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Hepatoprotective activity of alcoholic and aqueous extracts of leaves of *Anisochilus carnosus* (Linn.)Wall

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Abstract

The aim of the research is to find out new hepato-protective drug from indigenous plant which are potent and non-toxic or minimal toxic and to investigate the hepato-protective activity of alcoholic and aqueous extract of leaves of *Anisochilus Carnosus (L)*Wall against Rifampicin induced hepatotoxicity. Leaf powder of *Anisochilus Carnosus (L)* Wall was successively extracted with petroleum ether, alcohol and aqueous. Preliminary phytochemical tests were done and the LD50 values for both aqueous and alcohol extract determined. The hepatoprotective activity of the alcoholic extract (400 mg/kg P.O.) and aqueous extract (400mg/kg P.O.) were assessed in Rifampicin induced hepato toxicity in rats

Key words: Leaf extract, hepatoprotective activity, rifampicin

Introduction

God the almighty has blessed mankind with a treasure of medicinal plants. Numerous medicinal plants and their formulations are used for various disorders in ethno medical practices as well as traditional system of medicine in India. Simultaneously, the need for the basic scientific investigation of medicinal plants using indigenous medical system have became more interesting and relevant[(1)].*Anisochilus Carnosus (L)*Wall is a annual, erect,30-60 cm high, stem stout, bluntly, quadrangular, glabrous or finely pubescent. It's leaves are ovate, obtuse, some what fleshy & glabrous. Volatile oil of *Anisochilus Carnosus (L)*Wall contain Carvacrol (27.9%), Camphor (14.1%) and α-cis bergamotene(10.2%) [(2-3)]. The plant has been traditionally used as hepatoprotective agent, stimulant, anti ulcer, anti inflammatory [(4-6)]. The fresh Juice of leaves mixed with sugar candy is given to children in coughs mixed with sugar and ginjelly oil and it forms a cooling liniment for the head. Hence, the present study was aimed to investigate the hepatoprotective activity of leaf extract of *Anisochilus Carnosus (L)*Wall in Rifampicin induced hepatotoxic model in wistar rat

Material and Methods

Silymarin was obtained from Micro labs Bangalore, the kits for all biochemical estimation were purchased from R.S.Enterprises, Jaipur, India. The solvent and chemicals used were of analytical grade. The leaves of *Anisochilus Carnosus (L)* Wall were collected during the month of July of 2007 from the fields of Thenmalai, Kaaripatti, Salem, Tamilnadu. The plant was authenticated by a botanist. The leaves were shaded dried at room temperature and the Petroleum ether extract was obtained using soxhlet apparatus. Alcoholic extract was obtained with ethyl alcohol 95% w/v for 18 hrs using soxhlet apparatus. The aqueous extract was prepared with the remaining mass by maceration process for 7 days. The extract was dried at 55 ºC in a water bath. The Percentage yield of alcoholic and aqueous extract were 5.27% and 7.03% respectively.(Table no.1).

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The preliminary phytochemical screening of Alcoholic and aqueous extract were carried (Table no.2) out as described by khandelwal [7]. Male & Female Swiss albino mice weighing between 20-25 gm and male wistar rat weighing between 150-220 gm were used for this study. The animals were obtained from animal house of sri Venkateshwara Enterprises, Bangalore, India. On arrival the animal were placed randomly and allocated to treatment groups in propylene cages with paddy husk as bedding. Animals were housed at a temperature of 24± 2ºC and relative humidity 45-55% with 12:12 hours light/dark cycle. The animals had free assist to food and water. The animals were habituated to laboratory conditions for 48 hours prior to the experimental protocol to minimize any non-specific stress. All the experimental process and protocols used in this study were reviewed by the institutional animal ethical committee (Pharmacology project no. 19/2007) and were in according with the guideline of the CPCSEA.

Acute oral toxicity of alcoholic and aqueous extract were determined using nulliparous, non-pregnant female mice. The animals were fasted for 3 hours before experiment and were administered a single dose of extract dissolved in 2% w/v tween 80 and observed for mortality upto 48 hours . Based on short term toxicity the dose of next animal was determined as per OECD guideline [8]. All the animals are also observed for long tern toxicity.

Experimental protocols:-

Test compound: - The alcoholic & Aqueous extract of *Anisochilus Carnosus(L)*Wall leaves (400mg/kg body weight) and standard drug silymarin (25 mg/kg body weight) were used**.** The following chemicals were obtained from the indicated commercial Rifampicin (R-cin, Lupin Ltd, Aurangabad, Maharashtra), Silymarin (Silybon suspension, Micro Lab Ltd, Bangalore)

Experimental setup

Wistar rats (150-200 gm) used in the present studies were procured from listed suppliers of Sri Venkateshwara Enterprises, Bangalore, India. The animals were fed with standard pellet diet (Hindustan lever Ltd. Bangalore) and water *ab libitum.* All the animals were acclimatized for a week before use. The alcoholic & aqueous extract of *Anisochilus Carnosus(L)*Wall was dissolved in gum acacia.

The rats were divided into 7 groups of 6 animals in each.

Group I : Received vehicle control gum acacia (5 mg/ kg.p.o)
Group II : Received Rifampicin control (1 gm/ kg p.o) every 72 hrs.
Group III : Received Silymarin (25 mg/ kg p.o) for 10 days,
Simultaneously Rifampicin 1gm/kg body weights every 72 hrs.
Group IV : Received alcoholic extract of *Anisochilus Carnosus(L)*Wall (400mg/kg p.o), Simultaneously Rifampicin 1gm/kg body weights every 72 hrs..
Group V : Received aqueous extract of *Anisochilus Carnosus(L)*Wall(400mg/kg p.o) simultaneously Rifampicin 1gm/kg body weights every 72 hrs.

The blood samples were collected and allowed to clot and serum was separated by centrifuging at 2500 rpm for 15 min and analyzed for various biochemical parameters.

Assessment of hepatoprotective activity [9-11]:

Morphological parameters

These parameters were studied by recording the liver weight and volume. In damaged liver due to the fatty changes. The volume and weight of the liver is increased as compared with control group.

Biochemical parameters

Biochemical parameter i.e., Serum glutamate pyruvate transminase (SGPT), Serum glutamate Oxaloacetate Transaminase (SGOT), Alkaline Phosphtase (ALP), Billirubin, Total Protein, γ Glutamyl Transpeptidase (GGTP), were analyzed according to reported methods.

Liver Histopathology

After withdrawing the blood from the animals they were sacrificed. The liver was removed, fixed in 10% formalin, embedded in paraffin, sectioned and stained with hematoxylin and eosin, the studies were read by pathologist who was not aware of the treatments.

Statistical Interpretation

The analysis of data was done by ANOVA followed by Dunnett multiple comparison test.The values were expressed as mean ± SEM . The statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Dunnet's 't' test . P values <0.05 were considered to be significant.

Result:

Preliminary phytochemical studies reveled the presence of carbohydrates, glycosides, phenolic compound, tannins, alkaloids and flavonoids (Table no.2). The leaf extract of *Anisochilus Carnosus (L)* Wall was found to be non toxic. Treatment of rats with Rifampicin produce an increase in the weight and volume of rat liver(Table no.3). Aqueous and alcoholic extract shows significant decrease in liver weight and volume compare to (control group). Rifampicin administration resulted increase of SGOT, SGPT, ALP, GGTP and bilirubin direct , while total protein were found to be decrease compare to normal control group (Table no.4). Pretreatment with silymarin aqueous and alcoholic extract significantly prevented the biochemical changes induced by rifampicin. Aqueous extract treatment offered greater hepatoprotective effect than alcoholic extract(Table no.4). Hepatocytes of control group showed a normal histology of liver . Rifampicin treated group showed sever and macro vasicular fatty changes, severe inflammation and fatty degeneration. Silymarin treated group showed normal hepatocytes and no evidence of hepatic damage. Alcoholic extract treated group showed mild fatty degeneration and mild chronic inflammation and also show mild focal rearrangements of cells. Aqueous extract treated group showed normal lobular architecture of the liver with no evidence of inflammation.

Discussion:

In the present study rifampicin was selected as hepatotoxicant to induce liver damage . Rifampicin is one of the most common drug used in the treatment of tuberculosis which caused hepatotoxicity. The assessment of liver function can be made by estimating the activity of serum enzyme such as SGOT, SGPT, ALP, ALT etc. During hepatic damage the enzyme level changes in serum. So, toxicity produced by the toxicant. The present study has demonstrated that aqueous extract exhibited significant dose dependent hepatoprotective activity in comparison to alcoholic extract against liver injury induced by rifampicin. Rifampicin also alters the metabolic activity of hepatocytes [(10)]. Thereby, inducing hepatic damage which cause macrovascicular fatty changes, inflammation, fatty degeneration, evidence of inflammation etc. Both the extracts prevented the histological changes indicating these hepatoprotective activity.[11-13] All the histological changes observed were in the correlation with the physical, biochemical, and functional parameters of the liver. It can be concluded that *Anisochilus Carnosus (L)* Wall leaf extracts possess a protective effect against rifampicin induced hepatotoxicity in rat, as evidenced by the physical, biochemical, functional and histological parameters

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Table No.: Data Showing the Extractive Values of Leaves of *Anisochilus carnosus (L)* Wall.

Plant name	Part used	Method of extraction	Yield in percentage		
			Petroleum Ether extract	Alcohol extract	Aqueous extract
Anisochilus Carnosus(L)Wall	Leaves	Continuous hot percolation	4.05	5.27	7.03

Table No. 2: Data showing the preliminary phytochemical screening for the leaves extracts of *Anisochilus carnosus* (L) Wall

S. No.	Constituents	Tests	Pet. Ether extract	Ethanolic extract	Aqueous extract
1.	CARBOHYDRATE	Molish's test	+	+	+
		Fehling's test	+	+	+
2.	GLYCOSIDE	Legal's test	-	+	+
		Borntrager's test	+	+	+
		Baljet test	—	—	+
3.	FIXED OIL AND FATS	Spot test	+	+	+
		Saponification test	-	—	-
4.	PROTEINS & AMINO ACIDS	Million's test	—	—	-
		Ninhydrin test	—	—	-
		Biuret test	—	—	-
5.	SAPONINS	Foam test	+	+	-
6.	PHENOLIC COMP. AND TANINS	$FeCl_3$ test	—	+	+
		Gelatin test	—	—	+
		Lead acetate test	—	+	+
7.	PHYTOSTEROL	Salkowiski test	—	—	-
		Libermannburchard test	—	+	-
8.	ALKALOIDS	Dragendroff's test	+	+	+
		Mayer's test	—	+	+
		Wagner's test	—	—	-
		Hager's test	—	+	+
9.	GUM & MUCILAGE	Swelling test	—	—	-
10.	FLAVONOIDS	Aqueous NaOH test	—	+	+
		Con. H_2SO_4 test	—	+	+
		Shinoda's test	—	+	

Table No. 3: Liver weight of animals after 9 days treatment against Rifampicin induced epatotoxicity in Rats

Groups	Treatment	Dose (mg/kg)	Liver wt (gm)	Percentage increase
I	Control	-	5.41 ± 0.02	-
II	Rifampicin treated	1000	6.93 ± 0.15[a]	28.09
III	Silymarin	25	5.88± 0.13[b]	8.68
IV	Alcoholic extract of Anisochilus carnosus	400	6.03± 0.01[b]	11.46
V	Aqueous extract of Anisochilus carnosus	400	5.46± 0.09[b]	0.92

Values are represented as mean ± S.E.M (n=6)
One-way ANOVA followed by Student-Newman-Keuls post test (P< 0.001) is used.
a-*vs* group I and b-*vs* group II.

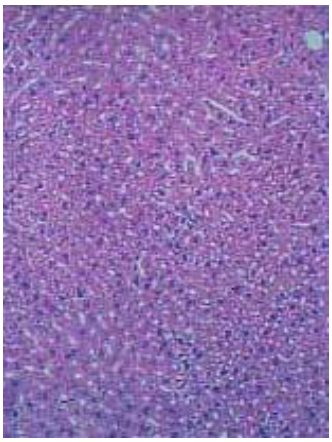
Table no.4: Effect of extract of *Anisochilus Carnosus*(L)Wall on biochemical parameter against Rifampicin induced hepatotoxicity in rats

Groups	Treatment	Dose (mg/kg)	SGOT (U/L)	SGPT (U/L)	SALP (U/L)	TOTAL PROTEIN	GGPT (U/L)	BILIRUBIN DIRECT (MGS %)
I	Control	-	112.8 ± 1.24	80 ± 0.80	337 ± 0.07	6.5 ± 0.08	150.16 ± 1. 3	0.49 ± 0.06
II	Rifampicin treated	1000	162. 33± 1.05	141 ± 0.80[a]	427 ± 0.6[b]	5. 8 ± 0.06[a]	244.83± 1. 3	0.79 ± 0.04[a]
III	Silymarin treated	25	117.16 ± 2.15[b]	81. 6 ± 0.67[b]	352± 0.7[b]	6.3± 0.03[b]	176.83 ± 2.0[b]	0.53 ± 0.04[b]
IV	Alcoholic extract treated	400	140.33 ± 1.05[b]	112. 8± 1. 24[b]	393 ± 0.8[b]	5.9 ± 0.03[b]	235.33± 1.3[b]	0.72 ± 0.03[b]
V	Aqueous extract treated	400	133 ± 0.80[b]	109 ± 0.80[b]	383. 16±0.96	6.2 ± 0.03[b]	226.17± 0.5[b]	0.67± 0.01[b]

Values are represented as mean ± S.E.M (n=6)
One-way ANOVA followed by Student-Newman-Keuls post test (P< 0.001) is used.
a-*vs* group I and b-*vs* group II.

References

1. Hara,H., An enumeration of the flowering of plants of Nepal.1978-1982.
2. SIRSI M, RAO RR, Indian J med Res.1956 Apr: 44(2), 283-7
3. J.ravikumar ; H Y Santosh ,Pharma info.net ,Volume 45,979-981,dec.2008.
4. Grover J.K., Adiga G., Vats V., Rathi s.s.; Extracts of Anisochilus carnosus(L)Wall prevent development of experimental inflammation. J. of Ethanopaharam.; 2001;78;159-164.
5. Khandelwal K.R, "Practical pharmacognosy technique and experiments " 9th edition, Nirali prakashan, Pune, 2000.
6. OECD 2001 guideline on acute oral toxicity (AOT),Enviromental health and safety monograph series on testing and adjustment.
7. Luna LG, Manual in histology and staining methods. McGraw Hill ; New York;1999.
8. Goodman, gillman's. the pharmacological basis of therepeutics;10th edition;1563-1596.
9. D.R. Laurence, P.N. Bennett. Clinical pharmacology; 7th Edition; 581-590.
10. Subramoniam A , Pushpangadan P. Development of phytomedicines for liver diseases,Indian J Pharmacol 1999;31:166-7
11. Lim HK, Kim HS, Choi HS, Oh S , Choi J.Hepatoprotective effects of bergenin , major constituents of Mallotus japonicus on CCl4 intoxicated rats, J Ethanopharmacol 2000;72;469-74.
12. Miller, LC and Trainter ML. proc Soc Exptl Med Biol., 1944; 57:261.
13. Kumar A.; Vimalavathini R. Annorectic effect of methanolic extract of *Anisochillus carnosus*(l)Wall in swiss albino mice. ; Indian J. of pharmacol. ; 2004; 36(6):348-350.



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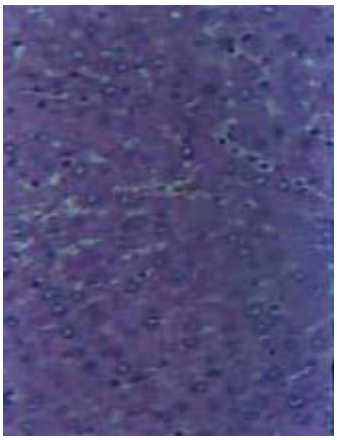

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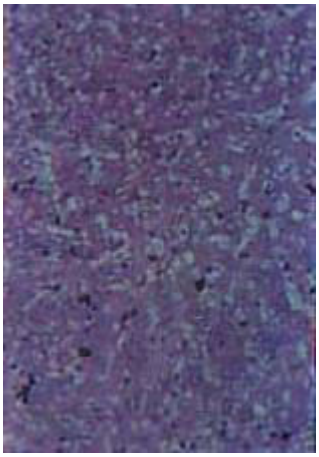

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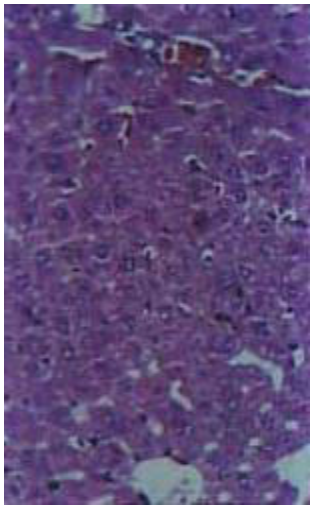

G-5

Figure-1 Normal Histopathology of rat liver showed normal histology of liver
Figure-2 Histopathology of Silymarin treated rat liver showed normal haepatocytes, no evidaence of hepatic damage
Figure-3 Histopathology of Rifampicin treated rat liver showed severe macro vasicular fatty changes, severe inflammation and fatty degeneration
Figure-4 Histopathology of Alcoholic extract treated rat liver showed mild fatty degeneration and mild chronic inflammation and also show mild focal rearrangements of cells
Figure-5 Histopathology of Aqueous extract treated rat liver showed normal lobular architecture of the liver with no evidence of inflammation.