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Lipotropic activity of Natchol, a polyherbal formulation in albino Wistar rats

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Abstract:

The present study evaluates the lipotropic activity of Natchol, a polyherbal formulation, on carbon tetrachloride (CCl_4) induced fatty liver in male albino Wistar rats. Fatty liver was induced by intraperitoneal injection of CCl_4 at 0.1 ml/kg b.w. on 7th and 8th day of treatment. Natchol was administered orally at 100 mg/kg b.w. while choline chloride was given at the dose of 200 mg/kg b.w. for seven days prior to CCl_4 treatment. The administration of Natchol and choline chloride was continued till 72 hours after the second dose of CCl_4. Natchol treatment significantly reduced ($p \leq 0.05$) the hepatic triglycerides level and ameliorated the reduction in weight gain induced by CCl_4. The histopathological examination of liver further corroborated the lipotropic activity of Natchol and it was found to be comparable with that of choline chloride, the positive control. Thus the present study revealed the lipotropic effect of Natchol in hepatic steatosis.

Keywords: Lipotropic activity, Choline chloride, Natchol, *Solanum nigrum, Citrullus colocynthis, Bacopa monnieri, Sida cordifolia, Boerhaavia diffusa.*

1. Introduction

Choline, formerly known as Vitamin B_4, is an essential factor in the normal development and health of animals, especially for the metabolism of fat. It is a major dietary source of methyl group and is a precursor for biosynthesis of membrane phospholipids like phosphatidylcholine, sphingomyelins and lysophosphatidylcholine and neurotransmitter acetylcholine [1, 2]. Thus choline plays an important role in many physiological processes.

In poultry, the choline deficiency is characterized by fatty degeneration of the liver and kidney, reduced growth rate, perosis, decrease in egg production and hatchability. In swine, the deficiency results in reduced growth rate, uncoordinated movements, reduced number of total live piglets per litter, fatty degeneration of the liver [3]. Due to these, the commercial practical diets for poultry and swine must be supplemented with choline and the most common source of choline for poultry and swine diets is choline chloride, available commercially. Choline is oxidised in mitochondria to betaine aldehyde and further to betaine. Betaine is also used as a feed supplement which acts as a methyl donor and ameliorates pathologic states

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induced by reductive and oxidative stress. The experimental studies have shown that the lipotropic agents like choline and betaine can effectively prevent or treat hepatic steatosis.

Numerous medicinal plants and their formulations are used for liver disorders in ethnoveterinary practice as well as in traditional system of medicine in India. It has been demonstrated that indigenous medicinal plants like *Solanum nigrum, Citrullus colocynthis, Bacopa monnieri, Sida cordifolia* and *Boerhaavia diffusa*, possess hepatoprotective, free radical scavenging and hypolipidemic activities [4-10].

Natchol, a polyherbal formulation, containing the above mentioned herbs as the major ingredients is expected to have choline and/or betaine like lipotropic activity and claimed to replace the feed additive choline chloride, in the poultry diet. Hence, the present study was undertaken to evaluate the above assumptions using appropriate scientific model.

Carbon tetrachloride, chlorinated methane which causes liver injury, has become an important experimental hepatotoxin over the past 50 years. The effects of CCl_4 on hepatocytes, depending on dose and duration of exposure, are manifested histologically as hepatic steatosis (e.g., fatty infiltration), centrilobular necrosis and ultimately cirrhosis [11]. In this study, CCl_4 has been used to induce fatty liver and to study the lipotropic effect of Natchol in adult male albino Wistar rats.

2. Materials and Methods

2.1 Plant material and chemicals

Natchol (*Batch No.:* 66*; Date:* 24/12/04) containing sun dried powder of *Solanum nigrum* Linn. (F: Solanaceae), *Citrullus colocynthis* (L.) Schrad (F: Cucurbitacea), *Bacopa monnieri* (L.) Pennel (F: Scrophulariaceae), *Sida cordifolia* Linn. (F: Malvaceae) and *Boerhaavia diffusa* Linn. (F: Nyctaginaceae) as the major ingredients, was manufactured by Natural Remedies Pvt. Ltd., Bangalore, India. CCl_4 was purchased from Ranbaxy laboratories Ltd, India.

2.2 Animals

Thirty two male albino Wistar rats (180-230 g) were procured from central animal facility of Natural Remedies Pvt. Ltd. and housed four animals per cage with free access to pelleted feed and UV purified and filtered tap water *ad libitum*. Paddy husk was used as the bedding material. The animals were acclimatized to 12 hour light-dark cycle and were maintained under standard laboratory conditions. The animals were randomly allotted to four groups of 8 each and treated as follows:

Group I : Vehicle control (0.5% carboxy methyl cellulose)

Group II : CCl_4 control

Group III : CCl_4 treatment + Choline chloride at 200 mg/kg b.w.

Group IV : CCl_4 treatment + Natchol at 100 mg/kg b.w.

The animals were given test substance/vehicle once a day orally for 11 days. The body weight and feed intake were recorded daily during the treatment period. All the animals except those from group I were injected with CCl_4 at 0.1 ml/kg b.w. intraperitoneally on 7^{th} and 8^{th} day of the experiment following 2 hours of test substance administration. Seventy two hours after the last dose of CCl_4 (11^{th} day), the animals were sacrificed.

2.3 Body weight gain, feed intake and liver weight

The body weight and feed intake of the experimental animals were recorded and percentage body weight gain was calculated before and after CCl_4 treatment. The absolute liver weight was recorded on the day of sacrifice and relative liver weight was calculated.

Table 1: Effect of Natchol on mean body weight (g)

Treatment Groups	Day of experiment					
	Day 1	Day 7	% gain	Day 11	% gain	Overall % gain
Vehicle Control	203.63 ± 4.44	215.88 ± 8.26	6.02	238.13 ± 6.20	10.31	16.94
CCl_4 control	203.13 ± 4.09	230.13 ± 5.52	13.29	230.00 ± 6.35	-0.05	13.23
Choline chloride	205.13 ± 4.58	229.63 ± 3.96	11.94	234.38 ± 4.77	2.07	14.26
Natchol	203.38 ± 5.27	226.88 ± 5.11	11.55	239.63 ± 5.57	5.62	17.82

Values are expressed as mean ± SEM; n=8.

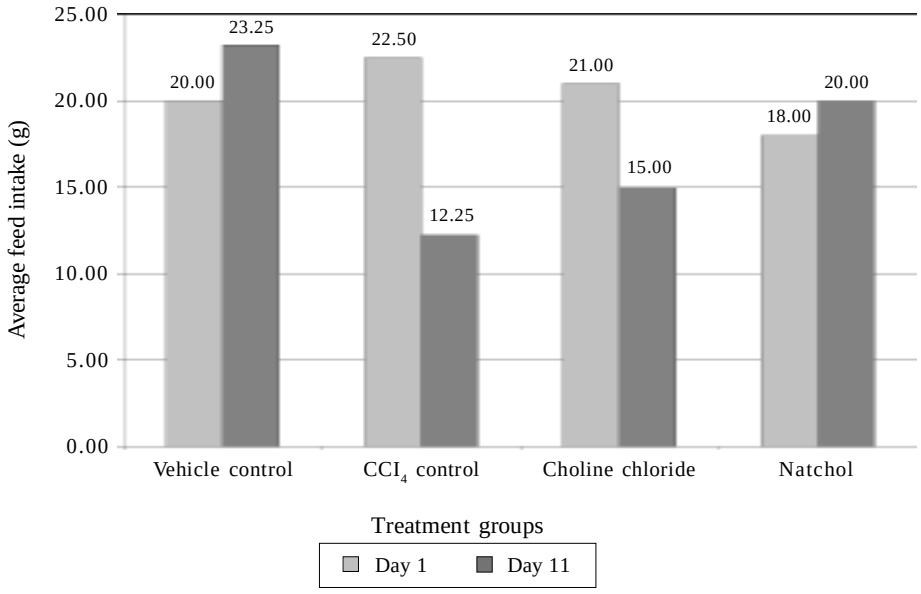

Fig. 1.
Effect of Natchol on average feed intake (g)

Table 2.
Effect of Natchol on relative liver weight and hepatic lipid profile

Treatment Groups	Relative Liver weight (g/100 g b.w)	Triglycerides (mg/g liver)	Phospholipids (mg/g liver)
Vehicle control	3.95 ± 0.21	6.45 ± 0.68	5.25 ± 0.44
CCl_4 Control	3.89 ± 0.19	15.05 ± 0.58#	7.50 ± 0.25#
Choline chloride	3.90 ± 0.15	8.44 ± 0.80*	8.03 ± 1.07
Natchol	3.95 ± 0.12	8.10 ± 0.55*	7.53 ± 0.67

Values are expressed as mean ± SEM; n=8.

$p \leq 0.05$ compared to vehicle control

* $p \leq 0.05$ compared to CCl_4 control

Table 3.

Intensity of fatty changes on histopathological observation of liver after treatment

Treatment	Intensity of fatty changes	
Groups	Nil to Mild	Moderate to Severe
Vehicle control	8	0
CCl_4 Control	4	4
Choline chloride	6	2
Natchol	6	2

Nil ⇒ No fatty changes in liver parenchyma.

Mild ⇒ Presence of few fat cells around the central vein and in liver parenchyma.

Moderate ⇒ Presence of small fat cells as patches around the central vein and in liver parenchyma.

Severe ⇒ Presence of bigger fat cells as patches around the central vein and in liver parenchyma, sometimes presence of vacuolated cells also.

2.4 Hepatic lipid measurements

The total hepatic lipids were extracted from samples of liver kept under deep freezing using chloroform ($CHCl_3$): methanol (2:1) [12]. Triglycerides and phospholipids were estimated using GPO-PAP and ammo-nium molybdate methods respectively.

2.5 Histopathology

A portion from each lobe of the liver was preserved in 10% neutral buffered formalin and embedded in paraffin and stained using hematoxylin and eosin (H and E). The steatosis, inflammation and necrosis in the liver tissues were evaluated under light microscopy [13]. The hepatic changes were graded histologically according to the following criteria.

Normal (0) : Nil - No fatty changes in liver parenchyma.

Grade 1 (+) : Mild - Few small fat cells around the central vein and in liver parenchyma.

Grade 2 (++) : Moderate - Small fat cells as patches around the central vein and in liver parenchyma.

Grade 3 (+++) : Severe - Bigger fat cells as patches around central vein and in liver parenchyma, sometimes presence of vacuolated cells.

2.6 Statistical analysis

The data were analyzed using one way ANOVA and Dunnet's T3 method as the post-hoc test. All values were reported as mean ± SEM. Statistical signifi-cance was set at $p \leq 0.05$.

3. Results

3.1 Body weight gain, feed intake and liver weight

The experimental animals exhibited normal body weight gain prior to CCl_4 challenge. After CCl_4 administration, the weight gain was lowered in all the CCl_4 treated animals. However, choline chloride and Natchol administration ameliorated reduction in weight gain induced by CCl_4 (Table 1). There was no significant variation in feed intake (Fig. 1) and relative liver weight (Table 2) among different treatment groups.

3.2 Hepatic lipid measurements

Natchol showed significant reduction in the liver triglycerides level as compared to the CCl_4 control group. The phospholipid content among different treatment groups did not show any significant variation (Table 2).

3.3 Histopathology

Under light microscopy, liver section from CCl_4 control group showed marked fatty changes, cell swelling, biliary hyperplasia in addition to lymphocyte and neutrophil infiltration at moderate to severe degree. In vehicle control group, there were no pathological changes

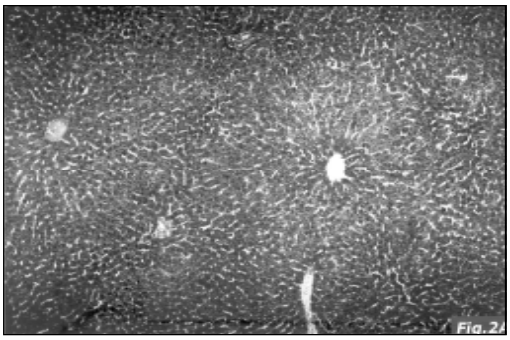

Fig. 2 A.

Liver section of vehicle control rat showing normal liver architecture (H & E Stain, 10X).

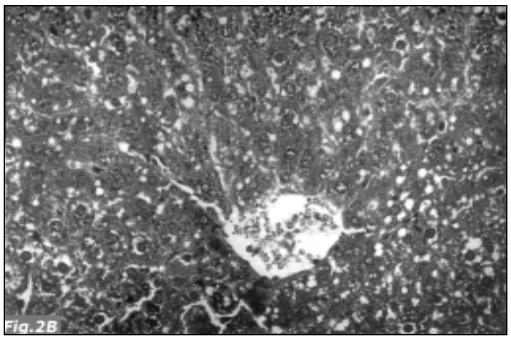

Fig. 2 B.

Liver section of CCl_4 control rat showing moderate to severe degree of steatosis (H & E Stain, 40X).

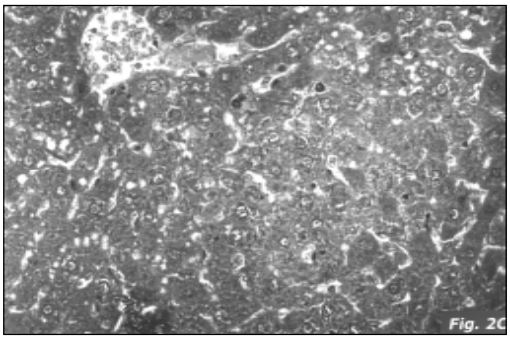

Fig. 2 C.

Liver section of CCl_4 + Choline chloride treated rats showing mild to moderate degree of steatosis (H & E Stain, 40X)

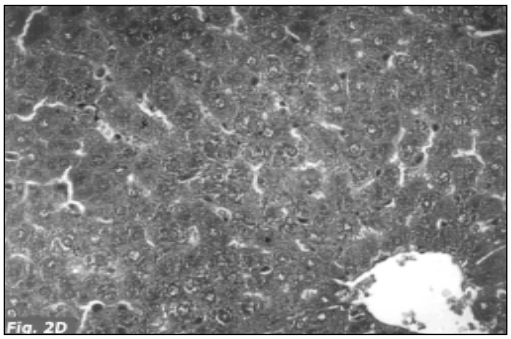

Fig. 2 D.

Liver section of CCl_4 + Natchol treated rats showing mild degree of steatosis (H & E Stain, 40X)

whereas the choline chloride and Natchol treated groups showed alleviated fatty changes from severe to mild or moderate degree (Table 3 and Fig. 2).

4. Discussion

It is well known that hepatic triglycerides accumulation, resulting in fatty liver, occurs in rats [14, 15]. Fatty liver (hepatic steatosis) is a common clinical finding observed by accumulation of lipid droplets within individual hepatocytes, which is characterized by steatosis and inflammation, may progress to necrosis and ultimately fibrosis and cirrhosis [13].

The fatty liver caused by the administration of CCl_4 in rats is believed to be associated with a block in the passage of triglycerides from the liver to the plasma and marked fall in the rate of formation of the protein moiety in the liver [16-18].

The inhibition of the synthesis of plasma-lipoproteins after administration of CCl_4 is of particular interest since it may explain the subsequent changes in the concentrations of lipid in the liver. Lipoproteins being the carrier for lipids from the liver, any reduction in their formation will result in a failure to remove lipid

from the liver at a normal rate and may hence be the cause for the subsequent increase in concentrations of lipid in the liver [19].

In the present study, CCl_4 treatment has reduced body weight gain, feed intake and induced fatty liver as evidenced by significant increase in liver triglycerides as compared to the vehicle control. The histopathological observation further corroborated the above findings.

Choline is a major dietary lipotrope essential for the metabolism of fat and in the absence of choline, triglycerides get accumulated in the liver and may result in fatty liver [20]. Choline chloride is a common feed supplement in poultry diet [3].

Betaine which is a metabolite of choline is rapidly absorbed and utilized as an osmolyte. It acts as a source of methyl group, there by helps to ameliorate pathologic changes induced by reductive and oxidative stress [21]. Betaine can replace choline chloride in its lipotropic function [22] and has shown to reduce liver necrosis induced by CCl_4 in mice after oral treatment with betaine [23].

The lipotropic effect of betaine, choline and methionine as well as their roles in transmethylation reactions are already well proven [11]. The findings of the present study revealed that, Choline and Natchol have a preventive effect on the development of hepatic steatosis as evidenced by reduction of hepatic triglycerides level from that of CCl_4 control.

The above treatments also ameliorated the reduction in weight gain induced by CCl_4 administration. A comparative histopatholo-gical observation of the liver from different groups further supported the lipotropic activity of the above treatments. According to histological observations, CCl_4 treatment has induced moderate to severe degree of fatty changes, infiltration of inflammatory cells, swelling of hepatocytes and biliary hyperplasia that were found to be in concordance with that reported in literature [24].

Moreover, the animals treated with Natchol showed nearly normal hepatic architecture with mild to moderate degree of fatty changes along with minimal inflammatory changes indicating the preventive effect on hepatic steatosis.

Similarly choline chloride which served as positive control also prevented the intensity of fatty changes to a milder degree, showing the lipotropic activity. Thus, the present study indicated the lipotropic effect of Natchol and choline chloride by alleviating the hepatic lipidosis induced by CCl_4 in male albino Wistar rats.

In the present study, Natchol seems to have better lipotropic activity than choline chloride. Therefore, further studies are required to elucidate the exact mechanism by which Natchol produces the lipotropic activity. In conclusion, the results showed a remarkable lipotropic effect of Natchol in CCl_4 induced hepatic steatosis.

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