

Hepatoprotective Effect of Sarvakalp Kwath Against Carbon Tetrachloride Induced Hepatic Injury in Albino Rats

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ABSTRACT

Modern medicine, even in the modern era, lacks effective medications in its armamentarium which could be useful to treat or prevent hepatic ailments. However, Indian Ayurvedic system claims to have many indigenous plants and herbal preparations which provide therapeutic benefit in the treatment of liver diseases. One of such herbal preparations claiming to be of benefit in liver diseases is Sarvakalp Kwath by Divya Pharmacy, Haridwar. This study was undertaken to explore the hepatoprotective effects of Sarvakalp Kwath in animal model using albino rats. The animals were administered aqueous extract of the preparation in the dose of 120 mg/kg for seven days followed by carbon tetrachloride (CCl₄) challenge. Animals were then sacrificed. Hepatic damage and protection from the damage was assessed by biochemical parameters viz. alanine transaminase, alkaline phosphatase, total bilirubin and albumin as well as histological studies. It was observed that aqueous extract of Sarvakalp Kwath offered significant hepatoprotection as reflected by improvement in all biochemical and histological parameters as compared to saline controls. In conclusion, Sarvakalp Kwath offers significant hepatoprotection as is observed in this study. Further studies are warranted to establish its therapeutic efficacy in humans.

Key words: Hepatoprotective, Sarvakalp, Kwath, CCl₄, Divya Pharmacy, Ayurveda

INTRODUCTION

Liver is the largest gland of the human body and also the seat of metabolism for endogenous as well as xenobiotics. The fact that the liver is constantly exposed to xenobiotics, which also include harmful entities, makes it vulnerable to ailment. Although, despite having a remarkable reserve and regeneration capacity, continued insult to the liver by toxins, alcohol, infectious agents or cardiac decompensation result in liver disease.

Despite gigantic strides have been taken in the field of modern medicine, unfortunately, no significant drug has yet been developed for liver diseases.

The indigenous system of Ayurvedic medicine claims to have a wealth of solutions for

the liver problems. Various indigenous plants have been scientifically screened for hepatoprotective activity and quite a few have been found to possess significant activity in this regard^{1,2,3,4,5}. Also, there are some formulations which have been established as hepatoprotective agents both in experimental as well as clinical studies. An outstanding example of one such formulation is Liv.52, a product of The Himalaya Drug Company⁶.

One of such formulations to claim hepatoprotective action is "Sarvakalp Kwath" (Divya Pharmacy, Haridwar). Up till now, no scientific study has been performed to evaluate these claims. Therefore, this study was undertaken to explore the hepatoprotective activity of this formulation in animal models with experimentally induced hepatotoxicity.

MATERIALS AND METHODS

Study Drug

Sarvakalp Kwath is a proprietary preparation of Divya Pharmacy, Haridwar. It is available as a crude powder and is claimed to be beneficial for liver disorders. Each 5g powder contains:

- *Boerhavia diffusa* 2.50g
- *Phyllanthus urinaria* 1.25g
- *Solanum nigrum* 1.25g

The instructions for consumption are as follows

Boil 5-10g powder in 400 ml water till only 100 ml volume of the mixture remains. Filter and consume.

Sarva Kalp Kwath was procured from Divya Pharmacy authorized vendor. Working on the same lines as instructions on the Sarvakalp Kwath pack (which leads to the preparation of an aqueous extract), aqueous extract of the preparation was obtained using Soxhlet extraction apparatus followed by evaporation of the extract over water bath to obtain a solid product which served as the test compound.

Experimental Animals

The study was undertaken after due permission of the institutional animal ethics committee.

Wistar strain albino rats of either sex and weighing 150-200g were utilized for the main study while acute toxicity study was carried out in female rats only of the same strain and same weight range.

The animals were housed in cages under controlled conditions of temperature (25°C) and alternating 12 hour cycle of light and darkness and the animals had free access to standard rat pellet diet (Lipton India Ltd.) and tap water *ad libitum*.

After one week of acclimatization, the animals were considered suitable for study.

Acute Toxicity Study

The acute toxicity study was performed according to the Organisation for Economic Co-operation and Development (OECD) guideline 425.

A dose limit of 2000 mg/kg of the test compound was administered in five healthy female adult Wistar rats. Rats were fasted overnight from food, but not water, prior to dosing and weighed before the extract was administered orally. A dose of 2000 mg/kg was given to one animal, and this rat was observed for mortality and clinical signs (behaviours: unusual aggressiveness, unusual vocalisation, restlessness, sedation and somnolence; movements: twitch, tremor, ataxia, catatonia, paralysis, convulsion, fasciculation, prostration and unusual locomotion) for the first hour, then hourly for 3 h and, finally periodically until 48 h. If the animal survived, then four additional animals were given the dose 2000 mg/kg sequentially at 48 h intervals. All of the experimental animals were maintained under close observation for 14 days, and the number of rats that died within the study period was noted. The LD50 was predicted to be greater than 2000 mg/kg if three or more rats survived⁷.

Study Groups

These animals were divided into three groups of six animals each.

Group-I: This group was given normal saline, once daily, per orally for seven days followed by 1 ml/kg intraperitoneal injection of normal saline.

Group-II: This group was given normal saline, once daily, per orally for seven days followed by 1 ml/kg of a 50% v/v solution of Carbon tetrachloride (Nice Chemicals Pvt. Ltd., Cochin) in olive oil intraperitoneally.

Group-III: This group received the test compound in dose of 120 mg/kg, once daily, per orally for seven days followed by carbon tetrachloride intraperitoneally as in Group-II.

Per oral administration of normal saline or the test compound were carried out by gavage method with animals fasted 3-4 hours prior and 1 hour after administration to ensure proper absorption. Intraperitoneal normal saline (Group I) or carbon tetrachloride (Groups II, and III) dose was given concomitantly with the last (seventh day) corresponding oral dose of the group (normal saline for groups I and II; test compound for group III). Food was completely restricted after the corresponding intraperitoneal administration in each group. Animals of all the groups were fasted for 24 hours

(during which duration water remained freely available) after which they were sacrificed under Ketamine (75 mg/kg) and Diazepam (10 mg/kg) anaesthesia given intraperitoneally⁸.

Blood was collected from the anaesthetized animals from retro-orbital plexus. After blood collection, the animals were sacrificed to obtain the liver.

Biological Study Parameters

After a standing time of half an hour, the collected blood was centrifuged at 2500 rpm for 10 min. The serum so obtained was used to estimate the biochemical study parameters viz. Alanine transaminase (ALT), Alkaline phosphatase (ALP), Total bilirubin and Albumin which were all estimated spectrophotometrically via commercially available reagent kits based on established methods^{9,10,11,12}.

The liver was excised from the animals and washed with the normal saline. About one cm piece was cut and fixed in 10% neutral formalin for 12-24 hours. It was then dehydrated and cleared with ethanol and xylene respectively followed by embedding in paraffin wax from which blocks were prepared. Sections of 5m thickness were taken from the blocks using a microtome¹³. These were processed in alcohol-xylene series and were stained with Harris haematoxylin and eosin stain¹⁴ and subjected to histopathological examination.

Statistics

Mean \pm SD was calculated for each group to observe the general trend of the group. Wilcoxon Rank Sum Test was applied to test the significance of the results. P-values were estimated by referring to appropriate tables.

RESULTS

No symptoms or signs of toxicity or death were observed in the experimental rats in the acute toxicity study. Hence the LD50 of aqueous extract of Sarvakalp Kwath was considered to be greater than 2000 mg/kg.

In this study, Group I provided the baseline values of the studied parameters viz. normal biochemical parameters (Table 1) and normal histological study (Fig 1). In Group II, which was challenged with CCl₄ without any hepatoprotection, extensive hepatic injury was observed. With the exception of albumin, all the biochemical parameters were significantly raised. The histological study of Group II confirmed hepatic damage wherein classical centrilobular hepatic necrosis was observed (Fig 2). The Group III, which was challenged with CCl₄ after one week administration of Sarvakalp Kwath, showed evidence of hepatic injury as compared to Group I. However, the extent of derangement of biochemical parameters was significantly lower as compared to Group II and this was statistically significant with p-value less than 0.01 (Table 1). The histological study of Group III showed a remarkable level of hepatoprotection potential of Sarvakalp Kwath from the CCl₄ assault (Fig 3). No necrosis was observed and the extent of liver injury was limited to fatty changes in the hepatocytes. Normal architecture of the liver was largely preserved.

DISCUSSION

Liver has a pivotal role in regulation of several vital body functions such as metabolism, storage, secretion etc. Liver diseases are common

Table. 1: Biochemical parameters in the studied groups

	ALT (IU/L)	ALP (IU/L)	Total Bilirubin (mg/dl)	Albumin (g/dl)
Group I (Saline control)	20.69 \pm 5.94	78.89 \pm 12.54	0.15 \pm 0.17	5.24 \pm 0.13
Group II (Saline + CCl ₄)	428.78 \pm 40.49 [^]	602.37 \pm 34.19 [^]	1.76 \pm 0.69 [^]	5.20 \pm 0.12
Group III (Test compound + CCl ₄)	173.28 \pm 13.04 [*]	253.70 \pm 14.23 [*]	0.51 \pm 0.10 [*]	5.17 \pm 0.12

[^] p<0.01 as compared to group I

^{*} p<0.01 as compared to group II

and usually come to be noticed when they are well advanced. In spite of the tremendous advances made in modern medicine, no effective hepatoprotective medicine has yet emerged.

There are numerous plants and polyherbal formulations in ayurvedic system of medicine which have been claimed to have hepatoprotective actions and many of these have been proved by scientific studies as well. Most

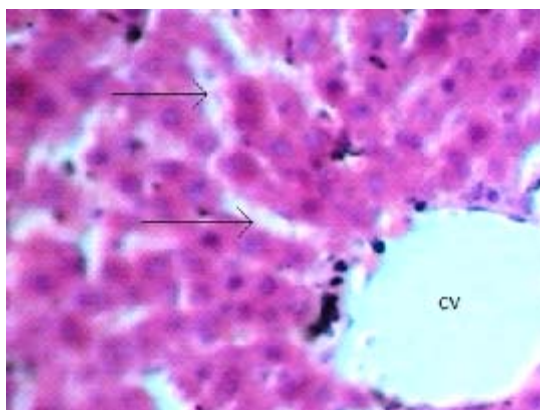


Fig. 1: Group I (Saline control) – Microscopic features of liver. Normal hepatic study. Hepatocytes and their nuclei are well visible (H & E stain 400x) CV: Central Vein; Arrows: Sinusoids

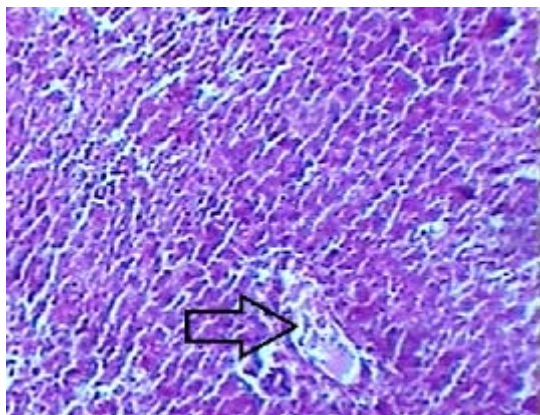


Fig. 2: Group II (CCl₄ control) – Microscopic features of liver. Extensive necrosis apparent. No nucleated hepatocytes can be demarcated. (H & E stain 400x) Arrow: Central vein

scientific studies on hepatoprotective plants/formulations have been carried out by chemically inducing liver damage in rodents.

The injury of CCl₄ has been ascribed to its metabolite – trichloromethyl radical (CCl₃^{*}) which is generated by microsomal enzymes namely CYP2E1, CYP2B1 or CYP2B2, and possibly CYP3A. This metabolite and not CCl₄ per se is toxic to the hepatocytes. This radical can also react with oxygen to form the trichloromethylperoxy radical (CCl₃OO^{*}), which initiates the chain reaction of lipid peroxidation¹⁵. This affects the permeabilities of mitochondrial, endoplasmic reticulum, and plasma membranes resulting in elevated levels of transaminases, alkaline phosphatase, bilirubin and other biochemical parameters¹⁶. Observed albumin levels were similar in all the groups because of the half life of albumin is 15 to 20 days.

Based on the mechanism of injury of CCl₄, the plausible mechanism of hepatoprotective action of Savakalp Kwath can be due to microsomal enzyme inhibition, free radical scavenging action or anti-oxidant action on lipid peroxidation or combination of two or more of the above actions. Also it is quite possible that the hepatoprotection of Sarvakalp Kwath may be due to a mechanism which is yet to be identified.

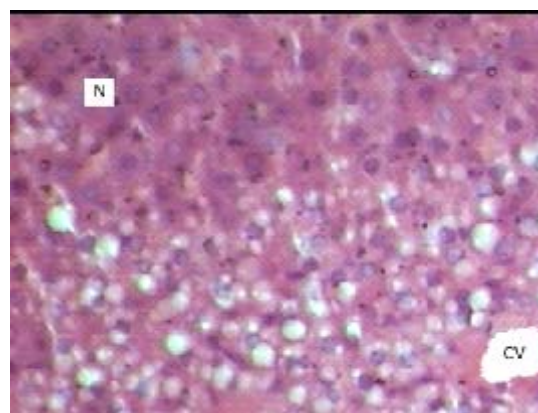


Fig. 3: Group III (Sarvakalp Kwath) – Microscopic features of the liver. Centrilobular hepatocytes show fatty changes. No necrosis is seen. Normal hepatocytes are visible in the periphery of the hepatic lobule. (H & E stain 400x) CV: Central vein; N: Normal hepatocytes

CONCLUSION

Sarvakalp Kwath, a proprietary product of Divya Pharmacy, Haridwar, offers significant preventive hepatoprotection against CCl₄ as

observed in this animal study. Although it is reasonable to extrapolate these results to the human population for which this medicine is intended, only clinical trials of Sarvakalp Kwath can firmly establish its role in therapy of liver diseases in humans.

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