ORIGINAL ARTICLE

HEPATOPROTECTIVE ROLE OF n-HEXANE FRACTION AND CRUDE METHANOLIC EXTRACT OF WITHANIA COAGULANS IN BALB C MICE

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Background: Withania coagulans is one of the most important medicinal herbs due to its wide range of biological activities. The aim of this study was to compare the hepatoprotective activity of crude methanolic extract versus n- hexane fraction of fruit of Withania coagulans in CCl4 induced liver toxicity **Methods:** This study was done on 36 Balb c mice in Department of Anatomy of Khyber Medical College Peshawar. **Results:** The mean serum of Group 1(control Group) was 33.41±1.82U/L, for Group 2 (CCl4 treated Group) was 89.01±7.51 U/L, for Group 3(low dose Group) was 49.91±3.48 U/L and for Group 4 (High dose Group) was 50.86±4.87 U/L. There was significant difference in the readings of Group1 and Group 2 which indicated CCL4 induced hepatotoxicity in two groups. There was significant difference in the values of Group 3 and Group 4 (*p*-value .000), showing the hepatotoxicity in these Groups was further enhanced. The mean AST at the end of six weeks for Group1 was 26.80±3.21U/L, for Group 2 was 149.01±13.63U/L, for Group 3 (including both low doses) was 70.81±7.92U/L and for Group 4 (High doses group) was 51.01±11.05U/. **Conclusion:** Withania coagulans both fractions have hepatoprotective effect against CCL4 induced hepatic toxicity in high and low doses in Balb c mice.

Keywords: Withania Coagulans; CCL4; Liver; Mice; Hepatoprotective

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INTRODUCTION

Plants have been used by human beings as food, shelter, fuel, for decoration, tools, honey collections and as well as for health maintenance. In remote areas plants are used as medicines due to easy availability, less cost and less side-effects.[1] About 80% of population of the world depends on plants for health care.[2] Withania coagulans is distributed in East of Mediterranean Region prevailing in South Asia.[3] Withania coagulans is one of the medicinal herbs, which belongs to Solanacea of night shade family, native to Afghanistan and Indian Sub-continent. Withania coagulans is commonly known as 'vegetable rennet', the local name is 'spiubajja' in Afghanistan 'khamjira' in Punjabi, ‘punier band' or 'punier-ja-fota' in Sindhi, Indian cheese maker in India and 'Thukhme-kaknaje-hidi' in Persian and is commonly known as `panir booti` in Punjab. Withania coagulans dunal is a wild, rigid, grey under shrub 60–120 cm high and natural regeneration is from the seed.[4] Withania is thought to have been named after a British geologist Henry Witham.[5] Its different parts have been reported to possess a variety of biological active compounds such as: its fruits and leaves are rich in alkaloids, esterase’s, free amino acids and phenolic compounds.[6] Withania coagulans is a potential herb and rich in withanolide, (17S, 20S, 22R-14a, 15a, 17B, 20B-tetrahydroxy-1-oxowitha-2, 5, 24-trienolide) named coagulanolide, 1–3 and 5.[7] Withania coagulans has antifungal activity[8], anti-hyper Lipid-emic effect[9], anti-diabetic effect[10], wound healing activity[11], antioxidant effect[12], anthelmintic[13], antinociceptive[14]. Liver is the vital organ involved in physiological functions as well as in all biochemical pathways concerned with growth, detoxification of drugs, resistance against diseases and nutrient supply.[15] It is susceptible to injury from drugs, herbs, toxic chemicals due its close anatomical relation with gastrointestinal tract (GIT) and unique metabolic function. Susceptibility of the liver to toxins depends on age, race, gender, nutritional status, pre-existing liver disease, renal function, pregnancy, duration and dosage of drugs and drug to drug interaction. Hepatotoxins damage the mitochondria as well as produce a toxic metabolite (N-acetyl-P-benzoquinone) under the effect cytochrome P-450 enzyme in the liver. Normally toxic metabolite is detoxified when conjugated with glutathione which decreases with ageing.[16]

Carbon tetrachloride (CCl4) is a known hepatotoxic chemical compound in experimental animals. In the liver, microsomal cytochrome P-450 which is an enzyme causing degradation of drugs, converts carbon tetrachloride into Trichloromethyle radical (CCl3). This CCl3 in combination with oxygen produces Trichloromethyle peroxy radical, which affects the unsaturated fatty acids in the cell

membrane causing their peroxidation and finally leads to necrosis.[17]

Many studies supported the role of Withania coagulans as hepatoprotective agent but so for no study regarding crude methanolic extract and n-hexane fraction of Withania coagulans has been seen. Present study was conducted to highlight the hepatoprotectivity of Withania coagulans in above mentioned fractions. My study was carried out to highlight the hepatoprotective effect of Withania coagulance in our part of world as much work has not been done over here.

Furthermore, to study the comparison of n- hexane fraction and crude methanolic extract of Withania coagulans in chemically induced hepatotoxic by using CCl4 in albino mice.

MATERIAL AND METHODS

It was an experimental study carried out at the Department of Anatomy, Khyber Medical College Peshawar for a period of 6 months. Thirty-Six Balb C mice were divided into six group, each group having six mice. CCL4 was administered through oral route. Then histological study of their liver was carried out. The inclusion criteria was mice having average body weight 25–30 gm, male mice and not diseased. The exclusion criteria was animals which are in a disease state, animals which are underweight and female mice.

The freshly collected shade dried fruit was powdered, extracted by maceration in 90% methanol for 10 days (3x50 L). The methanolic extract was filtered with muslin cloth, evaporated and concentrated under vacuum by rotary evaporator (at 40 °C). The viscous extract for fractionation was dissolved in water and partitioned between n-hexane, ethyl acetate, chloroform and butanol by using 5000 ml capacity separating funnel.

Animals grouping and dosing

In each group the number of mice was 6.

Group 1: Was administered with normal saline (5%), 1.5 ml/kg body weight served as a control.

Group 2: Animals was treated with CCL4 1.5 ml/ kg body weight.

Group 3: Was given CCl4 followed by crude hydro-methanolic extract at low dose 150 mg/kg.

Group 4: Was given CCl4 followed by crude hydro-methanolic extract at high dose 300 mg/kg.

Group 5: Was given CCl4 followed by n-hexane fraction at low dose 150 mg/kg.

Group 6: Was given CCl4 followed by n-hexane fraction at high dose 300 mg/kg.

Behaviour, diet, hygiene was noted on daily basis. At the end of 6 weeks blood samples were taken from vein. Weight of animals was noted before and after the experiment. Animals were to be sacrificed by cervical dislocation and dissected for morphological studies. Liver was be fixed in 10% formalin solution and was be processed for histological studies using haematoxylin and eosin and Masson Trichrome stain.

One-way ANOVA test was used to find out the statistical variation among the means of experimental groups, using SPSS version 23. The level of significance was $p \leq 0.05$.

RESULTS

Cellular leakage results in elevated levels of these enzymes. Administration of fruit extract of W. coagulance showed remarkable recovery of enzymes and liver texture.

The serum ALT for six randomly taken mice was 31.01±1.01 U/L. While mean first serum AST was 25.21±1.31 U/L.

There was no significant difference in the values of ALT and AST (*p* value .890). The mean serum of Group 1(control Group) was 33.41±1.82 U/L, for Group 2(CCl4 treated Group) was 89.01±7.51 U/L, for Group 3(low dose Group) was 49.91±3.48 U/L and for Group 4 (High dose Group) was 50.86±4. 87 U/L. There was significant difference in the readings of Group1 and Group 2 which indicated CCL4 induced hepatotoxicity in two groups. There was significant difference in the values of Group 3 and Group 4 (*p* value .000), showing the hepatotoxicity in further groups were enhanced. The mean AST at the end of six weeks for Group1 was 26.80±3.21 U/L, for Group 2 was 149.01±13.63U/L, for Group 3 (including both low doses) was 70.81±7.92 U/L and for Group 4 (High doses group) was 51.01±11.05 U/L. All this shows that there was significant difference in values of all Groups. Finally, low and high dose of W. coagulans both fruit extract has an equal hepatoprotective effect.

The carbon tetrachloride caused extensive vascular degenerative change, congestion of centrioles and macro vesicular steatosis. The fruit extract both methanolic and n-hexane fraction showed better effects against carbon tetrachloride to mutable scores; with the maximum dose seeming most effective.

Histological studies of Group 2 revealed fatty accumulation, inflammation and damaged hepatocytes. However, all these changes were found in reverse in the Groups treated with W. coagulans both extracts.

Table-1: Groups comparisons in methanolic extract using Roenigk Grading

Changes	Dose 150 mg	Dose 300 mg	CCL4 1.5 mg	Control	*p* value
Fatty	1.07	2.003	1.002	1.02	0.01
Necrosis	2.05	4.13	4.13	1.05	0.003
Fibrosis	4.01	8.09	4.01	2.03	0.001

Table-2: Groups comparisons in n-Hexane fraction using Roenigk Grading

Changes	Dose 150 mg	Dose 300 mg	CCL4 1.5 mg	Control	P value
Fatty	1.06	2.005	1.001	1.02	0.02
Necrosis	2.03	4.19	4.19	1.05	0.008
Fibrosis	4.07	8.07	4.01	2.03	0.001

DISCUSSION

The incidence of liver diseases rises now due to acquired issues particularly chemicals used in Work place.[18] CCl4 is common chemical used for grease-removal in industries.[19] It is hepatotoxic, after inhalation converted to CCL3 which produced Lipid peroxidation, oxidative damage to nucleic acids, alter the cell membrane integrity of mitochondria and endoplasmic reticulum. It finally leads to oxidative stress, change in permeability of cell membrane of hepatic and bile duct results raised enzymes.[20]

The same was observed in present study where CCl_4 treated hepatotoxic control group II showed elevated levels of enzymes. Further groups showed the cell protective effect of these fruits' extracts. This was consistent with our study. Liver is the main organ of metabolism, its illness results weight loss. This is similar in our study, group11 (hepatotoxic) showed marked weight loss.

The demand of herbal drugs is increasing internationally because of low incidence of side effects as compare to synthetic drugs. W. coagulans has wide medicinal use as antifungal, antihyperglycemic, antimicrobial, hypolipidimic, anti-inflammatory, depressant of central nervous system, antitumor and hepatoprotective activity.

The methanolic extract and n-hexane fraction of w. c has hepatoprotective effect as its glucocorticoids exerts anti-inflammatory effect. Withanolide are steroidal lactones and most of them have many important pharmacological activities. As 3-hydroxy-2,3 dihydrowithanolide F has been mark off for its hepatoprotective effect against the CCL4 persuaded toxicity as compared hydrocortisone.

Strengths of the study is that it was rigorously done with the limitations that it was carried out on small number of mice.

CONCLUSION

W. Coagulance methanolic extract and n-hexane fraction have important hepatoprotective effect on CCL4 induced hepatotoxicity both in low and high doses.

AUTHORS' CONTRIBUTION

FN, HI, SJ: Conceptualization of the study design, proof reading. ZR, SI, WA, JH, SA: Literature search, data collection, analysis, data interpretation.

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