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Research Article

HEPATOPROTECTIVE ACTIVITY AND SUB ACUTE TOXICITY STUDY OF WHOLE PART OF THE PLANT ANOECTOCHILUS FORMOSANUS HAYATA (ORCHIDACEAE)

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ABSTRACT

Objective: The present investigation aimed at phytochemical screening of the whole plant of *Anoectochilus formosanus* Hayata (Orchidaceae) after successive extraction followed by hepatoprotective activity of its aqueous extract. The research work also focused on the sub acute toxicity study of aqueous extract of the same plant.

Methods: Successive extraction was carried out with petroleum ether, chloroform, methanol and water respectively. Hepatoprotective activity of its aqueous extract was investigated in carbon tetrachloride, ethanol and paracetamol induced hepatotoxic rat models and compared with silymarin (20 mg/kg) as reference standard. Sub acute toxicity study was elicited by studying the effect of *Anoectochilus formosanus* on the lipid profile, biochemical parameters and hematological parameters in rats and compared with standard silymarin (20 mg/kg).

Results: Phytochemical screening revealed the presence of anthraquinone glycosides, cardiac glycosides, reducing sugars, carbohydrates, phenolic compounds, tannins, flavonoids and saponins in the aqueous extract of the plant under investigation. Two way analysis of variance study of the estimated biochemical parameters for instance, aspartate aminotransferase, alanine amino transferase and alkaline phosphatase were revealed that there is significant difference (p-value < 0.0001) exists between the different treatment groups supported by least significant difference test of various biochemical parameters, which was also evident from the histopathological study of liver sections. Sub acute toxicity study revealed no significant toxic effects.

Conclusion: Aqueous extract of *Anoectochilus formosanus* (200 mg/kg) had shown significant hepatoprotective activity as compared to standard silymarin. Sub acute toxicity studies had concluded that it might be considered safe for a longer duration of time.

Keywords: Alanine aminotransferase, Alkaline phosphatase, *Anoectochilus formosanus*, Aspartate aminotransferase, Least significant difference, Phytochemical screening, Silymarin

INTRODUCTION

Hepatotoxicity refers to liver dysfunction or liver damage that is associated with an overload of drugs or xenobiotics [1]. Chemicals that cause liver injury are called hepatotoxins or hepatotoxicants. Hepatotoxicants are exogenous compounds of clinical relevance and may include overdoses of certain medicinal drugs, industrial chemicals and natural chemicals like microcystins, herbal remedies and dietary supplements [2]. Certain drugs may cause liver injury when introduced even within the therapeutic ranges. Hepatotoxicity may result not only from direct toxicity of the primary compound but also from a reactive metabolite or from an immunologically-mediated response affecting hepatocytes, biliary epithelial cells and/or liver vasculature [3].

The hepatotoxic response elicited by a chemical agent depends on the concentration of the toxicant which may be either parent compound or toxic metabolite, differential expression of enzymes and concentration gradient of cofactors in blood across the acinus [4]. Hepatotoxic response is expressed in the form of characteristic patterns of cytolethality in specific zones of the acinus. Hepatotoxicity related symptoms may include a jaundice or icterus appearance causing yellowing of the skin, eyes and mucous membranes due to high level of bilirubin in the extracellular fluid, pruritus, severe abdominal pain, nausea or vomiting, weakness, severe fatigue, continuous bleeding, skin rashes, generalized itching, swelling of the feet and/or legs, abnormal and rapid weight gain in a short period of time, dark urine and light colored stool [5]. *Anoectochilus formosanus* Hayata (Orchidaceae) is an indigenous and valuable Taiwanese medicinal plant and has been used popularly as a nutraceutical herbal tea in Taiwan and other Asian countries. This herbal plant is also called “King Medicine” because of its diverse pharmacological effects such as liver protection, cancer prevention, and diabetes and for treatment of cardiovascular diseases [6]. However, limited scientifically proven information is available on the bioactivity, physiological function, and specific clinical efficacy of this herbal orchid plant. *A. formosanus* possesses hepatoprotective effect on carbon tetrachloride (CCl_4) and acetaminophen induced acute hepatitis [7, 8]. Effect of *A. formosanus* on arachidonate metabolism has also been reported [9]. Aqueous extracts of *A. formosanus* inhibited cell damage induced by CCl_4 in primary cultured rat hepatocytes [10]. Recently, it was also confirmed that an aqueous extract of *A. formosanus* attenuated hepatic fibrosis induced by both CCl_4 and dimethylnitrosamine in rats [11, 12]. However, the hepatoprotective compounds in *A. formosanus* are still unknown. The plant contains kinsenone, and a number of known flavonoid glycosides also [13]. The purpose of this research investigation was to study the *in vivo* hepatoprotective activity of whole plant of *A. formosanus* after its successive extraction with petroleum ether, chloroform, methanol and water. Three different types of models are chosen in the present study, such as carbon tetrachloride (CT), ethanol (ET) and paracetamol (PT) induced hepatotoxicity in rats and compared with silymarin (SL) as reference standard. The research work also includes preliminary phytochemical screening and effect of *A. formosanus* on the lipid profile, biochemical parameters and hematological parameters in its subacute toxicity study.

MATERIALS AND METHODS

Plant materials and chemicals

Whole plant of *Anoectochilus formosanus* (AF) were collected in the month of September from Nilgir district, Tamilnadu and authenticated by Dr. N. K. Dhal, Taxonomist, Institute of Minerals and Materials Technology, Bhubaneswar, Odisha. Paracetamol (PT), obtained as a gift sample from Cadila Pharmaceuticals, Ahmedabad, Gujurat, India. Silymarin (SL), SGOT (Serum Glutamate Oxaloacetate Transaminase) Kit, SGPT (Serum Glutamate Pyruvate Transaminase) Kit and ALP (Alkaline Phosphatase) Kits were purchased from Scientific Corporation, Rasulgarh, Bhubaneswar, Odisha. Petroleum ether, chloroform, methanol, carbon tetrachloride and ethanol were

purchased from Merck. All the reagents used were of analytical grade and were used as received.

Animals

Albino rats of Wistar strain weighing 100-150 g of either sex were purchased from M/s Ghosh Enterprises, Kolkata and used for the study. The animals were housed individually in polypropylene cages at a temperature of 27 ± 2°C and 50 – 60 % RH with food and an unlimited supply of drinking water. Animals were kept on a standard light / dark cycle (12 hr/12 hr) with lights on at 7:00 AM. The animals are randomly selected, marked to permit individual identification, and kept in their cages for 7 days prior to dosing to allow for acclimatisation to the laboratory conditions. They were fed with Amrut Laboratory Animal Feed (Nay Maharashtra Chakan Oil Mills Ltd, Pune) and water ad libitum.

Extraction of plant materials

The collected plants (whole part of plant *Anoectochilus formosanus*) were washed thoroughly in water and chopped, dried for a week (35-40^{0}C) and pulverized in an electric grinder. The powder obtained was successively extracted in petroleum ether (60-80^{0}C), chloroform, methanol and distilled water. The extracts were then made to powder by using rotary evaporator under reduced pressure. Preliminary phytochemical screening was carried out on different extracts by using standard procedures to find out the nature of phytoconstituents present in the extracts. Accordingly, powdered aqueous extract of this plant was prepared in sufficient quantity and stored in a well closed tight container for further use.

Preliminary phytochemical screening

The plant extracts after each successive steps were subjected to qualitative chemical testing for preliminary screening of phytoconstituents. Phytochemical screening was performed using standard procedures [14, 15]. Phytochemical screening of AF extracts include test for alkaloids, saponins, glycosides and sugar, phenolic compounds and tannins, flavonoids and flavones, coumarin and its derivatives and triterpenoids.

Experimental design of *in vivo* hepatoprotective activity

In vivo hepatoprotective activity was evaluated on the basis of CT (2 ml/kg, i.p.), ET (2 ml/kg, i.p.) and PT (200 mg/kg, i.p.) induced liver damaged in rats. Total 96 rats were divided in to 16 groups (each group consists of 6 animals).

Three different doses of aqueous extracts of AF (50 mg/kg, 100 mg/kg and 200 mg/kg, orally) were chosen for the screening of hepatoprotective activity and compared with SL (20 mg/kg, orally). Detail of the experimental design was given in Table 1. After thirty six hours of CT/ET/PT/normal saline administration, blood was collected from all the groups of rats by cardiac puncture. Serum was separated by centrifugation at 2500 rpm at 37^{0}C for 15 min and analysed for various biochemical parameters like SGOT, SGPT and ALP using commercially available test kits and UV-Visible spectrophotometer (SHIMADZU 1700-JAPAN). The experimental protocol was approved by Institutional Animals Ethics Committee (CPCSEA Regd. No. – CPCSEA/C/990/2005).

Table 1: Experimental design for hepatoprotective activity of AF

Group code	Group name	Treatment
A	**Normal**	**Normal saline (2 mg/kg, i.p) once daily**
B1	Control CT	CT (2 ml/kg, i.p.) once daily for 3 days
B2	Control ET	ET (2 ml/kg, i.p. once daily for 3 days
B3	Control PT	PT (200 mg/kg, i.p.) once daily for 3 days
C1	Standard SL-CT	SL (20 mg/kg, orally) twice daily for 7 days followed by CT (2 ml/kg, i.p.) on the 8th day
C2	Standard SL-ET	SL (20 mg/kg, orally) twice daily for 7 days followed by ET (2 ml/kg, i.p.) on the 8th day
C3	Standard SL-PT	SL (20 mg/kg, orally) twice daily for 7 days followed by PT (200 mg/kg, i.p.) on the 8th day
D1	Test CT-AF 50	Aqueous extract of AF (50 mg/kg, orally) twice daily for 7 days followed by CT (2 ml/kg, i.p.) on the 8th day
D2	Test CT-AF 100	Aqueous extract of AF (100 mg/kg, orally) twice daily for 7 days followed by CT (2 ml/kg, i.p.) on the 8th day
D3	Test CT-AF 200	Aqueous extract of AF (200 mg/kg, orally) twice daily for 7 days followed by CT (2 ml/kg, i.p.) on the 8th day
E1	Test ET-AF 50	Aqueous extract of AF (50 mg/kg, orally) twice daily for 7 days followed by ET (2 ml/kg, i.p.) on the 8th day
E2	Test ET-AF 100	Aqueous extract of AF (100 mg/kg, orally) twice daily for 7 days followed by ET (2 ml/kg, i.p.) on the 8th day
E3	Test ET-AF 200	Aqueous extract of AF (200 mg/kg, orally) twice daily for 7 days followed by ET (2 ml/kg, i.p.) on the 8th day
F1	Test PT-AF 50	Aqueous extract of AF (50 mg/kg, orally) twice daily for 7 days followed by PT (200 mg/kg, i.p.) on the 8th day
F2	Test PT-AF 100	Aqueous extract of AF (100 mg/kg, orally) twice daily for 7 days followed by PT (200 mg/kg, i.p.) on the 8th day
F3	Test PT-AF 200	Aqueous extract of AF (200 mg/kg, orally) twice daily for 7 days followed by PT (200 mg/kg, i.p.) on the 8th day

Histolopathological study of liver

Immediately after sacrificing the rats, small pieces of livers tissues were fixed in 10% formalin and proceed for embedding in paraffin sections. Serial sections of 5 µm thickness were made, stained with hematotoxyline and eosin followed by examination under microscope for histopathological changes which include all necrosis fatty changes and infiltration of kuffer cell and lymphocytes. One normal group, all the three control groups, all the three standard SL treated groups and three AF treated groups (only 200 mg.kg doses) were subjected to liver section microscopic study in order to compare the standard SL with highest dose of aqueous AF extract as far as hepatoprotective activity is concerned.

Sub acute toxicity study

Thirty Wister albino rats were divided in to five groups (six animals in each group). Group 1 received no treatment and served as normal, whereas group 2 received distilled water as solvent (2 ml/kg). Group 3 (standard) was treated with silymarin. Group 4 and 5 were received 100 mg/kg and 200 mg/kg of the aqueous extract of AF

orally. The animals were treated for 20 days to carry out the sub acute study and on the 21st day lipid profile, biochemical parameters and hematological parameters are determined. Lipid profile included determination of total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL), low density lipoprotein (LDL) and very low density lipoprotein (VLDL).

Biochemical parameters studied in the sub acute toxicity study are aspartate aminotransferase (AST), alanine amino transferase (ALT), alkaline phosphate (ALP), total bilirubin (TB), DB, albumin, protein and globulin. Hematological parameters determined in the study are red blood corpuscles (RBC), white blood corpuscles (WBC), hemoglobin (HB), clotting time, neutrophils, eosinophil, basophil, lymphocyte and monocyte.

Statistical analyses

All values were expressed as mean ± SEM (standard error about mean), where n = 6. Data from *in vivo* experiments for hepatoprotective activity were treated by two-way analysis of variance (ANOVA) and followed by least significant difference (LSD) test using the following formula.

$$LSD = t_{df,0.025}\sqrt{\frac{2\ MSN}{N}}$$

Where $t_{df,\,0.025}$ = table value of "t" at degrees of freedom (df) of error term at 0.025 level of significance, MSE = Mean square error value, N= number of observations. Any difference between the mean values of different treatment groups exceeding the LSD is considered to be statistically significant [16]. Results of sub acute toxicity stud were treated by one-way analysis of variance followed by Dunnett's test. The significance level was set at p-value < 0.05, during comparison between the treatment values and control values.

RESULTS AND DISCUSSION

Preliminary phytochemical screening

Chemical tests with petroleum ether extract of *Anoectochilus formosanus* whole plants revealed that there is absence of almost all the phytoconstituents, whereas triterpenoids were found in chloroform extract of AF. Methanolic extract of AF had shown presence of cardiac glycosides and flavonoids. Lastly aqueous extracts of AF had shown the presence of anthraquinone glycosides, cardiac glycosides, reducing sugars, carbohydrates, phenolic compounds, tannins, flavonoids and saponins.

In vivo hepatoprotective activity

Serum enzyme levels of different biochemical parameters (SGOT, SGPT and ALP) were shown in Table 2 as mean ± SEM. Various biochemical parameters like SGOT, SGPT and ALP were significantly reduced after administration of aqueous AF extracts (p-value < 0.0001) as compared to their respective control groups.

Two way ANOVA study of the *in vivo* data revealed that there is significant difference (p-value < 0.0001) exists between the different treatment groups. LSD values for SGOT, SGPT and ALP are 3.63, 15.2 and 29.31respectively for CT induced hepatotoxicity. Difference between mean of control and any respective treatment value exceeds their corresponding LSD values, thus indicating statistical significant effect (p-value < 0.05) of the AF aqueous extracts at different doses. All the treatment groups had shown statistically equivalent activity (p-value > 0.05) as compared to standard SL treatment group. LSD values for SGOT, SGPT and ALP were 2.49, 4.24 and 12.86 respectively for ET induced hepatotoxicity, whereas these values were 3.33, 3.57 and 10.26 respectively in case of PT induced hepatotoxicity.

LSD values for ET and PT induced hepatotoxicity depicted that different treatment values were differ significantly from their respective control values but shown similarity with their respective standard SL treatment groups as far as SGOT, SGPT and ALP values were concerned. All the aqueous extracts of AF having dose at 200 mg/kg were proven to be statistically comparable (p-value > 0.05) with their respective SL treated standard groups in the context of biochemical parameter estimation, to illustrate, SGOT, SGPT and ALP.

Table 2: Serum enzyme levels of different biochemical parameters

Group code	Treatment	Serum Enzyme levels in IU/L (Mean ± SEM)		
		SGOT	SGPT	ALP
A	Saline 2 ml/kg i.p	21.05 ± 0.3	24.85 ± 0.19	106.5 ± 0.85
B1	CT 2ml/kg	84.44 ± 0.25	152.83 ± 0.15	425.5 ± 0.75
C1	SL 20mg/kg	21.62 ± 0.63	24.32 ± 0.37	120.5 ± 0.6
D1	AF 50 mg/kg	30.68 ± 0.68	48.33 ± 0.15	118.92 ± 0.96
D2	AF 100 mg/kg	27.18 ± 0.58	42 ± 0.4	113.23 ± 0.02
D3	AF 200 mg/kg	26.66 ± 0.39	35 ± 0.37	110.25 ± 0.25
B2	ET 2ml/kg	83.6 ± 0.05	126.8 ± 0.02	415.5 ± 0.02
C2	SL 20mg/kg	21.62 ± 0.63	24.32 ± 0.37	90.23 ± 0.6
E1	AF 50 mg/kg	29.2 ± 0.03	39.65 ± 0.02	269.85 ± 0.05
E2	AF 100 mg/kg	25.6 ± 0.03	30.02 ± 0.01	91.36 ± 0.01
E3	AF 200 mg/kg	19.3 ± 0.01	20.8 ± 0.03	87.63 ± 0.02
B3	PT 200 mg/kg	79.8 ± 0.3	138.3 ± 0.08	374.33 ± 0.6
C3	SL 20mg/kg	21.66 ± 0.83	25.26 ± 0.84	90.23 ± 0.8
F1	AF 50 mg/kg	32.11 ± 0.06	39.16 ± 0.13	275 ± 0.01
F2	AF 100 mg/kg	25.15 ± 0.2	29.18 ± 0.7	87.31 ± 0.7
F3	AF 200 mg/kg	22.9 ± 0.01	27.36 ± 0.5	85.4 ± 0.6

Histolopathological study of liver

Liver section of normal control rats is depicted in Figure 1A, which had shown normal hepatic cell with prominent nuclei, nucleoli and normal position of hepatic artery portal vein and bile ducts. CT induced fatty degeneration with severe liver necrosis of parenchyma cell mostly seen in central lobular region, sinusoidal congestion, broad infiltration of kupffer cells and loss of boundaries were observed in the liver section of control CT groups as portrayed by Figure 1B. It was well demonstrated by Figure 1C that the hepatic cells radiate outwardly from the central vein and constitute the parenchyma of the lobules. The portal tract consists of hepatic artery, portal vein and bile ducts. It is well observed in Figure 1D that the severe hepatic lesions induced by CT were remarkably lowered by the administration of AF 200 mg/kg which is also proven by the results of the biochemical analysis and is comparable to the liver section of standard SL treated groups (Figure 1C). Control ET groups had shown fatty degeneration with severe liver necrosis of parenchyma cell mostly seen in central lobular region, infiltration of kupffer cells and loss of boundaries (Figure 2A), whereas control PT revealed necrosis of parenchyma cells seen in central lobular region, sinusoidal congestion and broad infiltration of kupffer cells (Figure 2D). It was clearly indicated by Figure 2B and 2E that the hepatic cells radiate outwardly from the central vein and constitute the parenchyma of the lobules. At the same time it is also observed that severe hepatic lesions induced by ET and PT were remarkably lowered by the administration of AF 200 mg/kg to their respective groups, which is also proven by the results of the biochemical analysis and is comparable to the liver section of standard SL treated groups (Figure 2C and 2F).

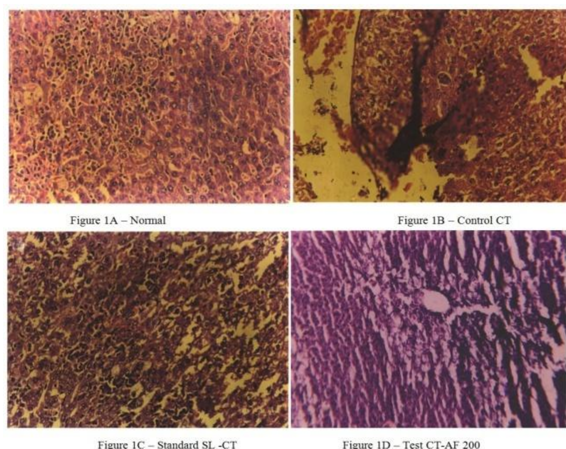

Fig. 1: Representative microphotographs for histopathological study of CT induced hepatotoxicity

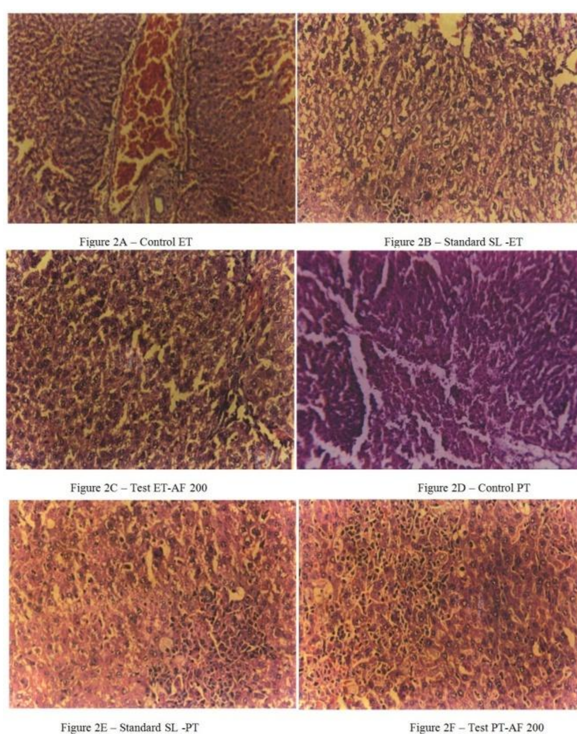

Fig. 2: Representative microphotographs for histopathological study of ET and PT induced hepatotoxicity

Sub acute toxicity study

Effect of AF on lipid profile in subacute toxicity study is shown in Table 3 (* marks indicate, p-value < 0.001). The extent of reduction in lipid profiles like TC, TG, HDL, LDL and VLDL at both the dose level (100 and 200 mg/kg) of test extract was dose dependent. In case of triglycerides (TG) level the test extract at tested dose levels and standard Silymarin (20 mg/kg) reduced significantly (p-value < 0.001) when compared with solvent control. However, the value of HDL is increased significantly (p-value < 0.001) at tested dose levels and standard SL treated group than that of solvent control, while the LDL & VLDL levels are lowered significantly (p-value < 0.001) in all the drug treated groups as compared to solvent control. The effect of AF on biochemical parameters in subacute toxicity study were given in Table 4. (Superscripts a, b and c denotes statistical significance at p-value < 0.001, p-value < 0.01 and p-value < 0.02 respectively).The test extract treated groups had shown reduced SGOT (p-value < 0.001 in case of standard SL and p-value < 0.02 in case of AF 200mg/kg), SGPT (p-value < 0.001) and ALP (p-value < 0.01) levels when compared with solvent control. The total protein content in serum was significantly lowered (p-value < 0.001) in all the drug treated groups as compared with solvent control. The value of albumin and globulin were decreased without any statistical significance as compared with standard SL treated group. The effect of AF on haematological parameters in subacute toxicity study was given in Table 5. (Superscripts "a" and "d" denotes statistical significance at p-value < 0.001, p-value < 0.05 respectively). The haematological parameters exhibited that the animals treated with standard drug silymarin and test extracts bear normal values in RBC count, WBC count and HB count, where as the clotting time is reduced significantly than normal values (p-value < 0.05). However animals treated with test extracts had shown a decreased value of WBC (p-value < 0.001) and increased HB content (p-value < 0.05) when compared with solvent control.

The neutrophil count appears to nearly equal with that of normal and solvent control values. The other haematological parameters like eosinophil, basophil, lymphocyte & monocytes in case of the standard drug and test extract treated were not significantly altered. Therefore, it might be suggested that the test extracts have not significant toxic effects on the biochemical parameters, haematological parameters and lipid profiles of rat and is evident for the safety use of the AF aqueous extract for a longer duration of time.

Table 3: Subacute toxicity study of AF (Effect on lipid profile)

Treatment	TC in mg/dl	TG in mg/dl	HDL in mg/dl	LDL in mg/dl	VLDL in mg/dl
Normal	140.15 ± 2.6	75.34 ± 2.59	52.5 ± 1.5	67.6 ± 1.3	16.15 ± 0.5
Solvent (2ml/kg)	241.15 ± 3.12	215.7 ± 5.2	32.6 ± 0.6	165.8 ± 3.9	44.25± 0.22
SL (20 mg/kg)	150. ± 2.7*	86.6 ± 2.55*	50.5 ± 1.25*	81.15 ± 2.5*	18.9 ± 0.5*
AF (100mg/kg)	220.9 ± 7.5*	118.6 ± 3.8*	35.75 ± 0.5*	155.9 ± 1.8*	25.3 ± 4.5*
AF (200mg/kg)	195.2 ± 3.5*	90.7 ± 3.12*	45.15 ± 0.45*	125.5 ± 1.29*	20.5 ± 0.35

* marks indicate, p-value < 0.001

Table 4: Subacute toxicity study of AF (Effect on biochemical parameters)

Treatment	SGOT IU/L	SGPT IU/L	ALP IU/L	TB mg/dl	DB mg/dl	Albumin gm/dl	Protein gm/dl	Globulin gm/dl
Normal	21.5 ± 2.7	28.1 ± 2.1	265.3 ± 10	0.95 ± 0.04	0.25 ± 0.06	3.25 ± 1.5	6.6 ± 0.15	1.5 ± 0.05
Solvent 2mg/kg	40.1 ± 2.45	50.3 ± 4.15	315.5 ± 10.05	1.2 ± 0.5	0.31 ± 0.02	5.11 ± 3.7	4.65 ± 0.15	1.0 ±0.35
SL (20 mg/kg)	24 ± 1.95[a]	30.35 ± 2.75[a]	262.7 ± 9.85[b]	0.7 ± 0.39	0.22 ± 0.02	9.25 ± 2.5	5.45 ± 0.15[a]	1.25 ± 0.35
AF (100mg/kg)	41.5 ± 3.1	32.5 ±2.5[a]	295.5 ± 6.1	1.41 ± 1.03	0.45 ± 0.06	4.45 ± 2.95	5.25 ± 0.15[a]	0.85 ± 0.1
AF (200mg/kg)	25.5 ± 3.5[c]	32.1 ± 3.15[a]	275.5 ± 8.1[b]	0.7 ± 0.03	0.21 ± 0.03	3.85 ± 1.41	5.45 ± 0.2[a]	1.5 ± 0.65

a denotes statistical significance at p-value < 0.001, b denotes statistical significance at p-value < 0.01, c denotes statistical significance at p-value < 0.02

Table 5: Subacute toxicity study of AF (Effect on haematological parameters)

Group & Treatment	RBC (million/ml)	WBC (Iwo/ml)	HB (g/dl)	Clotting Time (min)	Neutrophils (%)	Eosinophil (%)	Lymphocyte (%)	Monocyte (%)
Normal	4.5 ± 0.35	6.65 ± 0.27	11.23 ± 1.45	1.5 ± 0.25	28.5 ± 2.5	1.5 ± 0.95	68.0 ± 4.65	1.8 ± 0.89
Solvent (2ml/kg)	2.40 ± 0.11	7.5 ± 0.41	7.30 ± 2.25	1.5 ± 0.5	21.5 ± 1.15	4.5 ±1.1	72.25 ± 5.25	4.5 ± 1.5
SL (20mg/kg)	4.25 ± 0.25[d]	6.8 ± 0.3[a]	10.45 ± 3.15[d]	1 ± 0.45[d]	25.5 ± 2.75	1.5 ± 0.5	65.3 ± 5.5	2.2 ± 0.22
AF 100 ml/kg	3.25 ± 0.45	5.5 ± 0.26[a]	8.25 ± 3.4	2.15 ± 0.5[d]	15.5 ± 2.5	2.5 ± 0.2	80.5 ± 6.5	1.5 ± 0.12
AF 200kg/ml	3.65 ± 0.25	3.05 ± 6.4[a]	8.5 ± 2.5	0.45 ± 0.05[d]	32.5 ± 2.5	2.5 ± 0.3	65.4 ± 5.5	1.5 ± 0.4

a denotes statistical significance at p-value < 0.001, d denotes statistical significance at p-value < 0.05

CONCLUSIONS

In the present study successive extraction method was successfully explored for whole parts of the plant *Anoectochilus formosanus* and its hepatoprotective activity was studied against carbon tetrachloride, ethanol and paracetamol induced liver damage. Aqueous extracts of AF had shown the presence of anthraquinone glycosides, cardiac glycosides, reducing sugars, carbohydrates, phenolic compounds, tannins, flavonoids and saponins. Estimation of biochemical parameters like aspartate aminotransferase, alanine amino transferase and alkaline phosphatase were depicted that highest dose (200 mg/kg) of aqueous extract of *Anoectochilus formosanus* is indeed producing hepatoprotective activity comparable to that of standard silymarin at a dose of 20 mg/kg against carbon tetrachloride, ethanol and paracetamol induced hepatotoxicity. Equivalent hepatoprotective activity of *Anoectochilus formosanus* whole plant with standard silymarin had also been supported by histopathological study of liver sections. Sub acute toxicity studies of the *Anoectochilus formosanus* extract for 21 days had concluded that it might be considered safe for a longer duration of time as the above plant extracts have not significant toxic effects on the biochemical parameters, haematological parameters and lipid profiles of rat.

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