Research Article

"Hepatoprotective activity of Rudanti (Capparis moonii Wight) in ATT induce Hepato-toxicity in wistar rats"

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ABSTRACT

Objective: Drug induced liver injury is an associated adverse effect seen with most of the drugs and the most common cause for a drug to be withdrawn from the market. First line antituberculosis drugs (ATT) are one of them. Anti-tuberculosis drugs are effective to provide complete cure from Mycobacterium tubercle but drug induced hepatotoxicity is one of the frequent and potentially serious adverse effect associated which may lead to therapy discontinuation. The Anti-tuberculosis treatment regimen must be discontinued once liver injury occurs, which may result in TB relapse and or drug resistance and or TB-related death. Thus study was planned to evaluate the hypothesis "Hepatoprotective activity of Rudanti (*Capparis moonii* Wight) in ATT induced Hepato-toxicity in wistar rats.

Material and Methods: 24 healthy albino wistar rats was divided in 4 groups. Group A (Normal Control): 6 Healthy wistar albino rats had received distilled water 5 ml/kg P.O/day. Group B (Negative control): 6 hepato-toxicity induced wistar albino rats had received distilled water 5 ml/kg P.O/day. Group C (test Group): 6 hepato-toxicity induced wistar albino rats had received *Capparis moonii* 400 mg/kg/ P.O/day for 60 days. Group D (Standard Group): 6 hepato-toxicity induced wistar albino rats had received Silymarin 200 mg/kg / P.O/day for 60 days.

Results: After 60 days of treatment the liver function biomarker showed significant increase in the ALT, AST, ALP, Total Bilirubin levels in ATT treated groups as compared to control group with normal animals. Whereas, in group C (Rudanti) significant reduction noted proves the hepato-protective activity of test drug comparable to standard (group D Silymarine) comparator evident for its hepato-protective activity.

Conclusion: Fruit powder of Rudanti (*Capparis moonii* Wight) showed good hepatoprotective activity against ATT induced hepato toxicity. The effects are curative in nature and were further endorsed by the histopathological changes in the liver.

INTRODUCTION

Drug induced hepato-toxicity, a major concern of medical society and pharmaceutical industries. More than 900 drugs have been found guilty in causing liver injury and is the most common reason for a drug to be detached from the market. 'Liver Tox' the site available for reporting of drug induced liver injury identified total 671 drugs can cause hepato-toxicity.[1] As per the cases reported to 'Liver Tox' anti- tuberculosis drugs have highest potential to cause hepato-toxicity and are placed in the category 'A'.[2]

But drug induced hepato-toxicity is a one of the frequent and probably serious adverse effect associated with anti-tuberculosis treatment regimens (ATT).[3,4,5,6] Which can lead to chemo-therapy disruption with more severe morbidity and even mortality.[7] Once liver injury occurs, the anti–TB regimen must be amended or interrupted, which can result in TB relapse, drug resistance and TB related death. The incidence of anti-tuberculosis drug-induced hepato-toxicity (ATDH) ranges from 1% to 36%.[4,8,9,10] Highest rate of hepato-toxicity is reported to have in the Asian population especially in Indian patients.[11,12,13,14] Moreover, the risk of hepato-toxicity reported in Indian patients is more i.e., up to 11.5% than that of western population which is up to 4.3%.[10]

The pathogenesis of hepato-toxicity is not perfectly clear, but anti-tuberculosis drug induced hepato-toxicity (ATDH) may involve oxidative stress, lipid peroxidation, choline deficiency leading to lowering of phospholipids and protein synthesis with alteration in cell wall configuration, reduced glutathione level and activation of CYP2E1.[15] Thus oxidative stress and idiosyncratic reactions shows major role as a causative factor of hepato-toxicity due to ATT drugs.[6,14]

To reduce the incidence of hepato-toxicity in TB patients, various measures have been taken with revised recommendations for drugs and patient selection criteria. But until today no drug has been refined in conventional health science for prevention of hepato-toxicity. Disengagement of the therapeutic drugs is the only measure available for managing ATT induced hepato-toxicity and reintroducing the same after regularization of liver enzymes.[16,17,18,19] It has been observed that significant number of the patients suffering from Tuberculosis find difficulty to replete the recommended treatment regimen due to hepato-toxicity caused due to the ATT drugs. Thus, some adjuvant therapy is essential which could scale down the hepato-toxicity of the therapeutic drugs.

Many herbal and herbo-mineral drugs are being used for the treatment of Rajayakshma (PTB). Similarly, there is one drug named Rudanti (*Capparis moonii* wight.) which is widely used as an anti-tubercular drug, but till now no proper research has been done to evaluate the probable mode of its action. But many in-vitro and in-vivo studies have been done which shows that it has an immunomodulatory and anti-oxidant properties along with its hepato protective activity in CCl_4 induced hepatotoxicity.[20,21,22] As per available data, there is no systematic work available evaluating the effect of Rudanti on anti-tuberculosis drugs induced hepatotoxicity. Thus, present study was planned to evaluate the hypothesis

"Hepatoprotective activity of Rudanti (Capparis moonii Wight) in ATT induce Hepato-toxicity in wistar rats". The results of the study revealed that the Rudanti may be used to prevent ATT induced hepatotoxicity.

MATERIAL AND METHODS

Trial drug i.e**.** Rudanti (*Capparis moonii* Wight.) unripe fruits were collected from the forest of Dist. Sindhudurg, State- Maharashtra in the month of May-June-2018. Botanical Identification of the trial drug was done by Professor Kamal Nayan Dwivedi, Department of Dravyaguna, Faculty of Ayurveda, IMS, BHU. Sample of collected raw drug was kept in the museum of the department of Dravyaguna with specimen accession number DG/19-20/234.

A total of 24 adult albino rats (both sexes) of Wistar strain weighing 145-184 grams were used for the present study. Experiment was conducted in Bilwal Medchem and Research Laboratory Pvt. Ltd, H-9 SKS (Ext.) Reengus, RIICO Industrial Area, Reengus, Sikar, Rajasthan with CPCSEA Approval No: 2005/PO/RcBt/S/18/CPCSEA, IAEC approval number: BMRL/AD/CPCSEA/IAEC/6/1

The animals are randomly selected, marked with Picric acid H (Mark on head), B (Mark on Back), T (Mark on Tail), HT (Mark on head and Tail), HB (mark on head and back), BT (Mark on tail and back) for individual identification, and kept in their cages for at least 5 days prior to dosing to allow for acclimatization to the laboratory conditions. Indusial rat had been marked with picric acid. The temperature in the experimental animal room had been 22ºC (±3ºC). Although the relative humidity had been at least 30% and preferably not exceed 70% other than during room cleaning the aim should be 50-60%. Lighting should be artificial, the sequence being 12 hours light, 12 hours dark. For feeding, conventional laboratory diets may be used with an unlimited supply of drinking water.

Experimental Design

Animals were randomized into groups (n=4) and administered with ATT to induce hepato-toxicity. Sterile water was given to control group. Isoniazid was dissolved in distilled and orally administered for 30 days at dose 7.5 mg/kg.

24 healthy albino wistar rats was divided in 4 groups. Each group has 6 rats.

Group A (Normal Control): 6 Healthy wistar albino rats had received distilled water 5 ml/kg P.O/day.

Group B (Negative control): 6 hepato-toxicity induced wistar albino rats had received distilled water 5 ml/kg P.O/day.

Group C (test Group): 6 hepato-toxicity induced wistar albino rats had received *Capparis moonii* 400 mg/kg/ P.O/day for 60 days.

Group D (Standard Group): 6 hepato-toxicity induced wistar albino rats had received Silymarin 200 mg/kg / P.O/day for 60 days.

Assessment of Liver Function and Histopathological Analysis

Blood sample were collected under all aseptic precautions. All specimens had collected by orbital puncher in EDTA blood sample collection bottle for analysis of Alkaline phosphate, SGPT, SGOT, Total bilirubin. At the end of trial period, the animals were euthanized with excess dose of ketamine and xylazine and livers were removed, weighed

accurately, and fixed in 10% formalin. Tissue cross sections of the livers were prepared, stained, and studied under microscope.

Statistical Analysis

All the data related to different parameters studied will be subjected to statistical analysis to determine the validity of the results. The value will be expressed to ±S.E. Student t-test was applied for detecting the significance of difference between groups. P values of 0.05 or less were considered significant.

Observation and Results

Effect of study drugs on Alkaline phosphatase, Serum Alanine Aminotransferase (SGPT), Serum Aspartate transminase (SGOT) and Total Bilirubin on rats from various treatment groups are summarized in Table 1 as below

Table No. 1- Effect of study drugs on various Biochemical Parameters of Liver Function Test

Treatment group (n=4)	**Biochemical Parameters (Mean ± SEM)**							
	ALP (IU/L)		**AST (IU/L)**		**ALT (IU/L)**		**T. Bilirubin (IU/L)**	
Group A (Normal Control)	12.76 ± 0.823		43.30 ± 1.176		30.19 ± 3.388		0.24 ± 0.012	
Group B (Negative control)	77.54 ± 5.047		164.70 ± 4.379		149.93 ± 5.216		1.15 ± 0.034	
Group C (Test Drug)	27.79 ± 2.973		71.68 ± 3.192		62.90 ± 4.176		0.69 ± 0.033	
Group D (Standard Group)	41.03 ± 3.753		85.55 ± 2.620		72.82 ± 3.391		0.92 ± 0.019	
Inter group results	**Mean Diff.**	**95.00% CI of diff.**	**Mean Diff.**	**95.00% CI of diff.**	**Mean Diff.**	**95.00% CI of diff.**	**Mean Diff.**	**95.00% CI of diff.**
Group B vs. Group A	64.78	52.20 to 77.37	121.4	110.4 to 132.4	119.7	105.0 to 134.5	0.9073	0.8130 to 1.002
Group B vs. Group C	49.75	37.17 to 62.34	93.02	82.00 to 104.0	87.04	72.26 to 101.8	0.4582	0.3638 to 0.5525
Group B vs. Group D	36.51	23.93 to 49.10	79.15	68.13 to 90.17	77.11	62.33 to 91.88	0.2305	0.1361 to 0.3249

Dunnett's multiple comparisons test was used to test the significance between inter group results and Group B shows significant results when compare with other groups.

The groups treated with ATT showed significant high level of Alkaline phosphatase (ALP), serum Aspartate transaminase (AST/SGOT), Alanine Aminotransaminase (ALT/SGPT) and total bilirubin as compared to normal control group indicating raise in ALP, AST, ALT and Total Bilirubin level after induction of toxicity. Further standard comparator group C (Rudanti) and treated group D (Silymarine) showed significant reduction in ALP level when compared with ATT treated group. Although Test group (Rudanti) showed significant reduction in AST level compared to standard Silymarine.

Normal hepatic cells with central vein, sinusoidal dilation, liver lobules and portal areas were seen in normal control group. Whereas in negative control group B histopathological slides showed structural damage of central vein, ruptured and irregular hepatic plate, granulation in cytoplasm, necrosis, vacuolization, disruption of hepatocytes, mild increase of the periportal fibrosis and loss of liver architecture with areas of necrosis. In

test group C (Rudanti) and standard group D (Silymarine) showed reconstruction of hepatic plate, central vein.

DISCUSSION

In the study design, all the Animals were randomized into groups (n=4) and orally administered with Isoniazid (dissolved in distilled water) for 30 days at dose 7.5 mg/kg. Sterile water was given to control group. The test drug Rudanti powder (*Capparis moonii*) was given at the dose of 400 mg/kg/ P.O/day for 60 days. Silymarin, a reported conventional hepatoprotective drug used as a standard comparator and given at the dose of 200 mg/kg/ P.O/day for 60 days. Of all the biomarkers to assess liver function ALT & AST enzyme has utmost importance and are distinguishing hepatocyte injury. Aminotransferases transfer an amino group from a donor molecule to a recipient molecule. Aspartate aminotransferase facilitates the conversion of aspartate and α-ketoglutarate to oxaloacetate and glutamate, and vice versa, whereas alanine aminotransferase facilitates the conversion of alanine and α-ketoglutarate to pyruvate and glutamate, and vice versa. AST can be cytosolic and mitochondrial, whereas ALT is strictly cytosolic. These enzymes are intensively expressed in cells involved in physiologic protein metabolism, particularly hepatocytes and muscle cells. Elevated serum aminotransferase levels are nonspecific markers for hepatocellular damage. Thus, ALT is more specific to liver architecture injury whereas increase in AST can be seen in cardiac or muscular injury. ALP detects problem of the biliary system obstruction like blocked bile ducts. ALP is especially high in the edges of cells that join to form bile ducts. Total bilirubin level elevated in any condition that affects the processing and elimination of bilirubin or accelerates the breakdown of RBCs. After 60 days of treatment the liver function biomarker showed significant increase in the ALT, AST, ALP, Total Bilirubin levels in ATT treated groups as compared to control group with normal animals. Whereas, in group C (Rudanti) significant reduction noted in ALT and AST level proves the hepato-protective activity of test drug comparable to standard (group D Silymarine) comparator at the hepatocyte level evident for hepato-protective activity. Significant reduction was seen in ALP & Total bilirubin level in group C and D indicating effect on the biliary system. Similar changes were also seen in histopathological slides. Suggesting that the test drug is effective to prevent drug induced liver injury by ATT drugs at hepatocyte level.

CONCLUSION

In conclusion, the fruit powder of Rudanti (*Capparis moonii*) showed good hepatoprotective activity against ATT induced hepato toxicity. The effects are curative in nature and were further endorsed by the histopathological changes in the liver. It may be a suitable adjuvant formulation to avoid hepatotoxicity brought on by ATT medicines given the high incidence of TB and antituberculosis drug-induced hepatotoxicity (ATDH) in Asian populations, particularly Indian patients.

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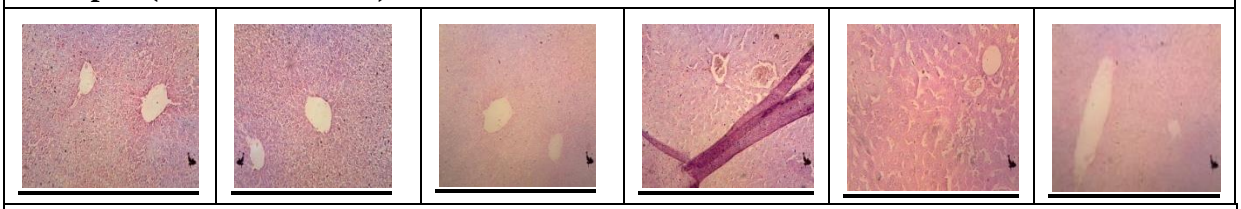
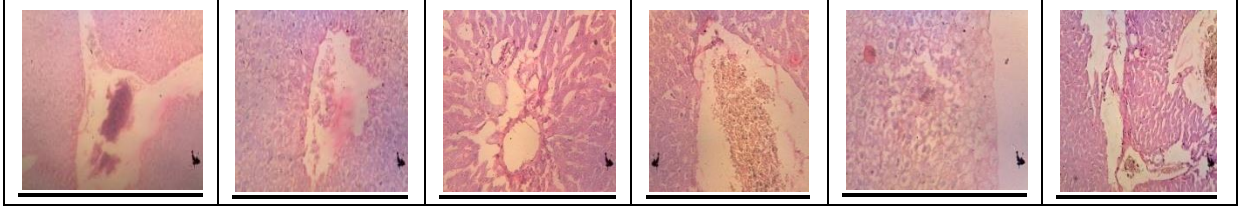
Histopathology of Liver

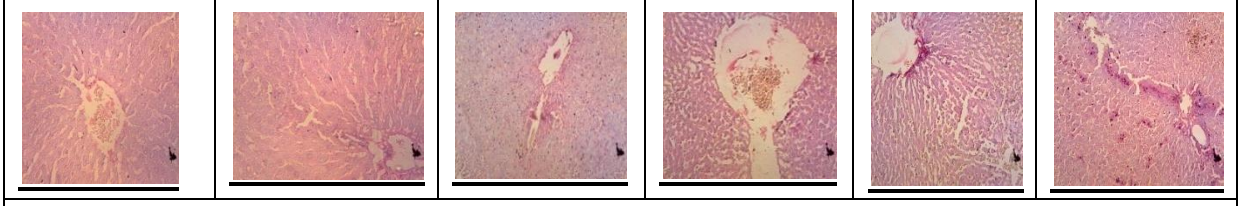
Group A (Normal Control)



Group B (Negative control)



Group C (Capparis moonii 400 mg/kg)



Group D (Silymarin 200 mg/kg)