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HEPATOPROTECTIVE ACTIVITY OF TUBEROUS ROOT OF *IPOMOEA DIGITATA* LINN. AGAINST CARBON TETRACHLORIDE INDUCED HEPATOTOXICITY.

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ABSTRACT

Hepatitis is major health problem in human being due to various chemical including therapeutic agent and environmental toxin and this can leads to illness like jaundice, which sometime may lead to even death. *Ipomoea digitata* Linn. is used in ayurvedic formulation for various disease conditions and commonly known as "Vidarikanda". The present study deals with hepatoprotective activity hydroethanolic extract of tuberous root of *Ipomoea digitata* Linn. against CCl_4 (i.p) induced liver damaged in female Wister rat. The drug was extracted and preliminary phytochemical screening of extract was done then extract was subjected to toxicity study. The hydroethanolic extract of tuberous root of *Ipomoea digitata* Linn. was administered orally at the dose of 250mg/kg and 500mg/kg .These shows significant protective effect on liver evidenced by lowering serum level of ALT, AST, ALKP, TB, DB, and Serum Triglyceride and also by histopathological study. All result obtain result are compared with standard (Silymarin) and control group. Thus it concludes that hydroethanolic extract of *Ipomoea digitata* Linn. shows significant hepatoprotective activity prevents chemically induced hepatic damaged in rat.

INTRODUCTION: The liver, in vertebrate body perform many vital function, including metabolic and detoxification activities. A number of chemical agent and routine drug produced cellular as well as metabolic liver injury, therefore the most drug withdrawn from market [1]. Hence several hundreds of plants are examine for use in wide variety of liver disorder.

The tuberous root of *Ipomoea digitata* Linn. is belonging to family Convolulaceae. Traditionally in ayurveda this plant is used as tonic, aphrodisiac, galactogogue, immunomodulator [2]. These drug also shows good antidiabetic and anti inflammatory activity [3]. Phytochemical investigation of hydroethanolic extract showed presence of alkaloids, flavonoids, glycoside, saponins, and hence, present study deals with hepatoprotective activity of hydroethanolic extract of *Ipomoea digitata* Linn. against CCl_4 induced hepatotoxicity.

MATERIAL AND METHOD:

Plant material: Dried tuberous root of *Ipomoea digitata* Linn. belonging to family Convolulaceae commonly known as Vidarikanda, this plant was collected in month of August 2010 from Santosh Ayurvedic supplier, Mazzid Bandar, Mumbai. The plant material was taxonomically identified by the Dr. Ganesh Iyer, Department of life science, Ramnarayana Ruia College, Mantunga, Mumbai, and Maharashtra, India. The sample specimen was preserved in our laboratory. Dried roots of *Ipomoea digitata* Linn. was washed thoroughly in tap water, shade dried and powdered when passed through 40 mesh sieve.

Preparation of extract: 100 gm of the dried and pulverized tuberous root was under goes hydroethanolic (1:1) extraction in soxhlet apparatus for 36 hrs. The solvent was removed from extract under vacuum rotator dryer. Then finally dried extract was used for assessment of phytochemical screening and in vivo activity.

Animal: Female Wister albino rat, weighing 150-200g maintain under standard husbandry condition were used for all experiment. Animal allowed feed on standard laboratory feed and water. The protocol no.- RP-94 was approved by animal ethical committee constituted for the purpose at National Toxicological Center, Pune and all study was carried out at NTC, Pune.

Chemical: Carbon tetrachloride was procured from SD Fine Chemical Ltd. (Mumbai), Silymarin was obtained as gift sample from Micro Lab. All the biochemical parameters are estimated by using kits of Crest biosystems. All other chemical used are of analytical grade.

Toxicity studies: Acute oral toxicity was performed using female mice as per OECD (Organization for economic co-operation and development) Guideline 423. The most widely used parameter for determination of acute toxicity is LD_{50}. The acute oral toxicity test aims at establishing the therapeutic index, which is the ratio between the pharmacologically effective dose and lethal dose on the same strain and same species.

CCl_4 induced hepatotoxicity [4]: The present study was carried out to evaluate the prophylactic effect of extract against CCl_4 induced liver injury. Female Wister albino rat were divided into give group of animal containing six in each group were used for study.

Group I- (Vehicle Control): received 5ml/kg water per oral (p.o) for 8 days and olive oil (0.5ml/kg) i.p on 7^{th} day, 30 min after administration of water.

Group II- (CCl4 Control): received 5ml/kg water per oral (p.o) for 8 days and CCl_4 (1ml/kg) in olive oil (1:1) i.p on day 7^{th}, 30 min after administration of water.

Group III- (Standard control): received 25mg/kg of Silymarin once a day orally for 8 days and CCl_4

(1ml/kg) in olive oil (1:1) i.p on day 7th, 30 min after administration of Silymarin.

Group IV- (Test- 1): received 250mg/kg of extract once a day orally for 8 days and CCl_4 (1ml/kg) in olive oil (1:1) i.p on day 7th, 30 min after administration of extract.

Group V- (Test- 2): received 500mg/kg of extract once a day orally for 8 days and CCl_4 (1ml/kg) in olive oil (1:1) i.p on day 7th, 30 min after administration of extract.

On 9th day was sacrificed by decapitation under Urethane anesthesia and blood sample was collected. The blood sample were kept at room temperature for 1 hr for clotting, serum was separated by centrifuged at 2500 rpm at room 37°C for 15 mins and following biochemical parameter were estimated [4].

1. Alanine aminotransferase [5]
2. Aspartate aminotransaminase [6]
3. Alkaline phosphates [7]
4. Direct bilirubin [8]
5. Total bilirubin [8]
6. Serum triglycerides [9]

Histopathological observation: Histopathology of liver was carried out by modified luna [15]. Liver tissues collected were used for the preparation of histopathological slide by using microtome. The CCl4 induced histopathological changes in liver were confirmed. Silymarin and test extract reversed the liver to normalcy (**Fig. 1**).

Statistical analysis: Results are expressed as mean ±SEM for six rats in each group. Total variation present in set of data was estimated by one-way analysis of variance (ANOVA) followed by Dunnett's test.

RESULT:

Toxicity study: The LD_{50} determination was done in female mice as per OECD guideline 423 and LD_{50} of *Ipomoea digitata* Linn. was determined. The oral administration of hydroethanolic extract of tuberous root caused neither any behavioral change nor up to 2000mg/kg, so LD_{50} of *Ipomoea digitata* Linn. was found to be more than 2000mg/kg (**Table 1**).

TABLE 1: LD_{50} VALUE OF *IPOMOEA DIGITATA* LINN.

No. of Swiss albino Mice	Dose (mg/kg)	Acute oral toxicity study- 24 hrs*	Acute oral toxicity study -14 days*
3	175	0	0
3	550	0	0
3	1250	0	0
3	2000	0	0

*(X= died, 0= survival)

Since drug was non toxic, then dose of drug selected was 250mg/kg and 500mg/kg.

Phytochemical investigation: The phytochemical investigation of extract *of Ipomoea digitata* Linn. shows presence of resin, carbohydrate, tannin, alkaloid, saponins and flavonoids in acetone, ethanolic, chloroform and hydroethanolic extract. Hydroethanolic extract shows presence of most of these compounds. Hence hydroethanolic extract were selected for these study [10] (**table 2**).

TABLE 2: PHYTOCHEMICAL INVESTIGATION OF DIFFERENT EXTRACTS OF *IPOMOEA DIGITATA* Linn.

Name of Extract	Acetone Extract	Chloroform Extract	Ethanol Extract	Hydroethanolic Extract
Alkaloid's	+	-	+	+
Carbohydrate's	-	-	+	+
Glycoside's	-	-	-	+
Saponins	-	-	-	+
Fat and Oil's	-	-	-	-
Phytosterols	-	+	+	+
Resin's	-	-	+	+
Flavonoids	-	-	+	+
Tannin's	-	-	-	+
Protein's	-	-	+	+

+ = Presence; - = Absence

Biochemical Parameter: Carbon tetrachloride in rat showed a significant hepatic damaged as observed from elevated serum level of hepato- specific enzyme as well as sever alteration in different liver parameter. The group treated with hydroethanolic extract of *Ipomoea digitata* Linn. showed significant decrease in level of various liver biochemical parameters like ALT, AST, ALP, TB, DB and Serum Triglycerides as compared to CCl_4 treated group of animal. The higher dose (500mg/kg) of extract showed maximum protection against CCl_4 toxicity of liver (**table 3 & 4**).

TABLE 3: EFFECT OF HYDROETHANOLIC EXTRACTS OF *IPOMOEA DIGITATA* LINN. ON LIVER WEIGHT AND LIVER VOLUME

	Liver weight (gm)	Liver volume (ml)
Vehicle Control	7.71±0.35[c]	6.26±0.23[c]
CCl_4 Control	11.56±0.22[c]	10.13±0.21[c]
Silymarin (25mg/kg)	8.21±0.32[c]	7.21±0.18[c]
Test 1 (250mg/kg)	9.91±0.26[c]	8.28±0.27[c]
Test 2 (500mg/kg)	9.78±0.19[c]	7.58±0.18[c]

n= 6 animal in each group. Values are expressed as mean ± SEM. One way ANOVA followed by Dunnett's test when compared with CCl4 control [a]p<0.05, [b]p<0.01, [c]p<0.001

TABLE 4: EFFECT OF HYDROETHANOLIC EXTRACT OF *IPOMOEA DIGITATA* LINN. ON BIOCHEMICAL PARAMETERE IN CCl_4 INDUCED HEPATIC INJURY

Treatment	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	DB (mg/dl)	TB (mg/dl)	Serum Triglyceride (mg/dl)
Vehicle Control	72.33±3.42[c]	55.16±5.26[c]	81.00±7.72[c]	0.13±0.05[c]	0.65±0.05[c]	61.16±4.02[c]
CCl_4 Control	176.16±5.13	155.0±9.37	249.66±8.87	1.66±0.08	4.03±0.15	133.16±6.51
Silymarin (25mg/kg)	91.31±2.81[c]	72.83±7.81[c]	114.0±4.74[c]	0.56±0.10[c]	1.67±0.11[c]	81.83±4.24[c]
Test 1 (250mg/kg)	116.83±4.57[c]	107.50±8.77[c]	196.16±8.96[c]	1.04±0.06[c]	2.96±0.20[c]	111.33±3.62[b]
Test 2 (500mg/kg)	105.16±1.90[c]	92.66±6.17[c]	136.0±7.42[c]	0.94±0.08[c]	1.96±0.14[c]	93.83±2.08[c]

n= 6 animal in each group. Values are expressed as mean ± SEM. One way ANOVA followed by Dunnett's test when compared with CCl4 control [a]p<0.05, [b]p<0.01, [c]p<0.001

Histopathological studies: The CCl_4 induced histopathological changes in liver were confirmed, silymarin and *Ipomoea digitata* Linn. reversed the liver to normalcy (**fig. 1**). Histological examination of liver Vehicle control rat showed normal hepatic cell, central vein, sinusoids with normal texture (**fig. 1 a**). Disarrangement of normal hepatic cells with centrilobuler necrosis, vacuolization of cytoplasm and fatty degradation was observed in CCl_4 intoxicated animal (**fig. 1 b**). The liver section of rat treated with silyamarin and test extract observed regeneration of hepatic cell, central vein, and nucleus (**fig. 1 c-e**).

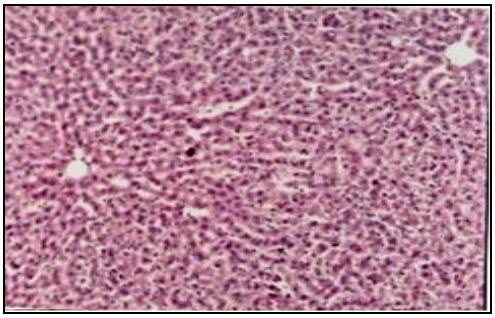

FIG. 1A: VEHICLE CONTROL RAT

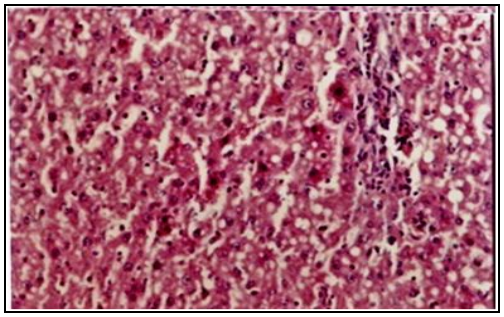

FIG. 1B: CCL_4 TREATED RAT

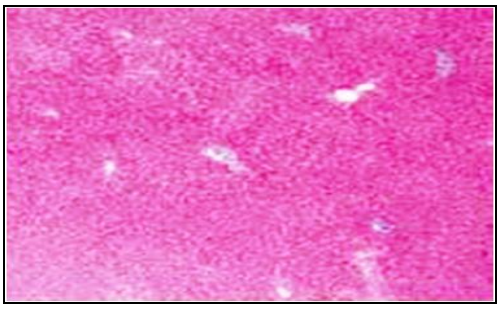

FIG. 1C: SILYMARIN (25MG/KG

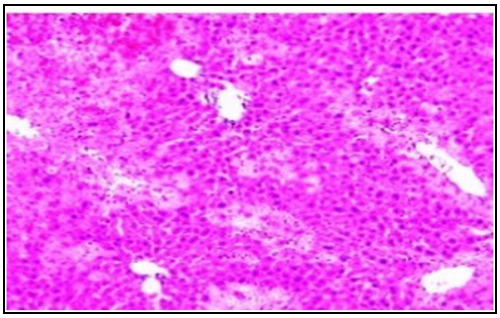

FIG. 1D: TEST 1 (250MG/KG)

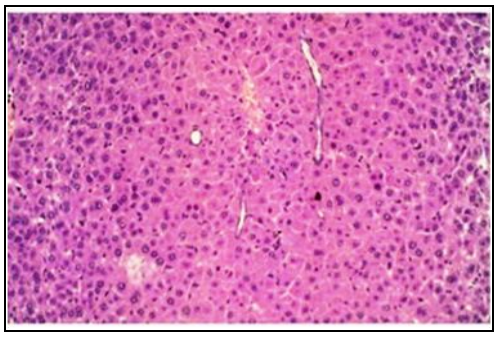

FIG. 1E: TEST 2 (500MG/KG) TREATED RAT

DISCUSSION: Liver damage induced by CCl_4 is commonly used model for screening of hepatoprotective activity of drug [11]. By using hepatic cytochrome p450s, CCl_4 is converted into reactive metabolite. The lipid peroxidative degeneration of biomembrane is one of the most important caused of CCl_4 toxicity. This evident from an elevation in marked enzyme level like ALT, AST, ALP, Bilirubin both direct and total and also serum triglycerides [12].

The hydroethanolic extract of tuberous root of *Ipomoea digitata* Linn. administered prophylactically exhibited significant protection against CCl_4 induced liver injury, which is confirmed by reduction in toxin mediated rise in serum level of various biochemical liver parameters in rats. The qualitative phytochemical investigation of different extracts of *Ipomoea digitata* Linn. showed positive on test for carbohydrates, triterpenes, steroids, tannins and flavonoids. The hydroethanolic extract of *Ipomoea digitata* Linn. showed positive for flavonoids by $ZnCl_2$, alkaline reagent and shinoda test, further it has been reported that flavonoids constituents of plant posses antioxidant activity properties [13] and it was found to be useful in treatment of liver damaged [14, 15].

CONCLUSION: Hydroethanolic extract of tuberous roots of *Ipomoea digitata* Linn. showed significant hepatoprotective activity against CCl_4 induced hepatic damaged. It is worthwhile to isolate the bioactive principle which are responsible which are responsible for hepatoprotective activity. These finding are important from clinical point of view and further studies to revel mechanism of action. It also indicate the necessity of explore experimentally similar other herbal plant described in ancient texts.

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