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Research Article

ABROGATION OF CARBON TETRACHLORIDE (CCL_4) INDUCED HEPATOTOXICTY BY AROGYAVARDHANI IN WISTAR RATS

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ABSTRACT

From long back Arogyavardhani, a herbo-mineral preparation is used in the affections of liver & spleen disorders as an ayurvedic preparation. The present study was aimed to evaluate the hepatoprotective effect of Arogyavardhani in carbon tetrachloride (CCl_4) induced liver damage in wistar rats. In the present study Arogyavardhani A (65 mg/kg, p.o) and Arogyavardhani B (65 mg/kg, p.o) were used to screen the hepatoprotective activity. Hepatotoxicity was induced by the CCl_4 (3 ml/kg, p.o), and silymarin (50 mg/kg, p.o) was taken as a standard. Biochemical parameters like serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate trasaminase (SGPT), alkaline phosphatase (ALP), total bilirubin and direct bilirubin levels were estimated. Histapathological examination of liver samples were also done. CCl_4 treated groups showed the elevated levels of biochemical parameters like SGOT, SGPT, ALP, total bilirubin, and direct bilirubin levels. In-case of Arogyavardhani treated groups significantly (p<0.01) prevented this hepatotoxicity. Histopathological examinations revealed the post-treatment of Arogyavardhani exhibited the protection of liver tissue from CCl_4 induced hepatotoxicity. The observed results strongly support the hepatoprotective activity of Arogyavardhani against CCl_4 induced hepatotoxicity.

Keywords: Arogyavardhani, CCl_4, Hepatotoxicity, SGOT, SGPT.

INTRODUCTION

Liver was called as a chemical factory of body, because most of the biochemical and metabolic pathways are continued here [1]. So, liver is a major affected organ for the different types of chemical toxicities [2]. Many liver diseases are now challenging to treat in the modern scientific community [3]. Now-a-days, there are different types of plant derived and allopathic medicines are used in the management of hepatic disorders [4,5].

Ayurveda is a one of the widely practiced system of traditional medicine in India. It mainly recommends the use of plant based medicines, mineral based medicines such as sulfur (S), mercury (Hg), arsenic (As), lead (Pb), copper sulfate (CuSO4) and gold (Au) for treating wide variety of disease conditions. The main specialty of Ayurveda practice of mineral, metallic compounds and herbal medicines use is known as rasa shastra [6-8]. Arogyavardhani is an ayurvedic herbo-mineral formulation and it is used in the treatment of affections of hepatic & Spleen disorders, Jaundice, Pachana (Indigestion), Durdharsha Kshutprapartini (Loss of appetite) and Kushtha (Skin diseases). It consists of Shodhit Parada, Shodhit Gandhaka (purified sulfur), Loha bhasma (iron compound), Abhraka bhasma (mica), Tamra bhasma (copper), Shodhit Shilajatu (Asphaltum), choorna of Haritaki (*Terminalia chebula*), Bibhitaka (*Terminalia belerica*), Amalaki (*Emblica officinalis*), Shodhit Guggulu (*Commiphora weightii*), Chitraka (*Plumbago zeylanica*) root, Katuka (*Picrorhiza kurroa*) rhizome, and processed in Nimba (*Azadirachta indica*) leaf swaras [9].

Carbon Tetra Chloride (CCl_4) is a prototype of chemical used to induce the hepatotoxicity widely [10]. CCl_4 induced hepatotoxicity is mainly due to its intermediate reactive metabolites such as trichloromethyl radical (CCl_3•) and its derivative trichloromethyl peroxy radical (CCl_4OO•), generated by cytochrome P_{450}2E1(CYP_{450} 2E1). In addition, CCl_4 intermediates also induce–the production of reactive oxygen species (ROS), which play an important role in pathogenesis of different degenerative disease like atherosclerosis, liver disorders, lung , aging and diabetes mellitus [11]. Moreover, these ROS and intermediates may activate the reactive nitrogen species (RNS), which are also being involved in the hepatotoxicity production. These reactive species are thought to interact with membrane lipids leading to their peroxidation.

Inducible nitric oxide synthase (iNOS) along with other inflammatory pathways resulted in the tissue damage by CCl_4. Another proposed mechanism was reactive oxygen species (ROS) are interact with the antioxidant sulfhydrayl group of antioxidant system such as glutathione and others. CCl_4 induced hepatotoxicity characterzied by increasing in the liver biomarker enzymes like aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and γ-glutamyltransferase (γ-GT) and histopathological changes like steatosis and centrilobular necrosis due to membrane lipid peroxidation [12].

Although wide range of antioxidants and other allopathic medicines are currently used in the treatment of hepatic disorders. In this present study we evaluated the hepatoprotective effect of Arogyavardhani in CCl_4 induced hepatotoxicity in rats.

MATERIALS& METHODS

Two Samples of Arogyavardhani A (ERM Machine) and Arogyavardhani B (AVD Machine) were collected from the Shree Dhoothapapeshwar Limited (SDL), Mumbai.

Chemicals

All the chemicals required for the experiment were of analytical grade procured from the Sigma- Aldrich, Mumbai.

Animals

30 adult albino rats of wistar strain of both sexes weighing between 180-200g were used in this study, procured from the Haffkine Biopharmaceutical Corporation Ltd., Mumbai. The animals were kept in standard conditions like 22°c ± 2°c and relative humidity 55 ± 15 %. Rats were freely accessible to food and water *ad libitium* with a 12 hours and 12 hours light and dark cycle. All animals were acclimatized for at least 1 week before start of the study. All the

experimental protocols were approved by Institutional Animal Ethics Committee and performed according to the CPCSEA guidelines for the care and use of animals (SDARF/PC/2013/02).

Experimental Design

Induction of experimental hepatotoxicity

Hepatotoxicity was induced by administration of CCl_4 at a dose of 3 ml/kg, p.o body weight at first day of the study [13].

Preparation of solutions

CCl_4 was diluted with olive oil in 1:1 ratio, and Arogyvardhini A & B were suspended in 2% CMC solution.

Evaluation of hepatotoxicity

Animals were divided into the five groups, consisting of six animals per each group.

Group I : Normal control (2% CMC 5 ml/kg, p.o)
Group II : Negative control (CCl_4-3 ml/kg, p.o)
Group III : Arogyavardhani A (65 mg/kg, p.o) + CCl_4 (3 ml/kg, p.o)
Group IV : Arogyavardhani B (65 mg/kg, p.o) + CCl_4 (3 ml/kg, p.o)
Group V : Sylimarin (50mg/kg) + CCl_4 (3 ml/kg, p.o)

All the groups were treated for 28 consecutive days. At the end of the day blood samples were collected from retro-orbital technique using CO_2 asphyxiation and sent to the JDC diagnostic center, kharghar, Mumbai for the biochemical & histopathological estimations.

Histopathological examination:

A portion of liver tissue samples was collected from the each group and preserved in 10 % neutral formalin solution for the histopathological examination. For the histopathological examination liver samples were sectioned at 5µm and haemotoxylin, eosin (H&E) staining were used.

Statistical analysis

Values were represented as mean ± SEM. Data were analysed by the one way analysis of variance (ANOVA) followed by the Tukey's test using graph pad prism 5.1 version. $p<0.05$ was considered as significant.

RESULTS

Biochemical parameters:

The animals treated with CCl_4 exhibited a significant ($p< 0.01$) rise in SGPT, SGOT levels and incase of ALP, total bilirubin levels were also increased significantly ($p< 0.001$).This was significantly ($p<0.01$) reduced after treatment with Arogyavardhani treated groups, which was almost similar to silymarin treated groups as shown in Table 1.

Table 1: Average Biochemical parameters (Average ± SEM, *n*=6)

	ALT (SGPT)	AST(SGOT)	ALP	Total Bilirubin	Direct Bilirubin
Group I	48.4 ± 3.98	124.26±4.38	247.48±9.68	0.185±0.02	0.076±0.005
Group II	77.06±4.57[a]	175.11±8.44[a]	458.81±12.25[b]	0.245±0.008[ns]	0.138±0.005[b]
Group III	50.33 ±4.0***	128.7 ±4.46***	267.78±7.73***	0.191±0.006*	0.096±0.003***
Group IV	47.81±4.3***	126.13±6.11***	255.66±11.48***	0.182±0.007**	0.091±0.005***
Group V	46.5±4.34***	124.71 ±3.49***	246.73±12.84***	0.174±0.009**	0.081±0.004***

Statistical comparisons of the data for groups were done by Student's test followed by the Tukey's test.
***$p<0.0001$ vs. Positive control, **$p<0.001$ vs. Positive control, *$p<0.01$ Vs Postive Control, [a]$p<0.01$ Vs Negative control, [b]$p<0.001$ Vs Negative control, ns=Non-significant.

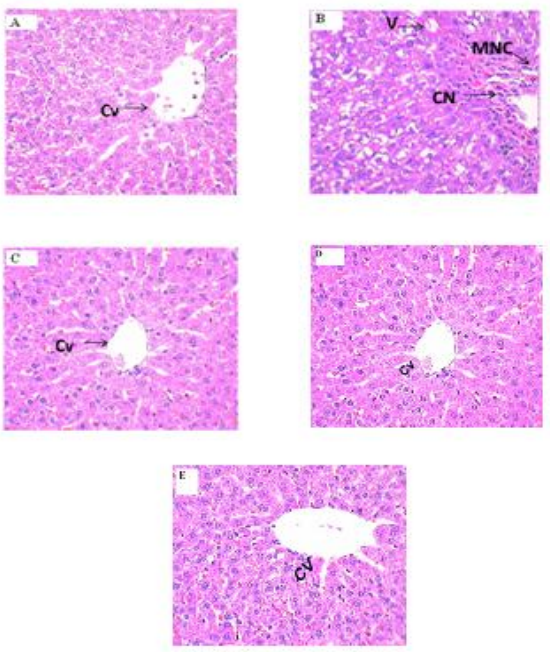

Figure: 1 Phtomicrograph showing the histopathology of liver samples.

a) Normal control rat showing normal architecture of liver sample.
b) Negative control group (CCL4 3ml/kg)showing the centrilobular necrosis (CN), mononuclear cells infiltration (MNC) and Vacuolation (V).
c)Arogyavardhani A (65 mg/kg), d) Arogyavardhani B (65 mg/kg), e) Silymarin (50 mg/kg) treated groups showing normal artchitecture.

Histopathological Examination

Histopathological evaluation of liver reveled CCl_4 induced changes in liver parenchyma viz., centrilobular necrosis, mononuclear cells infiltration and vacuolation in negative control group (Group II) and these changes were not seen in treatment group [Figure: 1b]. Liver also showed degenerative changes in negative control group and these changes were not seen in treatment group. This clearly demonstrated that protection of liver from the CCl_4 induced hepatotoxicity [Figure: 1C, D and E].

DISCUSSION

Ayurvedic preparations were used in the treatment of various disorders from long back onwards and are widely being used. Now-a-Days, there is a growing interest on Indian traditional system of medicine [14]. The present study was performed to evaluate the protective effect of Arogyavardhani against CCl_4 induced hepatotoxicity. In this study, CCl_4 was given first and separate groups were treated with the Arogyavardhani.

Animals from the standard control were too active and respond very quickly as compared with the negative control group. This may be probably due to the CCl_4 is anaesthetic agent and may cause the slowing of reflexes [15].

In the estimation of liver toxicity by the CCl_4 the biomarker enzyme levels are mainly estimated. Serum SGOT, SGPT, ALP, and bilirubin levels are mainly increased in the hepatic damage, because these enzymes are cytoplasmic in nature. CCl_4 induced hepatotoxicity is prototype of hepatotoxicity induced model. Increased levels of SGOT,

SGPT, ALP, and total bilirubin clearly indicate the CCl_4 induced hepatocellular damage and there are number of studies supporting it. Decreased values of enzymes levels in Arogyavardhani treated groups significantly (p<0.01) showed their ability to normalize the status of hepatic damage.

Histopathological assessment of the liver of the normal control group showed the normal architecture of hepatocytes with portal traid. Liver sections derived from hepatotoxic rats showed centrilobular necrosis, mononuclear cells infiltration and vacuolation. These findings were observed in the livers of all rat belonging to Group II (CCl_4). Rats from group III, IV and V treated had far less centrilobular necrosis and vacuolation than the negatitive control rats (Group II). This could be due to the formation of highly reactive oxygen species, caused by the administration of CCl_4. But, post treatment with Arogyavardhani prevented these effects. Hence, histopathological studies revealed the post treatment of Arogyavardhani clearly exhibited the significant protection of liver cells, which confirmed the above values. So, Present results support the Dange SV et al. [16] as mentioned that CCL_4 increased the serum SGOT, SGPT, ALP, total bilirubin, direct bilirubin levels and Arogyavardhani significantly reduced the CCL_4 induced liver damage.

CONCLUSION

However, results of the present study clearly demonstrated the protective effect of Arogyavardhani against CCl_4 induced hepatotoxicity in rats and supports the notion of Arogyavardhani in the treatment of affections of hepatic & spleen as per traditional system of medicine and claims it's effect as per ancient scripts.

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