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PROTECTIVE EFFECT OF AQUEOUS EXTRACT OF *ANISOCHILUS CARNOSUS* WALL. IN CARBON TETRA CHLORIDE –INDUCED HEPATOTOXICITY IN ALBINO RATS

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ABSTRACT

The interest in traditional systems of medicine has rejuvenated in the recent times due to the increasing problems with drug resistant microorganisms, side effects of modern synthetic drugs and increasing number of emerging diseases with no proper medication is available in modern medicine. Curative, preventive and nutritive properties of the medicinal plants provide the necessary power and energy to fight the disease and assist the action of the restorative agents in the herbal drug. Liver is highly susceptible to the toxicity of the materials ingested. The role of the liver in maintaining and regulating the internal environment of the body makes it predisposed to such changes. Plants have been used in the treatment of liver diseases since the Vedic period. Hence the present study was designed to assess the hepatoprotective efficacy of a plant drug source *Anisochilus carnosus* Wall. which would provide an insight to the codified language of traditional medicine, paving way for a healthier world. Aqueous extract of *Anisochilus carnosus* was prepared using the protocol mentioned in Ayurvedic Formulary of India. The Wistar strains of albino rats used as the experimental models. Animals were divided into six groups each comprising of six rats each. Hepatotoxicity was induced using CCl_4. The selected plant drug was administered to the animals orally for a period of 21 days. Serum marker enzymes SGOT, SGPT, SALP & SGGT, Biochemical parameters-S.bilirubin, S.protein, hepatic glycogen, hepatic hydroxy proline and S.urea levels were assessed. The induction of liver injury with CCl_4 resulted in significant raise in the serum marker enzyme level, serum bilirubin, hepatic hydroxyl proline content and a decrease in serum protein, urea and tissue glycogen levelsThe biochemical picture and the marker enzyme levels were brought near normal on treatment with the aqueous extract of *Anisochilus carnosus.* The results obtained clearly portray the efficacy of *Anisochilus carnosus* against CCl_4 induced hepatotoxicity. The defensive activity may be attributed to the membrane stabilizing activity of the plant.

Keywords: Hepatotoxicity, *Anisochilus carnosus*, Hydroxyl proline, Carbon tetrachloride

INTRODUCTION

The interest in traditional systems of medicine has rejuvenated in the recent times due to the increasing problems with drug resistant microorganisms, side effects of modern synthetic drugs and increasing number of emerging diseases with no proper medication is available in modern medicine. Curative, preventive and nutritive properties of the medicinal plants provide the necessary power and energy to fight the disease and assist the action of the restorative agents in the herbal drug [1, 2]. Liver is the internal regulator of homeostasis and is highly susceptible to the toxicity due to the substances ingested. The role of the liver in maintaining and regulating the internal environment of the body makes it predisposed to such changes. Plants have been used in the treatment of liver diseases since the Vedic period [3]. Hence the present study was designed to assess the hepatoprotective efficacy of a plant drug source *Anisochilus carnosus* Wall., which would provide an insight to the codified language of traditional medicine, paving way for a healthier world.

*Anisochilus carnosu*s belongs to the family Lamiaceae (Mint family). It is an annual herb, found in the Western Ghats. Stems are erect, 30-60 cm tall, robust and branched [4]. The plant has been used for the treatment of gastrointestinal disorders, cough, cold, fever and ulcer [5]traditionally. Antiulcer activity of *Anisochilus carnosus* leaf extract in pylorus ligated rats has been reported [6]. Hence the present study focuses on the hepatoprotective activity of the aerial parts of the plant in CCl_4 induced toxicity.

MATERIALS AND METHODS

Collection of plant material

The aerial parts of *Anisochilus carnosu*s was collected in and around Trichy identified with the Flora of Presidency of Madras and authenticated with the voucher specimen deposited at the Rapinet herbarium of St.Joseph's college, Trichy.

Preparation of aqueous extract

The plant was shade dried and coarsely powdered. The powder was mixed though roughly with 6 times the volume of water and stirred continuously until the volume reduced to 1/3rd. The extract was filtered with muslin cloth. The residue was re extracted. The filtrate was mixed and evaporated in a water bath till it reaches a thick consistency. The extract was stored in refrigerator till further use.

Animal models

Wistar strains of Albino rats of both sexes weighing 150-200 g were selected and used for the present study. Animals were housed in well ventilated cages in the CPCSEA approved animal house. The protocol was approved by the Institutional Animal Ethics committee. Animals were allowed to take standard laboratory feed and water. They were acclimatized to the laboratory conditions for a week before experiments.

Experimental design

The rats were divided into 6 groups, consisting of six rats each. Group 1 served as the untreated normal control. Group 2served as disease control (Administered CCl_4 at a dosage of 0.5 ml/150 g bw in olive oil in the ratio1:1) orally for three days at the beginning of the experimental period [7]. Group 3,4 &5were induced with CCl_4 (at the above mentioned dose) and treated with the aqueous extract of *Anisochilus carnosu*s at a dose level of 100mg,200mg,300mg /kg bodyweight respectively. The plant extract was administered orally for 21 days. Group 6 was induced with CCl_4 (at the above mentioned dose) and treated with silymarin at a dose of 25mg/kg body weight.

At the end of experimental period the animals were sacrificed by cervical decapitation. The blood was collected and serum separated. The liver was washed in ice cold saline and homogenized. It was used for various experiments.

Biochemical parameters studied

Aspartate transaminase [8]

The assay mixture containing1ml of substrate and 0.2 ml of serum was incubated for 1 hr at 37ºC. To the control tubes serum was added after the reaction was arrested by the addition of 1 ml of DNPH.The tubes were kept at room temperature for 30 min. Added 0.5 ml of NaOH and the color developed was read at 540 nm. The

activity of AST was expressed as µmoles of pyruvate formed /min/mg of protein.

Alanine transaminase [8]

The assay mixture containing1ml of substrate and 0.2 ml of serum was incubated for 1 hr at 37ºC. Added 1 ml of DNPH and kept at room temperature for 20 min. Serum was added to the control tubes after the reaction was arrested by the addition of 1 ml of DNPH. Added 5 ml of NaOH and the color developed was read at 540 nm. The activity of ALT was expressed as µmoles of pyruvate formed /min/mg of protein.

Alkaline phosphatase[8]

The reaction mixture containing 1.5 ml carbonate buffer, 1 ml Di sodium phenyl phosphate, 0.1 ml Magnesium Chloride and 0.1 ml of serum was incubated at 37 ºC for 15 min. The reaction was arrested by the addition of Folin's phenol reagent. Control tubes were also treated similarly but serum was added after the reaction was arrested with Folin's phenol reagent. Added 1ml of Sodium Carbonate. The color developed was read after 10 min at 640 nm. The activity of ALP was expressed as µmoles of phenol liberated /min/mg of protein.

Gamma Glutamyl transferase [9]

The incubation mixture contained 0.5ml of substrate, 1ml of Tris Hcl, 2.2 ml of Glycyl glycine and 0.2 ml of homogenate.The total volume was made upto 4ml with water. After incubation for 30 min at 37 ºC the samples were heated at 100 ºC for 5 min and centrifuged. The amount of p-nitroaniline in the supernatant was measured at 410 nm.

Serum bilirubin [10]

For the determination of total bilirubin 0.2 ml of serum was taken and made up to 2 ml with water. Then added 0.5 ml of diazo reagent, 2.5 ml of methanol. To the blank 0.2 ml serum was added and made up to 2 ml with water and added 0.5 ml of diazo blank and 2.5 ml methanol. The color developed was read at 540nm. The values were expressed as mg/dl.

Total protein [11]

Aliquots of the suitably diluted serum (0.1ml to 10ml by two serial dilutions) was made up to 1.0 ml with water and 4.5 ml of alkaline copper reagent was added to all the tubes including blank, containing 1.0ml water and standards containing aliquots of standard BSA and made up to 1ml with water. The tubes were incubated for 10 min at room temperature. 0.5 ml was added to all the tubes and incubated for 20 min at room temperature. The blue color developed was read at 640nm. The protein content was expressed as g/dl.

Liver Glycogen [12]

A weighed amount of the tissue was subjected to alkali digestion in a boiling water bath for 20 min. The tubes were cooled and 3ml of absolute ethanol and a drop of ammonium acetate were added. The tubes were then placed in a freezer overnight so as to facilitate precipitation of glycogen. The precipitated glycogen was collected after centrifugation. The precipitate was washed thrice with alcohol and dissolved in 3 ml of water. Aliquotes were taken and made upto 1 ml with water. 4ml of anthrone was added to the tubes kept in an ice bath, mixed and heated in a boiling water bath for 20 min. The green colour developed was read at 640 nm.

Hepatic Hydroxy Proline [13]

Standards of varying concentrations were taken. Sample and standards were treated simultaneously. 1 ml of chloramines T was added. The contents of the tube were mixed thoroughly and allowed to stand for 20min at room temperature. Added 1ml of perchloric acid and incubated at room temperature for 5min. Finally added 1ml of PDAB. The colour developed was read at 557 nm.

Serum Urea [14]

To 0.1 ml of blood 3.3 ml of distilled water, 0.3ml of 10% sodium tungstate and 3.3 ml of 2/3 N H_2SO_4were added and centrifuged for few min at 3000rpm. To 2ml of the supernatant was added 2ml of distilled water, 0.4ml of DAM reagent and 1.6 ml H_2SO_4 - H_3PO_4 reagent. The tubes were incubated in a boiling water bath for 20 min. The pink colour developed was read at 480nm using a blank.

Stastical analysis

The data obtained was expressed as mean ± SEM. The data were subjected to one way ANOVA. The p value < 0.05 was considered statistically significant.

RESULTS

The reaction of the liver to the toxicity inducing agent and the plant drug source was evaluated and the results were tabulated in table 1,2 & 3.

Table 1: Effect of the aqueous extract of *A.carnosus* on the level marked enzymes in carbon tetrachloride intoxication

Gp/parameter	GOT (U/L)	GPT (U/L)	ALP (U/L)	GGT (U/L)
Normal	7.12 ± 0.28	12.40 ± 0.23	523.39 ± 0.96	31.46 ± 0.31
CCl4 control 0.5ml/ 150g	154.3 ± 2.41*	268.03 ± 1.8*	1002.46 ± 0.97*	66.93 ± 0.09*
ACAE100mg/kg	117.6 ± 1.21	186.33 ± 1.17	875.9 ± 0.71	54.86 ± 1.01
ACAE200mg/kg	47.7 ± 1.18	66.44 ± 0.56	754.54 ± 1.3	46.52 ± 0.89#
ACAE300mg/kg	13.1 ± 0.32	22.28 ± 1.24 #	635.55 ± 0.49	37.71 ± 1.31
Silymarin25mg/kg	7.67 ± 0.27	11.93 ± 0.49#	525.8 ± 4.31#	33.4 ± 1.4#

Data expressed as Mean ± SEM, n=6; P< 0.05, Enzyme activity expressed as * p<0.05 statistically significant when compared with normal control, # p< 0.05 statistically significant when compared with CCl_4 treated group

Table 2: Effect of the aqueous extract of *A.carnosus* on the serum protein, urea and bilirubin levels

Gp/parameter	S. Protein (mg/dl)	S. Urea (mg/dl)	S. Bilirubin (mg/dl)
Normal	6.95 ± 0.07	28.51± 0.26	0.95 ± 1.02
CCl4 control 0.5ml/ 150g	2.38 ± 0.08*	12 ±0.39*	5.29 ± 0.11*
ACAE100mg/kg	4.29 ±2.09	17.37 ± 0.58	3.88 ± 0.97
ACAE200mg/kg	3.43 ±0.02	24.47 ±1.44#	2.90 ±0.68#
ACAE300mg/kg	6.10 ± 0.11#	31.34 ±0.71	1.46 ±0.58
Silymarin25mg/kg	6.90 ± 0.12	30.17 ± 0.84#	1.01 ± 1.06#

Data expressed as Mean ± SEM, n=6; P< 0.05, * p<0.05 statistically significant when compared with normal control, # p< 0.05 statistically significant when compared with CCl_4 treated group

Table 3: Levels of hepatic glycogen and hydroxyl proline in the plant drug and disease control groups

Gp/parameter	Hepatic Glycogen (mg/g wet tissue)	Hepatic hydroxyl proline (mg/g wet tissue)
Normal	38.31± 0.85	3.09 ± 0.21
CCl4 control 0.5ml/ 150g	11.71 ± 0.42*	6.71 ± 0.15*
ACAE100mg/kg	24.38 ± 0.78	6.95 ±0.11
ACAE200mg/kg	32.05 ± 0.75#	5.95 ± 0.13
ACAE300mg/kg	34.22 ± 0.42	5.49 ± 0.13#
Silymarin25mg/kg	36.10 ± 0.96	3.73 ±0.17#

Data expressed as Mean ± SEM, n=6; P< 0.05, * p<0.05 statistically significant when compared with normal control, # p< 0.05 statistically significant when compared with CCl_4 treated group

DISCUSSION

The marker enzymes are located in the cytoplasm of the hepatocytes. The levels of these enzymes maintain a low circulating level. When a cell is damaged, the enzyme leaks into the blood, dramatically increasing their levels in the serum [15]. The cells lining the biliary ducts of the liver synthesise the enzyme Alkaline phosphatase (ALP). Bile duct obstruction or diseases of the liver result in an elevated level of ALP in the plasma. The increased activity of alkaline phosphatase, may be due to *de novo* synthesis by liver cells and is a reliable marker of hepatobiliary dysfunction. An elevation in the ALP levels along with an elevation in the GGT levels is a clear indication of a persisting liver damage. Measurement of these enzyme levels has proved to be powerful tools in the assessment of hepatotoxicity [16]. GPT and GOT serve as markers and their levels are high in the disease control groups. More than 10 fold increase in the activity of ALP with a consequent increase in the activity of GGT after CCl_4 intoxication clearly indicated severity of damage in liver. In the present study it was observed that the administration of the selected plant drug brought back the enzyme activities to near normal. (Table1) The results suggested the ability of the plant to contribute in the restoration of the marker enzymes by maintaining the structural integrity of the cellular membranes of the hepatocytes.

Liver plays a major role in the synthesis of amino acids and proteins. Hepatotoxins impair the ability of liver to synthesize protein. CCl_4 intoxication inhibits the addition of the amino acid to the growing polypeptide chain [17]. Due to the toxic effects of CCl_4 the number of viable hepatocytes available is less which in turn leads to a decrease in the synthetic function of the liver. In the present study also CCl_4 treated animals showed reduction in the total protein concentration in the serum due to impaired synthetic function of the liver indicating liver damage. On treatment with the aqueous extract of *A. carnosus* the protein levels were restored to normal, (Table 2) indicating the potential of the plant to regenerate viable hepatocytes to participate in the process of protein synthesis.

Bilirubin is synthesized in the liver. The increase in the levels of serum bilirubin reveals the intensity of liver disease. It is an important marker of hepatic functioning. An increase in the serum bilirubin indicates the inefficiency of the liver to conjugate bilirubin and excrete it into the bile. The hyperbilirubinemia may be due to the impaired hepatic uptake of the unconjugated bilirubin from the circulation [18]. In the present study the bilirubin concentrations were elevated in the disease control group (Table 2). The CCl_4 induction caused perivenular changes preventing the uptake of unconjugated bilirubin. The elevated levels of bilirubin is thus depicts the extent of liver damage. Administration of the aqueous extract of the selected plant drugs showed a significant reduction in the bilirubin levels thereby reducing the depth of the disease.

Transaminases are concerned with the inter conversion of a pair of amino acids and a pair of keto acids. The process is important because biosynthesis of urea depends upon the transamination, oxidative deamination, transport of ammonia and the reactions of the urea cycle. Ammonia synthesis an important function of the liver in the excretion of toxic material from the body requires glutamate as a source of nitrogen. Depletion in the transaminase levels in the hepatic tissue affects the synthesis of urea [19]. As indicated in Table 2, the present study also showed a decrease in the serum urea level in the group 2 animals which was restored to normal in the plant treated groups indicating the restoration of the normal liver functioning.

Liver is the metabolic seat of the body and is actively involved the synthesis and storage of glycogen. Glycogen is the major energy reserve of our body. Administration of toxins results in tissue necrosis and a greater rate of glycogenolysis [20]. The active hepatocytes which are involved in the synthesis of glycogen are reduced due to intoxication with CCl_4 which in turn leads to a significant reduction in the glycogen synthesis in the disease control group of the present study. A marked increase in the glycogen stores of the liver was noticed in the animals treated with the plant extracts (Table 3). The increase in the glycogen stores may be due to the capacity of the plants to rejuvenate the damaged tissue and thereby mounting the synthetic function of the liver.

A common pathological process of hepatic disease which can lead to irreversible cirrhosis, involving multiple cellular and molecular events is hepatic fibrosis. This ultimately results in the accumulation of collagen and extra cellular matrix protein. Oxidative stress plays an important role in the etiopathogenesis of hepatic fibrosis. As the fibrosis of the hepatic tissue progresses a proportionate increase in the hydroxy proline content of the liver is also noticed, an appropriate indicator of fibrosis [21]. The increase in the hydroxy proline content of the liver can be attributed to the reduced content of Cyt P450 in liver. During drug induced liver injury the levels of Cyt P450 reduces. As the damage progresses the solid liver tissue becomes fibrotic and hence the collagen content of the tissue gets hydrolysed to hydroxy proline thereby resulting in the accumulation of hydroxy proline. In the present study the CCl_4 induced groups showed an elevation in the hydroxy proline content On treatment the hydroxy proline content reduced, indicating the efficacy of the selected plant drugs due towards preventing degradation thereby arresting hepatic fibrosis (Table 3).

All the results obtained were in par with that of the silymarin treated animals. The group 6 animals also showed a marked decrease in the serum marker enzymes and serum bilirubin levels with a subsequent increase in the serum urea, protein, hepatic glycogen and hydroxyl proline levels.

CONCLUSION

The results obtained clearly portray the efficacy of *Anisochilus carnosus* against CCl_4 induced hepatotoxicity. The defensive activity may be attributed to the membrane stabilizing activity of the plant.

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