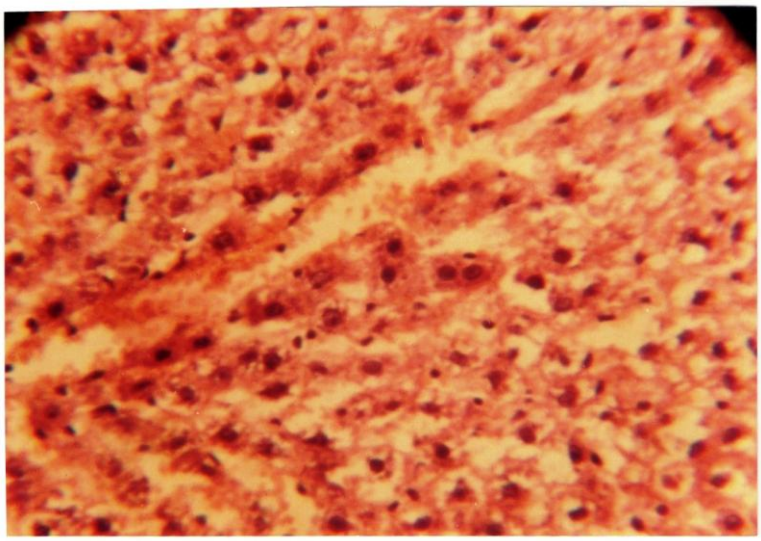

Fig-III, Group-III (200 mg/kg Petroleum Ether Rhizome Extract).

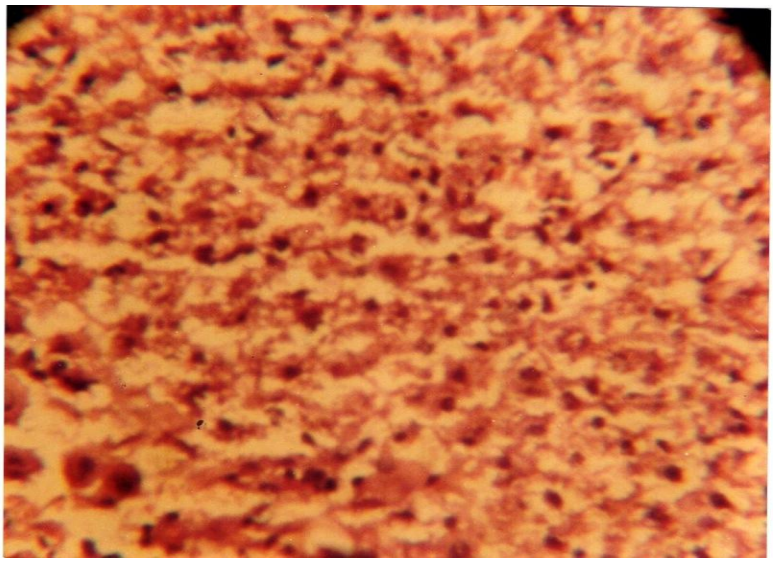

Fig-IV, Group-IV (200 mg/kg Ethanolic Rhizome Extract).

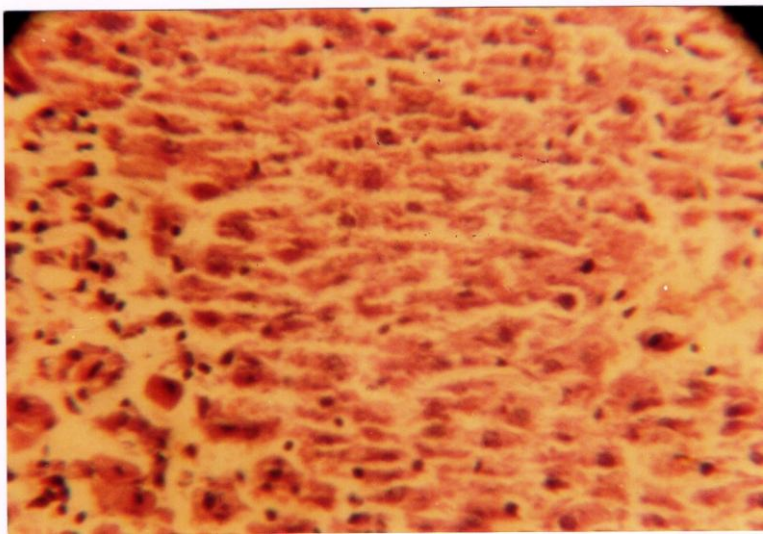

Fig-V, Group- V (Silymarin-standard reference).

TABLE-1: EFFECT OF ETHANOLIC AND PERROLEUM ETHER RHIZOME EXTRACT OF *Kyllanga triceps Rottb.* ON CCL4 INDUCED HEPATOTOXICITY IN RATS.

Treatment	SGOT(U/I)	SGPT (U/I)	ALP (TU/L)	ACP (U/L)	Bilirubin (mg/100ml of blood total)
Control	93.8 ± 3.28	36.18 ± 2.1	15.82 ± 0.82	11.8 ± 0.064	0.40 ± 0.40
CCl_4 (0.3 ml/kg)	188.2 ± 9.1	133.2 ± 8.2	99.3 ± 3.8	38.8 ± 2.3	0.88 ± 0.40
Alcohol extract 100 mg/kg	128.2* ±9.1	73.8* ± 3.3	61.5* ± 5.3	25.8* ± 1.2	0.73 ± 0.08
Alcohol Extract 200mg/kg	102.3** ± 7.89	42.2** ±2.3	38.4** ± 2.9	16.0* ± 0.24	0.47 ± 0.04
Petroleum ether extract 100mg/kg	148.64* ± 5.92	72.4* ± 6.8	68.9* ±5.8	25.4* ± 0.95	0.78 ± 0.06
Petroleum ether extract 200mg/kg	126.8** ± 8.28	47.5** ± 4.1	43.6** ± 3.4	16.5* ± 0.18	0.59 ± 0.04
Silymarin (25mg/kg)	108.2** ± 5.8	45.9** ± 2.8	33.8** ± 1.3	16.3* ± 0.8	0.43* ± 0.02

Data are expressed as mean ± S.E., n=6

*p<0.01 Vs Control

** P<0.001 Vs Control

TABLE-2: ANTIOXIDANT ACTIVITY OF ETHANOLIC AND PETROLEUM ETHER RHIZOME EXTRACTS OF *Kylling triceps* Rottb.

Treatment	Glutatione	SOD Liver Protein)	Catalase (mg liver protein)	TBARS (nmol malondiadehyde
Control	0.99 ± 0.08	77.83 ± 5.3	288.3 ± 13.8	1.28 ± 0.35
CCL_4 (0.3 ml/kg)	0.63 ± 0.02	49.31 ± 3.1	181.25 ± 12.8	1.92 ± 0.13
Ethanolic extract (100 mg/kg	0.89* ± 0.07	61.37* ± 0.64	262.52* ± 6.97	1.57 ± 0.12
Ethanolic extract (200 mg/kg	0.93* ± 0.03	88.52* ± 5.9	283.2* ± 18.1	1.31* ± 0.12
Petroleum Ether extract 100 mg/kg	0.89* ± 0.07	67.73* ± 0.54	266.27* ± 8.74	1.63 ± 0.07
Petroleum Ether extract 200 mg/kg	0.92* ± 0.06	86.97* ± 0.75	281.38* ± 9.92	1.43* ± 0.06
Silymarin (25 mg/kg)	099* ± 0.08	88.3* ± 5.8	269.28* ± 19.3	1.26* ± 0.8*

Data are expressed as mean ± S.E., n=6

*P< 0.01 Vs control by student 't' test.

RESULTS

CCl4-intoxication to rats for the period of 60 days, resulted in a significant-rise in the levels of SGPT SGOT, ALP, ACP and total bilirubin. The effect of *Kyllinga triceps* rottb. Rhizome extract on serum SGOT, SGPT, ALP, ACP and total bilirubin in CCl4 induced rats was found to be reduced. Significantly. CCl4 treated group III with the dose level of 200mg/kg of *kyllinga triceps* was most effective but the attenuation of altered serum parameters was not as high as in silymarin treated rats of group VI.

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