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Indian Journal of Pharmacology 2003; 35: 308-311 **SHORT COMMUNICATION**

HEPATOPROTECTIVE ACTIVITY OF PANCHAGAVYA GHRITA AGAINST CARBONTETRACHLORIDE INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT

Objective: To investigate the hepatoprotective activity of Panchagavya Ghrita (PG) against CCl_4 induced hepatotoxicity.

Methods: The hepatoprotective activity of PG was tested against carbontetrachloride induced hepatotoxicity in albino rats. The degree of protection was determined by measuring levels of serum marker enzymes like serum glutamate oxaloacetate transaminase (SGOT) serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP) and acid phosphatase (ACP). The histological studies were also carried out. Silymarin was used as the standard drug for comparison.

Results: Administration of Panchagavya Ghrita (150-300 mg/kg, *p.o.*) markedly prevented CCl_4 induced elevation of levels of serum GPT, GOT, ACP and ALP. The results are comparable to that of silymarin. A comparative histopathological study of liver exhibited almost normal architecture, as compared to control group.

Conclusion: Treatment with Panchagavya Ghrita significantly reduced the CCl_4 induced hepatotoxicity. A comparative histological study of liver from different groups further confirmed the hepatoprotective activity of Panchagavya Ghrita.

KEY WORDS Marker enzymes CCl_4 liver enzymes

INTRODUCTION

Panchagavya is a term used in Ayurveda to describe five important substances obtained from cow namely urine, dung, milk, ghee and curd. A number of formulations mentioned in Ayurveda describes the use of Panchagavya components either alone or in combination with drugs of herbal, animal or mineral origin[1]. Recently US Patent was obtained by CSIR, India which claimed a novel pharmaceutical composition containing cow urine distillate and an antibiotic[2]. Cow urine concoction (CUC) is a popular Nigerian herbal preparation containing cow urine. Over fifty components have been identified in CUC. Its major pharmacological actions include anticonvulsant and hypoglycemic effects[3]. Fulzele *et al.* reported immunostimulant activity of Ashtamangal ghrita[4], immunomodulatory activity of Haridradi ghrita[5] and antiinflammatory activity of Jatyadi ghrita[6].

Panchagavya ghrita (PG) is also one of the formulations mentioned in Ayurveda which is prepared with all five components of Panchagavya *viz* cow milk, ghee, urine, dung and curd in equal proportions and is claimed to be useful against liver disorders, fever, inflammations, anemia and also as a rejuvenator[7]. However, scientific data are not available regarding pharmacological aspects of Panchagavya ghrita, nor are there any scientific data, which could corroborate the claims. Much work has not been carried out on biochemical, pharmacological and pharmaceutical aspects and also on chemistry of bioactive compounds in Panchagavya. Therefore it was thought worthwhile to investigate hepatoprotective activity of Panchagavya ghrita against CCl_4 induced hepatotoxicity.

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MATERIALS AND METHODS

Panchagavya ghrita (PG): The formulation was obtained as a gift sample for research from Go-Vigyan Anusandhan Kendra, Deolapar. The formulation was used as received for the present investigation.

Quality control parameters: The quality control parameters of PG were recorded by methods specified by Bureau of Indian Standards[8]. The parameters calculated were slip point, acid, free fatty acid, saponification, peroxide, iodine and ester values. The formulation appeared reasonably stable at room temperature.

Animals: Male albino rats of Sprague Dawley strain weighing 150-200 g were used for the study. The animals were housed in clean metabolic cages and maintained in controlled temperature (27±2°C) and light cycle (12 h light and 12 h dark). They were fed with standard pellet diet (Goldmohor brand, Lipton India Ltd.) and water *ad libitum*. The protocol was approved by Institutional Animal Ethics Committee constituted for the purpose.

Experimental: Rats were divided into 5 groups of 6 animals each as follows - Group I served as control and received subcutaneous administration of liquid paraffin (LP) only (3 ml/kg) on alternate day for one week. Liver damage was induced in remaining groups by administering CCl_4 subcutaneously in the lower abdomen in a suspension of LP in the ratio 1:2 *v/v* at the dose of 1 ml CCl_4/kg body weight of each animal. Group II rats received LP+CCl_4 on alternate days for a week by subcutaneous route. Group III received silymarin (100 mg/kg, *p.o.*) daily and LP + CCl_4 subcutaneously on alternate days for a week. Group IV and V were fed orally with Panchagavya ghrita (150 and 300 mg/kg respectively) daily and LP+CCl_4 (*s.c.*) on alternate days for a week. The doses of Panchagavya ghrita were selected on the basis of doses prescribed by expert Ayurvedic physicians and in Ayurvedic texts. On 8th day blood was withdrawn from all groups and serum separated for estimating the levels of marker enzymes *viz* glutamate oxaloacetate transaminase (GOT)[9] glutamate pyruvate transaminase (GPT)[9], alkaline phosphatase (ALP)[10] and acid phosphatase (ACP)[10].

After sacrificing the animals, the liver was rapidly excised and serially sectioned. The tissue was fixed in 10% formalin and consecutive sections were stained by haematoxylin and eosin for histological examination.

Table 1. Quality control parameters of Panchagavya ghrita.

Slip point	37.0 - 37.5°C
Acid value	2.06 ± 0.0035
Free fatty acid value	1.03 ± 0.0018
Saponification value	98.33 ± 1.147
Peroxide value	4.365 ± 0.048
Iodine value	35.55 ± 0.14
Ester value	96.275 ± 1.15

The values are mean of 5 readings ± SD.

Statistical analysis: The data obtained were analyzed using one-way ANOVA followed by Dunnett's test. The level of significance was set at $P < 0.05$.

RESULTS

Quality control parameters: The analysis of the formulation reveals the values (Table 1) for the respective parameters.

Serum marker enzymes: The level of marker enzymes *viz* - SGPT, SGOT, ACP and ALP were elevated in CCl_4 treated group as compared to control (Table 2). The group treated with PG showed significant decrease in the levels of serum marker enzymes as compared with CCl_4 treated group.

Histopathology: The CCl_4 induced histopathological changes in liver were confirmed. Silymarin and PG reversed the liver to normalcy (Figure 1a-d).

DISCUSSION

Rapid loss of a variety of microsomal enzymic activities accompany peroxidative decomposition of lipids of liver cell endoplasmic reticulum. Disintegration of lysosomes has been correlated with peroxidative decomposition of lysosomal lipids. Peroxidative decomposition of hepatocellular membrane lipids result in breakdown of the delicate and complex mechanisms responsible for formation of VLDL in the cell, their movement within the cell and eventual extrusion into the plasma. This supposition

Table 2. Effects of Panchagavya ghrita on different biochemical parameters in the serum of rats.

Treatment		GPT (units/ml)	GOT (units/ml)	Total acid phosphatase ACP (K.A. units)	Alkaline phosphatase ALP (K.A. units)
Control group		32.83± 4.88	46.33±9.72	4.16 ±0.25	11.12 ±0.53
CCl_4		126.33± 6.88#	114.33±6.88#	12.25 ±0.32#	21.21 ±0.53#
Silymarin (100 mg/kg orally) +CCl_4		47.00± 6.16*	59.33±6.31*	6.34 ±0.27*	13.86 ±0.50*
PG (150 mg/kg, orally)+CCl_4		111.50± 7.79*	86.66±11.36*	10.7 ±0.59*	18.96 ±0.54*
PG (300 mg/ kg, orally)+CCl_4		84.6± 6.31*	78.83±3.96*	7.77 ±0.41*	15.78 ±0.58*
One-way ANOVA	F	226.97	65.27	423.83	240.56
	P	<0.0001	<0.0001	<0.0001	<0.0001

Values are expressed as mean±SD of 6 animals in each group; df=4,25
#P<0.01 as compared with group I; *P<0.01 as compared with group II (Dunnett's test).

Figure 1. Effect of Panchagavya ghrita treatment on CCl_4 induced histopathological changes in rat liver.

Figure 1a. Normal control rat: Section of liver showing normal hepatic cells.

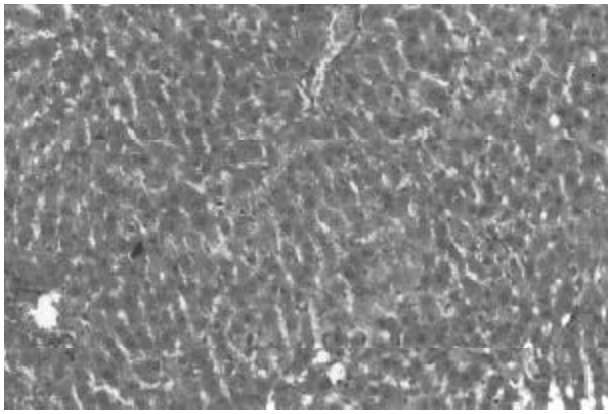


Figure 1b. CCl_4 treated rat: Section of liver showing centrilobular fatty degeneration, coludy swelling and necrosis of hepatic cells.

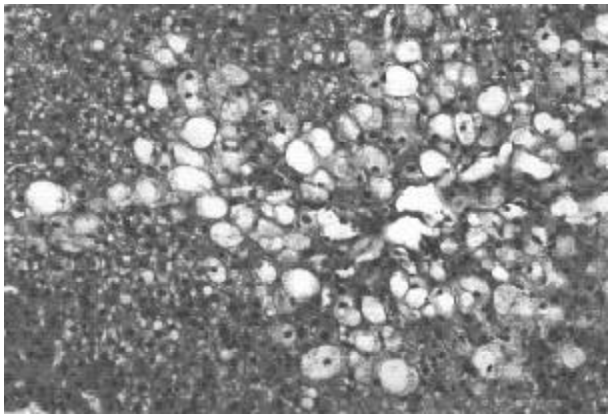


Figure 1c. Silymarin treated rat: Section of liver showing normalcy of hepatic cells, central vein and portal triad.

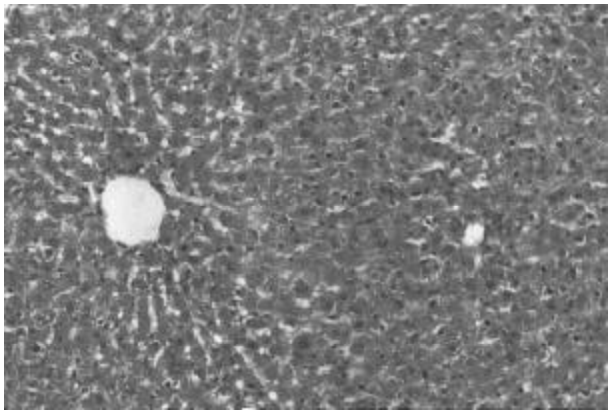
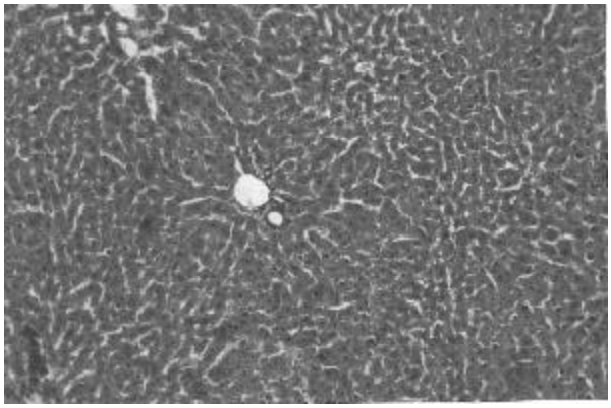


Figure 1d. Panchagavya ghrita (300 mg/kg) treated rats: Section of liver showing normalcy of hepatic cells.



is central to the lipoperoxidation theory of CCl_4 liver damage[11,12]. Carbon tetrachloride is biotransformed by cytochrome P-450 system to produce the trichloromethyl free radical which causes lipoperoxidation. The administration of the hepatotoxicant, CCl_4 increases the serum level of marker enzymes GPT, GOT, ACP and ALP indicating the induction of hepatotoxicity. Measurement of serum enzyme levels has provided a powerful tool for studies of hepatotoxicity. It has been employed in testing for toxicity of agents whose effects are unknown and in studying the circumstances and factors which influence the effects of known hepatotoxicants like CCl_4[13,14]. Treatment with PG in doses (150-300 mg/kg) significantly prevented the rise in the levels of serum marker enzymes *viz* GPT, GOT, ACP and ALP and the results obtained were comparable with those of silymarin treated group. The repair mechanisms are influenced by phospholipids coupled with a rise in the thymidylate synthetase and thymidine kinase levels in liver reaching a peak at 72 h indicating liver regeneration[15]. Both the damage and recovery proceeded simultaneously. However it is inhibited by repeated doses of CCl_4[16]. The comparative histo-pathological studies of liver from different groups further corroborated the hepatoprotective efficacy of PG. It would be reasonable to lay down the quality control parameters for such product since there is lack of standardized methodology in modern literature.

These findings are important from clinical point of view and need further studies to reveal the mechanism of action. It also indicates the necessity to explore experimentally similar other formulations described in ancient texts.

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