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Original Research Article

Hepatoprotective Activity of Methanolic Extract of Leaves Parts of *Chenopodium Album Linn* on Carbon Tetrachloride Induced Hepatotoxicity in Waster Rats.

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Abstract:
The liver is among the most complex and important organ in the human body. Its primary function is to control the flow and safety of substances absorbed from the digestive system before distribution of these substances to the systemic circulatory system. Carbon tetra chloride is well- known hepatotoxin which is widely used to induce hepatotoxicity in laboratory animals. *Chenopodium album Linn* also known as frost Bilte, Pigweed (Canada), found to have antipruritic and antinociceptic, sperm immoblizing agent, cryptomeridiol and 8- alpha-acetoxycryryptomeridiol as growth promoting activity, (patak and pandey) *Chenopodium album* has reported hepatoprotective activity, antifungal, antihelminitic, contraceptive, antipyretic and antinoceipetive activity. The present study was aimed to investigate hepatoprotective activity of alcoholic extract of leaves parts of *Chenopodium album Linn* on carbon tetrachloride induced hepatotoxicity in waster rats. The leaves of plant was dried under shed and then powdered. The Powder was packed into a Soxhlet apparatus, extract was concentrated in steam bath till the complete evaporation of the solvent. Administration of Carbon tetra chloride subcutanously (SC) in the lower abdomen, in a suspension of liq. paraffin (LP, 1:1 v\v) at the dose of 1ml\kg B.W. on alternate days of week showed significant increase in SGOT and SGPT. Methanol extract of *Chenopodium album Linn*. at dose of 200mg\kg B.W. P.O. (Group III) in a carbon tetra chloride treated groups cause significant decrease in SGOT and SGPT. Result of biochemical parameters of *Chenopodium album Linn.* (400 mg\kg B.W. P. O.), showed significantly arrestedin in rise of level of SGOT, SGPT was found in group IV. The standard drug Liv2R showed significant reduction in all parameters when compared to CCl_4 treated groups. This study concluded that methanolic extract of *Chenopodium album Linn.* has hepatotprotective effect in CCl_4 induced liver damage. This effect is evident especially with high dose of *Chenopodium album Linn.* (400 mg\kg B.W. p.o) because it caused drastic fall in serum markers as compared to other doses of Doses of *Chenopodium Album Linn.*
Abbreviations- serum glutamate oxaloacetatae transaminase (SGOT) and serum Glutamate Pyruvate Transaminase (SGPT)
Keywords- *Chenopodium album Linn*., methanolic extract, carbon tetrachloride (CCl_4), hepatotoxicity

1. INTRODUCTION

The liver is among the most complex and important organ in the human body. Its primary function is to control the flow and safety of substances absorbed from the digestive system before distribution of these substances to the systemic circulatory system[1]. A total loss of liver function leads to death within minute demonstrating the liver's importance. Carbon tetra chloride is well- known hepatotoxin which is widely used to induce hepatotoxicity in laboratory animals. The reactive metabolic tri-chloromethyl radical is formed from CCl_4 by the cytochrome p450 system. The free radical initiates the peroxidantion of membrane polyunsaturated fatty acids (PUFA), generates PUFA and covalently binds to microtonal lipids and proteins that finally result in cell necrosis and consequent cell death. Silymarin reduced oxidative stress and prevented liver necrosis in mice exposed to toxic agents[2]. Certain medicinal agents, when taken in overdoses (e.g. paracetamol) and sometimes even when introduced within therapeutic ranges (e.g. halothane), may injure the organ. Other chemical agents, such as those used in laboratories and industries, natural chemicals (e.g., microcystins), and herbal remedies (two prominent examples being kava, mechanism unknown, and comfrey, through its pyrrolizidine alkaloid content) can also induce hepatotoxicity[3]. More than 900 drugs have been implicated in causing liver injury (Zimmerman HJ (1999)) and it is the most common reason for a drug to be withdrawn from the market[4]. Hepatotoxicity and drug-induced liver injury also account for a substantial number of compound failures, highlighting the need for toxicity prediction models (e.g. DTI), and drug screening assays, such as stem cell-derived hepatocyte-like cells, that are capable of detecting toxicity early in the drug development process[5]. Chemicals often cause subclinical injury to the liver, which manifests only as abnormal liver enzyme tests. *Chenopodium album Linn* Synonyms- frost Bilte, Pigweed (Canada), Baconweed, Fat Hen, Bathuwa (Hindi), Chakwat (Marathi), Vastukah (sanskrti) and Pappukura (Telugu), found wild up to an altitiude of 4700 m and cultivated throughout India particularly Western Rajasthan, Kulu valley and Shimla. The species are cultivated as a grain or vegetable crop (such as in lieu of spinach), as well as animal feed in Asia (Gangaraju VK, Lin H (2009)) and Africa, whereas in Europe and North America, it is commonly regarded as a weed in places such as potato fields. *Chenopodium album* known as bathua sag are being used in traditional medicines. It has been found to have antipruritic and antinociceptic, sperm immoblizing agent, cryptomeridiol and 8- alpha-acetoxycryryptomeridiol as growth promoting activity, (patak and pandey) *Chenopodium album* has reported hepatoprotective activity, antifungal, antihelminitic, contraceptive, antipyretic and antinoceipetive activity[6-12].

2. MATERIALS AND METHODS

2.1 Plant material

The whole plant of *chenopodium album Linn*. was collected in the month of April from local area of Udaipur city (raj.) authenticated by rajasthan university botanical department (Jaipur). The plant authentification number is RUBL 211711 .

2.2. Plant extraction:-

The leaves of *Chenopodium album Linn*. plant was dried under shed and then powdered with a mechanical grinder and passed through sieve no. 40 and obtain a coarse powder. The Powder was packed into a Soxhlet apparatus and extracted with 100% Ethanol (60-800C) for 72 hrs. solvent was recovered by again using soxhelt apparatus. Lastly the extract was concentrated in steam bath till the complete evaporation of the solvent. The yield was 35.5% [13]

2.3. Experimental Animals:-

Albino rats (Wistar strain) either sex weighing between 175-225 g were used in the study. The animals were acclimatized for ten days under

standard laboratory condition. Animals were housed in polypropylene cages in controlled temperature (25+ 20C), relative humidity (60+5%) and 12:12 hrs. light\dark cycle. The animals were fed with rodent pellet diet and water ad libitum. The study protocol was approved from the Institutional Animal Ethics Committee (IAEC) before initiation of the experiments. The animlas were maintained as per CPCSEA guideline.

2.4. Determiniation of acute toxicity (LD50):-

The acute oral toxicity (AOT) of alcoholic extract of leaves parts of *Chenopodium album Linn* were determined by using female albino rats (Wistar strains), weighing between 180-220g. The animals were fasted 3 hrs prior to the experiment, up and down procedure (OECD Guideline no. 423). Animals were administered with single dose of extracts dissolved in DMSO and observed for its mortality during 48 hours study period (short term) toxicity. Based on short-term profile of drug, the dose of the next animals was determined as per as OESD guideline 423. All the animals were also observed for long term toxicity (14 days) . The LD50 of the test extract was calculated using AOT 423 guideline.[14]

2.5 Carbon tetrachloride induced hepatotoxicity:-

Liver toxicity was induced in rats by administrating CCl_4 subcutaneously (SC) in the lower abdomen, in a suspension of liquid paraffin (LP ; 1:1v\v) at the dose of 1 ml\kg body weight alternate days for one week. (Achilya et al.,2003). Rats were divided into I-V, each group consisting of six rats[15-18]:-

Group I of rats served as control and received subcutaneous administration of liquid paraffin (LP) only 1m\kg on alternate day for one week and vehicle (DMSO, 1 km\kg body weight) for two week orally. Group II (CCL_4 Control) rats were given CCl_4 subcutaneous in lower abdomen, in a suspension of liq. paraffin (1:1v\v) at the dose of 1ml\kg body weight on alternate days for a week and vehicle orally (DMSO, 1 ml\kg B.W.) for two week orally. Group III rats were given CCl_4 subcutaneous, in a suspension of liq. paraffin (1:1v\v) at the dose of 1ml\kg B.W. on alternate days for a week and *chenopodium album Linn*. (200mg\kgB.W.) for two week orally. Group IV rats were given CCl_4 subcutaneous, in a suspension of Liq. paraffin at the dose of 1ml\kg B.W. on alternate days for a week and *Chenopodium album Linn*. (400 mg\kg B.W.) for two week orally. Group V Rats were given CCl_4 subcutaneous, in a suspension of liq. paraffin at the dose of 1ml\kg B.W. on alternate days for a week and Liv 52R for two week orally.

2.6 Collection of blood.

Different doses of above mentioned drug, liquid paraffin and CCl_4 were administered to rats daily. On 15th daily the blood was collected from the rats by retro-orbital way and serum was separated by centrifugation and used for estimation of biochemical parameter[17-20].

2.7. Estimation of serum biochemical parameters- serum glutamate oxaloacetate transaminase (SGOT); Aspartate aminotransferse (AST); serum glutamate pyruvate transminase (SGPT); Alanine aminotransferase (ALT);

2.8. Estimation ofBiochemical parameters:- Cholesterol**,** serum alkaline phosphatise (SALP) **,** Bilirubin (direct and total)

2.9. Satistical analysis:-

The result were expressed as mean + SEM. The significance of difference between mean values for various treatment was tested using the impaired student "t" test. $p<0.01$ was considered as statistically significant. The data were subject to one-way analysis of variance (ANOVA).

3. **RESULT**

3.1. Determination of acute toxicity:-

Methanolic extract of *Chenopodium album Linn.* was given at dose of 5000 mg\kg, (P.O.). The extract was found to be non lethal (No mortality of the animals observed) at this given dose level. Hence, the extracts were found to be

safe up to the dose level of 5000 mg\kg. in this study the dose chose as 200mg\kg and 400mg\kg

3.2. Hepatoprotective activity:-

Administration of Carbon tetra chloride subcutanously (SC) in the lower abdomen, in a suspension of liq. paraffin (LP, 1:1 v\v) at the dose of 1ml\kg B.W. on alternate days of week showed significant increase in serum glutamate oxaloacetatae transaminase (SGOT) and serum Glutamate Pyruvate Transaminase (SGPT) as compared to group. Methanol extract of *Chenopodium album Linn*. at dose of 200mg\kg B.W. P.O. (Group III) in a carbon tetra chloride treated groups cause significant decrease in serum glutamate oxaloacetate transaminase (SGOT) serum Glutamate Pyruvate Transaminase. The result of biochemical paramenters of *Chenopodium album Linn.* (400 mg\kg B.W. P. O.) showed table 1-3 a significamt arrested rise in level of serum glutamate oxaloacetate transaminase serum Glutamate pyruvate transaminase (SGPT) was found in groupIV. Effect of *Chenopodium album Linn.* only for 15 days at moderate dose (400mg\kg B.W.P.O.) in group IV show remarkable changes in SGOT(235.7 U\L), SGPT (253.5 U\L), SALP(278.38U\L), cholesterol (140.8mg\dl), direct bilirubin (2.6mg\dl) and total bilirubin (6.7mg\dl), comparable to control group. The standard drug Liv2R showed significant reduction in all parameters when compared to CCl_4 treated groups.

Table : 1 Effect of *Chenopodium album Linn*. On serum biochemical parameters in Carbon tetrachloride hepatic damage in rat.

Groups	Treatment	Dose	SGPT (U\L+SEM)	SGOT (U\L+SEM)
1	Control	L.P 1ml\kg+vehicle	54.45+2.450	128.78+6.7
2	CCl_4 treated	1ml\kg	269.89+1.5+++	305.70+23.8+++
3	*Chenopodium album Linn.*+ CCl_4	200mg\kg+ 1ml\kg	255.60+3.57**	250.60+8.9***
4	*Chenopodium album Linn.*+ CCl_4	400 mg\kg+1ml\kg	253.45+4.5**	235.7+8.9**
5	LIV 52R+ CCl_4 treated	1ml\kg+1ml\kg	78.67+3.5*	207+9.6*

All values represented as Mean +SEM (n=6)
P value; *** <0.0001; **<0.001; * <0.01 when compared wit carbon tetra chloride group
P value; +++<0.0001; ++ <0.001; + <0.01 When compared with control group

Table 2: Effect of *Chenopodium album Linn.* On serum biochemical parameters in Carbon tetrachloride hepatic damage in rates.

Groups	Treatment	Dose	Cholesterol(mg\dl+SEM)	Alkaline
1	Control	L.P. 1mlg\kg vehicle	114.4+3.68	212+2.7
2	CCl_4Treated	1ml\kg	175.5+5.3+++	512.36+4.4+++
3	*Chenopodium album Linn.*+ CCl_4	200mg\kg+1ml\kg	152.37+5.2**	496.60+2.6**
4	*Chenopodium album Linn.*+ CCl_4	400mg\kg+1ml\kg	140.6+5.2**	476.36+4.2*
5	LIV 52R+ CCl_4 Treated	1ml\kg+1ml\kg	123.4+4.8*	222+4.3*

All values represented as Mean+SEM (n=6)
P value; *** < 0.0001; ** <0.001; * <0.001 when compared with carbon tetra chloride group
P value; +++ < 0.0001; ++ <0.001; + < 0.01 when compared with control group.

Table 3: Effect of *Chenopodium album Linn.* On serum biochemical parameters in Carbon tetrachloride hepatic damage in rats.

Groups	Treatment	Dose	Total bilirubin (mg\dl+SEM)	Direct bilirubin (mg\dl+SEM)
1	Control	L.P 1ml\kg+ vehicle	1.4+0.19	0.25+0.09
2	CCl_4 Treated	1ml\kg	4.25+0.14+++	1.57+0.33++
3	*Chenopodium album Linn*.+ CCl_4	200 mg\kg+1ml\kg	3.78+0.18***	1.1+0.08***
4	*Chenopodium alubm Linn*.+ CCl_4	400 mg\kg+1ml\kg	2.8+0.18***	0.67+0.09**
5	LIV 52R+ CCl_4 Treated	1ml\kg+1ml\kg	1.9+0.19***	0.28+0.05**

All Values represented as Mean+SEM(n=6)
P value; *** < 0.0001; ** < 0.001; * < 0.01 when compared with carbon tetra chloride group
P value; +++ <0.0001; ++<0.001; + < 0.01 when compared with control group.

4. Discussion

Liver is an important organ actively involved in metabolic function and is a frequent target of number of toxicants. Liver generate reactive oxygen species that induce oxidative tissue damage. These radical, which react with cell membranes and thus induce lipid peroxidation or cause inflammation, have been implicated as important pathological mediators in many clinical disorders such as heart disease, diabetes gout and cancer. A major defence mechanism is the antioxidant enzymes, which convert active oxygen molecule into non toxic compounds.

Hepatotoxicity implies chemical-driven liver damage. The liver plays a central role in transforming and clearing chemicals and is susceptible to the toxicity from these agensts. Certain medicinal agents when taken in overdoses and sometimes even when introduced within therapeutic ranges may injure the organ. Other chemical agents such as those used in laboratory and industries, natural chemicals (e.g. microsystins) and herbal remedies can also induce hepatotoxicity.

Liver diseases are remaining as one of the serious health problem. However we do not have satisfactory liver protective drugs in allopathic medical practices for serious liver disorders. Herbal drugs plays a role in the management of various liver disorder most of which speed up the natural healing processes of the liver.

Numerous medicinal plants and their formulation are used for liver disorders in ethanomedical practice as well as traditional system of medicine in India. More than 15 of these plants are evaluated for their hepatoprotective action in the light of modern medicine.

Hepatotoxicity is the major effect of exposure to carbon tetrachloride by any route in humans and animals. The hepatotoxic effect of CCl_4 is largely due to its active metabolite, tri-chloromethyl radical (free radical). These activated radicals bind covalently to the macromolecules and induce peroxidative degradation of membrane lipids of endoplasmic reticulum rich in poly unsaturated fatty acids. This leads to the formation of lipid peroxides, which in turn gives products like melon-di-aldehyde (MDA) that cause damage to membrane.

In this study CCl_4 toxicity elevated the levels of enzymes serum glumate oxaloceatate transaminase (SGOT), serum glutamates pyruvate transaminase (SGPT) and serum alkaline phosphatase (SALP). Biochemical parameters cholesterol, direct bilirubin and total bilirubin are sign of liver injury.

Chenopodium album Linn. is used as antipruritic, antinociceptic and sperm immobilizing agent etc. This study, observed that administration of 200 mg and 400 mg\kg B.W. p.o of *Chenopodium album Linn.* arrested in rise in serum glutamate oxalocetate transaminase (SGOT), , serum glutamates pyruvate transaminase (SGPT), and serum alkaline phosphatase (SALP) , cholesterol,

direct bilirubin andtotal bilirubin in a dose depended manner (table 5-7) by the CCl_4 induced elevated enzymes levelsin treated groups, it indicates antihepatotoxicity activity

The result of biochemical paramenters of *Chenopodium album Linn*. (400 mg\kg B.W. P. O.) showed in table 1-3 a significant arrested in rising level of serum glutamate oxaloacetate transaminase serum Glutamate pyruvate transaminase (SGPT) was found in groupIV.

Effect of *Chenopodium album Linn.* only for 15 days at moderate dose (400mg\kg B.W.P.O.) in group IV show remarkable changes in SGOT(235.7 U\L), SGPT (253.5 U\L), SALP(278.38U\L), cholesterol (140.8mg\dl), direct bilirubin (2.6mg\dl) and total bilirubin (6.7mg\dl), comparable to control group.The standard drug Liv2R showed significant reduction in all parameters when compared to CCl_4 treated groups

5. CONCLUSION

It is concluded that ethanolic extract of *Chenopodium album Linn.* has hepatotprotective effect in CCl_4 induced liver damage. This effect is evident especially with high dose of *Chenopodium album Linn.* (400 mg\kg B.W. p.o) because it caused drastic fall in serum markers as compared to other doses of Doses of *Chenopodium Album Linn.* Treatment of diseases associated with the liver is very vital, and must be done with importance and extensive care. Many herbal remedies for liver diseases are known but only a few of them have been pharmacologically assessed for their efficacy.

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