ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

The Effect Of *Wrightia tinctoria* Leaves On CCl4 Induced Hepatotoxicity.

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ABSTRACT

The present study has been designed to get better understanding about the actions of aqueous, alcohol extract and crude powder of *Wrightia tinctoria* leaves on hepatoprotective effect on wistar albino rats. In this study the efficacy of aqueous, alcohol extracts and crude powder of *Wrightia tinctoria leaves* for its hepatoprotective effect against CCl_4 induced liver damage was evaluated in *Wistar albino* rats**.** The treatment schedule and groups are as follows.

Group –I control group 1 ml-distilled water (vehicle) was administered for 21 days
Group –II was exposed to aqueous extract (200 mg/kg) in 1ml of vehicle for 21 days
Group–III was exposed to crude leaf powder (500mg/kg) in 1ml of vehicle for 21 days
Group–IV was exposed alcohol extract (200 mg /kg) in 1 ml of vehicle for 21 days.

At the end of the experimental period on 25th day, blood was collected from retro – orbital venous plexus and animals were sacrificed autopsied gross pathology examination of all organs and histopathology of liver also made, the blood samples were subjected biochemical and hematological investigations. The structural alterations observed in the histopathological examination of the liver tissue in animals treated with the alcoholic leaf extract after CCl_4 administration was strikingly lesser when compare to hepatic tissue damage of animals exposed to CCl_4 alone. This observation correlates well with the biochemical changes suggestive of lesser CCl_4 induced hepatic damage in animals treated with the leaf extracts. The extract of the leaves of *Wrightia tinctoria* have been found to protect experimentally induced liver damage.

Keywords: *Wrightia tinctoria,* Hepatoprotection, CCl 4 hepatotoxiciy, *Wistar albino* rats.

https://doi.org/10.33887/rjpbcs/2019.10.4.3

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ISSN: 0975-8585

INTRODUCTION

Medicinal plants are still considered as the source of untapped reservoir of drugs and the structure of the component molecules make them valuable sources of novel drug discovery. (Farn's Woryh, 1989).

One such medicinal plant "Wrightia tinctoria" quoted to possess a variety of medicinal properties, Himoliv® a polyherbal ayurvedic product (M/s. Emami Limited, Kolkata, India) containing aqueous extracts of 25 indigenous medicinal plants with *Wrightia tinctoria* as one of the ingredient is claimed to be useful in hepatitis, jaundice and biliary dysfunction, So in this study hepatoprotective effect of the plant is carefully evaluated.(Bhattacharyya D et al 2003)

Plant Description:

Division	: Plant Kingdom
Sub division	: Gamopetalae
Class	: Dicotyledones
Subclass	: Sympetalae
Serius	: Bicarpellatae
Order	: Gentianales
Family	: Apocynacae
Genus	: *Wrightia*
Species	: *tinctoria*

Scope Of The Present Study

The present study has been designed to get better understanding about the actions of aqueous, alcohol extract and crude powder of *Wrightia tinctoria* leaves on hepatoprotective effect.

MATERIALS AND METHOD

Collection Of *Wrightia tinctoria* Leaf Materials

Fresh leaves of *Wrightia tinctoria* were collected from vinny garden, Nachaloor near Trichy, TamilNadu, India.

Extraction From *Wrightia tinctoria* Leaves

Immediately after collection, the leaves were washed in tap water twice and in distilled water once to remove all the external dust, dirt, and unwanted materials. The leaves were than dried under shade for 72 hrs. Small bits of plants materials; petioles, mid ribs and twins were removed after shade drying. The dried leaves were crushed by hand to crude powder.

300 gm of this powder was soaked in 2 liters of rectified spirit for 1 days. The soaked leaves were allowed to undergo natural percolation under occasional shaking twice a day at an interval of 6 to 8 hours during daytime. The leaf extract was filtered using watman no.1 filter papers and the filtered extract was evaporated to dryness at 60 ° C over a water bath. The yield of the alcoholic leaf extract was between 3 to 5 percent. This extract was found to be sparingly soluble in water; but soluble in alkalized water (0.05N NaOH). This alcoholic leaf extract preparation was used in this study.

400 gm of this powder was soaked in double distilled water for 2 days. The soaked leaves were allowed to undergo natural percolation under occasional shaking twice a day at an interval of 6 to 8 hours during daytime. The leaf extract was filtered using watman no.1 filter papers and the filtered extract was evaporated to dryness at 95 ° C over a water bath. The yield of the aqueous leaf extract was between 8 to 12 percent. This extract was found to be soluble in water.

ISSN: 0975-8585

Animals

Healthy Wistar albino rats of both sexes not previously used for other studies were procured from central animal house facility are used in this study. All animal experiments were performed after getting prior approval from the Institutional Animal Ethical Committee (IAEC). IAEC NUMBER 2705.

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Study Of Hepatoprotective Effect

Liver is the one of the vital organ for human life. Basically it detoxifies all the chemicals and synthesizes enzymes and other important protein for normal physiological function. Liver is the first organ to be exposed to various drugs and other product after absorption from the GIT (gastro intestinal tract) via portal vein and is one of the important sites for first pass metabolism. Hepatic uptake, intracellular transport, biotransformation and excretion in bile are important processes that clear the drug in the body and terminate their pharmacological action (by transferring into water-soluble form and facilitating excretion via kidney) and prevent their toxic accumulation.

However, drugs and other chemicals induced hepatotoxicity result in deviation from normal structure and function of liver and it may lead to necrosis, cirrhosis, carcinoma and fatty infiltration accompanied by biochemical and physiological changes in this organ. Hepatocellular damage can be produced by the direct action of the drug itself or through their metabolites. E.g. Acetaminophen, Anti-tubercular drugs, Halothane, CCl_4 etc.

Scientific research on the use of medicinal plants is being carried out in large scale than ever before and the reason for this rapid development is to produce more specific and safe drugs for the treatment of various human ailments. It is generally believed that active principles extracted from plants are non-toxic and even harmless because of their natural origin (Chandrashekhar VM et al 2004). In this study plant principles extracted from the leaf of the herbal medicinal plant *Wrightia tinctoria* has been tested for their efficacy against CCl_4 induced hepatotoxicity. CCl_4 is a known hepatotoxic agent (Recknagel, 1967) and is commonly administered to induce hepatotoxicity for the evaluation of hepatoprotective properties of active principles extracted from medicinal plants (Ubale, Milind 2012). Hence, in this study the hepatoprotective efficacy of on aqueous extract, crude powder and alcohol extract of *Wrightia tinctoria* leaves against CCl_4 induced hepatocellular damage was evaluated in rats.

MECHANISM OF CCl_4 TOXICITY

It is hypothesized that hepatotoxicity induced by CCl_4 is brought about by its active metabolites, which are generated by involvement of cytochrome P450 dependent mixed function oxidase in the liver tissue. The primary step in CCl_4 metabolism is the reduction of CCl_4 by cytochrome P450 system. Yielding Trichloromethyl free radical ($CCl_3^{\bullet}$), which in turn reacts with oxygen to form peroxy radicals ($OOCCl_3$). (cameron GR et al)

Major pathway:

$$CCl_4 \xrightarrow{\text{Cyt.P450}} CCl_3^{\bullet} \longrightarrow OOCCl_3$$

Minor pathway:

$$CCl_4 \longrightarrow CCl_2 \text{ (less potent)}$$

The trichloromethyl free radical and peroxy radical are highly reactive and are capable of combining with cellular membrane and lipids in the presence of O_2 to induce LPO (lipid peroxidase) by $H^{\bullet}$ abstraction.(Johnston DE et al 1998)

Study Design

In this study the efficacy of aqueous, alcohol extracts and crude powder of *Wrightia tinctoria leaves* for its hepatoprotective effect against CCl_4 induced liver damage was evaluated in *Wistar albino* rats**.** The treatment schedule and groups are as follows.

Group –I control group 1 ml-distilled water (vehicle) was administered for 21 days
Group –II was exposed to aqueous extract (200 mg/kg) in 1ml of vehicle for 21 days
Group–III was exposed to crude leaf powder (500mg/kg) in 1ml of vehicle for 21 days
Group–IV was exposed alcohol extract (200 mg /kg) in 1 ml of vehicle for 21 days.

On 22nd, 23rd 24th days CCl_4 in liquid paraffin in 1:1 ratio was given subcutaneously at the dose of 2ml/kg . At the end of the experimental period on 25th day all the animals were anaesthetized under mild ether anaesthesia, and blood was collected from retro – orbital venous plexus and animals were sacrificed autopsied gross pathology examination of all organs and histopathology of liver also made, the blood samples were subjected biochemical and hematological investigations.

Statistical Analysis

The data were subjected to one way analysis of variance (ANOVA) and Tukey's multiple comparison test was done to evaluate the significance of difference of means of various treatment groups, using SPSS statistical package (version 10.5). The value are presented as mean ± SE and p value <0.05 was considered significant.

RESULTS AND DISCUSSION

No mortality was seen during the test period in all the groups, no abnormal external manifestation or behavioral change was seen in animals exposed to the test substances.

Biochemical Investigation

Rats treated with the aqueous extract and crude leaf powder did not show any significant difference in ALT after CCl_4 administration when compared to the distilled water treated control group, whereas rats exposed to alcoholic leaf extract had a significantly lower ($p<0.05$) ALT level as compared to distilled water treated control group . Rats exposed to aqueous extract and alcoholic extract had a significantly lower ($p<0.05$) ALP, AST levels as compared to distilled water treated control group. The level of the serum total protein was significantly higher ($p<0.05$) in rats treated with aqueous leaf extract as compared to control group, whereas animals treated with crude leaf powder and alcoholic leaf extract did not show any difference significantly when compared to the control group .

Hematology

Rats exposed to leaf powder had a significantly lower ($p<0.05$) circulating leukocyte count as compared to that of the control group . Rats exposed to alcoholic leaf extract had a significantly lower ($p<0.05$) erythrocyte count and haemoglobin content as compared to that of the control group . In the differential leukocyte count, rats exposed to alcoholic leaf extract had a significantly lower ($p<0.05$) lymphocyte count as compared to that of the control group. There was no significant difference in the clotting time.

Histopathology

The extent of cellular damage noticed in the groups treated with the powder or extracts of the leaves of *Wrightia tinctoria* after CCl_4 was less when compared to the distilled water treated control group.

Animals treated with the extracts of the leaves of *Wrightia tinctoria*, the CCl_4 induced increase in the serum enzyme levels has been found to be suppressed. Since these are intracellular enzymes released into serum during injury of the cells, in this case liver, it can be deduced that the administration of the leaf extracts of *Wrightia tinctoria* prevented cellular damage in liver. A highly significant suppression has been noticed with the alcoholic extract of the leaves. This indicates that the principle, responsible for the protective effect is present in a higher concentration in the alcoholic extract. The structural alterations observed in the histopathological examination of the liver tissue in animals treated with the leaf extract after CCl_4 administration was strikingly lesser when compare to hepatic tissue damage of animals exposed to CCl_4 alone. This observation correlates well with the biochemical changes suggestive of lesser CCl_4 induced hepatic damage in animals treated with the leaf extracts.

ISSN: 0975-8585

CONCLUSION

The extract of the leaves of *Wrightia tinctoria* have been found protect experimentally induced liver damage. The principle responsible for this effect seems to reside in the alcoholic fraction. This property can be explored for its therapeutic application

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