INTERNATIONAL AYURVEDIC MEDICAL JOURNAL

IAMJ

International Ayurvedic Medical Journal, (*ISSN: 2320 5091)* (November, 2017) 5(11)

HEPATO-PROTECTIVE ACTIVITY OF *ROHITHAKADYA LOHA* IN- VIVO ANIMAL EXPERIMENT

Akshatha Das A.V[1], Vinayachandra Shetty[2], Sudhakara[3]

[1]Final year P.G Scholar, Dept of P.G studies in Rasashastra and Bhaishajya Kalpana, Alva's Ayurveda Medical College, Moodbidri, Karnataka, India
[2]Principal and professor, Dept of P.G studies in Rasashastra and Bhaishajya Kalpana, Alva's Ayurveda Medical College, Moodbidri, Karnataka, India
[3]Research officer, Dept of PG studies in S.D.M. Centre for research in Ayurveda and Allied Science, Kuthpady, Udupi, Karnataka, India

Email: akshatha1899@gmail.com

ABSTRACT

Background: *RohithakadyaLoha* is selected from *RasendraChintamani*, *Udararogadhikara*, it is one among the formulations which cures liver disease and protects the liver. **Objective**: To evaluate hepato-protective activity of *RohithakadyaLoha* against paracetamol induced liver toxicity. **Method**: The hepato-protective activity of *RohithakadyaLoha*was tested against paracetamol induced liver toxicity in albino rats. The protection was determined by measuring the levels of serum Glutamic Oxalacitate Transaminase (SGOT), Serum Glutamic Pyuvic Transaminase (SGPT), Alkaline phos-phatase (ALP), Protien, Albumine and Globuline, Total and Direct Bilirubin, Creatinine, Body weight, Liver weight and Histopathological study. **Result:** Test drug slightly prevent the damage by reducing SGOT level, globuline, increase in Protein level, ALP and Direct Bilirubin. Histopathological study showed decreased degenerative changes when compared to standard drug. **Conclusion:** Acomparative Histopathological study confirms the moderate Hepato-protective activi-ty of the drug.

Keywords: *RohithakadyaLoha*, Paracitamol, *Udararogadhikara,* Oxalacitate Transaminase

INTRODUCTION

Liver disease is considered to be serious health problem as liver is an important organ for the detoxification and Metabolizing nutrients thus maintains the body functions. In present days life style has been changed with the habits of consuming alcohol, self medication, eating

unwholesome food and hepatic injury due to the long term intake of some medications which are the prime causes for hepatic disorders.[1] Injury or liver dysfunction is a major health problem that challenges not only health care professionals, but also the pharmaceutical industry and drug regulatory agencies.

Even with the advancement of medical science some diseases are yet challenging and hepatic disordersare one among them. In present days life style has been changed with the habits of consumingalcohol, self medication and eating unwholesome food, which are the prime causes for hepatic disorders.

In *Ayurveda Yakrit* is considered as Seat of *Ranjaka Pitta*[2] and *Moola* of *Raktavahashrotas*[3] any damage to it disturbs digestive system, which in turn is cause for all the diseases. In Ayurveda for the purpose of preservation from hepatic disorders more formulations and single drugs have been explained. *RohitakadyaLoha*[4] is one among them.

RohitakadyaLoha is a formulation which explained in *Rasendra Chinthamani*, *UdaraRogadhikara*. It is a potent formulation containing *Rohithaka*, *Triphala*, *Trimada*, *Trikatu* and *LohaBhasma* which is having hepatoprotective property.

Method

This study was conducted in the experimental pharmacology laboratory of SDM. Centre for Research in Ayurveda & Allied Sciences, Udupi, Karnataka. The study was duly approved by the Institutional Ethics Committee. In the present study, the hepato protective activity of the test drug *RohitakadyaLoha*was evaluated through Paracetamol induced hepato-toxicity in albino rats.

Wistar strain albino rats of either sex of body weight ranging from 200-300g were selected for the present study..A totalof 24 rats were taken for Paracetamol induced hepato-toxicity Model. These were distributed into four groups of 6 mice each, Group I served as a control, while Group II received Paracetamol, Group III received sylimarine as a standard drug and Paracetamol. Group IV received the test drug *RohitakadyaLoha* and Paracetamol.

Statistical analysis

The data obtained was analyzed by using analysis of variance (ANOVA) followed by Dunnett's't' test for determining the level of significance of the observed effects. A 'P' value of less than 0.05 was considered statistically significant.

Methodology

Hepato-protective study using paracetamol induced hepatotoxicity model in Albino Wistar rats of either sex weighing 200 – 300 g were selected and divided into six groups of six animals in each group (n = 6). Treatment was given as described for 10days.

Group I treated: animals received tap water.

Group II Paracetamol treated: animals received paracetamol (3g/kg, p.o.),0.5 % gum acacia and distilled Water.

Group III Standard drug treated: animals received Silymarin (50 mg/Kg, p.o.) in addition to paracetamol.

Group IV Test drug: animals received *RohithakadyaLoha*, TED (2.25mg /100 k, p.o.) in addition to paracetamol

The Test drug *RohitakadyaLoha* and reference drugs were administered orally for 10 consecutive days and two dose of the toxicant (paracetamol) were administered orally to each

group, except the water control group, on 8th and 10th day 1h after test drug administration. After 48 hours of toxicant Paracetamol, the blood was collected in the tubes and sent for biochemistry laboratory for biochemical investigations. All the animals were sacrificed by cervical dislocation. Liver is dissected out, cleaned to remove extraneous tissues, blotted to remove blood stain and weighed. The liver was preserved in 10% formalin for histopathological processing.

Serum was separated and serum level of biochemical parameters namely SGOT, SGPT, ALP, TP, Albumin, Globulin, TB, DB, Creatinine, were estimated as per standard procedure prescribed by manufacturer (AGAPPE diagnostics Ltd., Kerala, India) whereas serum level of ALP was estimated as per standard procedure described by manufacturer (Span diagnostics Ltd., Surat, India) of diagnostic kit. Pondral parameters Liver weight and Body weight by general method. Histopathological study followed fixation by formaline, tissue processing, sliding, staining and microscopic study.

Observation

Administration of Paracetamol leads to significant alteration in ALP and globulin. But statistically non-significant alteration was observed in SGOT, SGPT, Total Protein, albumin, total bilirubin, direct bilirubin and creatinine.

There were reduced degenerative changes in Histo pathological study of test drug when compared with control drug.

DISCUSSION

Serum parameters studied were SGOT, SGPT, ALP, total protein, albumin, globulin, total bilirubin, direct bilirubin and serum creatinine depict the effect of test drug . The activity profile obtained has been given in the form of consolidated form in table No1.

Serum levels of SGOT and SGPT are increased on damage to the tissues producing them. SGOT transaminase activity was found to be reduced by test drug, which is non significant shows that test drug is hepato-protective. SGPT activity changes do not correlate well with the above. If we consider in the light of the histopathological examination- it can be seen that toxicant induced severe degenerative changes which might have incapacitated the liver to synthesize more of the enzyme hence- no significant elevation was observed in the above marker enzymes.

In liver injury its level ALP gets elevated. Decrease was reversed it may be inferred that by attenuating the degenerative changes the test drug and reference standard may be restoring hepatobiliary damage induced by the toxicant**.**

Test drugs and reference standard group produced non-significant mild increase in total serum protein level when compared to control group. This may indicate the test drugs cytoprotective activity by potentiating the enzyme activities and altering metabolite flux and partitioning metabolites between different metabolic pathways. So the drug may be hapato-protective.

Albumin is a blood plasma protein synthesized in the liver. Whereas some globulins are produced in liver and some are from immune system. Nonsignificantly decreased in standard group and significantly decreased in test drug group. The observed increase may be due to toxicant induced inflammatory reaction, its reversal can be considered to represent the reversal of the toxicant's effect.

Globulin -So in present study there is a significant increase in paracetamol control group, whereas it is non-significantly decreased in standard group

and significantly decreased in test drug group. The observed increase may be due to toxicant induced inflammatory reaction, its reversal can be considered to represent the reversal of the toxicant's effect.

Albumin- moderate non-significant elevation was observed in paracetamol control group which was not influenced by either the standard drug or test drug. This activity profile did not allow drawing any inference.

Bilrubin, a breakdown product of the porphyrin ring of haem-containing proteins, is found in the blood into fractions- conjugated and unconjugated. In test group it is increased – this indicates increased turnover. Because of the complex nature of the activity observed and the histological observation showed moderate protection in the test drug administered group- no inference could be drawn.

Direct Bilirubin is a substance made when your body breaks down old red blood cells. it is significantly increased in test drug indicates that there may be increased breakdown of RBC. This may not be relevant for the inference related to hepato-protective activity.

The serum creatinine level is considered as marker of kidney function. In the present study significant increase in serum creatinine was observed by all the groups. Since creatinine is a bio-marker which indicates renal injury its increase indicates that renal function is probably affected. Thus the observed changes in this parameter could not be linked to possible hepato-protection. The serum creatinine level in the toxicant control group.

Ponderal Changes showed in table No 2. In paracetamol control group a modest 41% decrease in body weight was observed. In sylimarin treated reference standard group a 52% decrease was observed. In test drug treated group an increase in 47% was observed. The second parameter was liver weight. In this parameter administration of paracetamol lead to significant increase in liver weight. In sylimarin treated reference standard group a significant decrease was observed However, non-significant increase was observed in test group

Thus analysis of the ponderal parameters did not contribute to the hepato-protective efficacy of the test drug in an un-equivocal manner. Overall analysis show that the data generated provides a complex picture making it difficult to arrive at any un-equivocal inference.

Histopathological examination from the paracetamol group revealed marked degenerative changes in the form of cell depletion, fatty both macro and micro degenerative changes, balloon cell formation, mild cell infiltration, necrosis and increase in the apotopic changes in the hepatic parenchyma. Standard group revealed the severe degenerative changes observed in paracetamol control group were remarkably reduced in this group. Only mild microfatty changes and sinusoidal dilatation was observed at some sites. Test drug administered group examination revealed a sections from one rat exhibited severe degenerative changes similar to the toxicant control group. In another section moderate degenerative changes were observed. Cell depletion was much less. In the remaining sections mild fatty changes and bile stasis was observed. Thus this report provides true indication to the status of the organ clearly reveals marked protection in reference standard and moderate protection in the test drug administered group. The reasons for obtaining complex nature of activity with regards to biochemical parameters need careful consideration.

Probable Mode of Action

The pharmacological evaluation revealed that *RohithakadyaLoha* alleviates *Pitta*and

*Kapha*and has a *Srotoshodhana*(cleansing channels) .As it is a herbo-mineral formulation which contains *LohaBhasma* it penetrates deep and absorbed into the tissue. Thus action will be comparatively quicker than herbal drug.

Yakrit is the *Mula* for *RaktavahaSrotas* and is considered as *Pittaashaya*. *Yakritvikara*s are basically caused due to the vitiation of *Pitta* associated with *Kapha.* There will be *Avarodha* of the *Srotas* in *YkritPleehaRoga* due to the *Vikrutha Pitta* and *Kapha*. Here *Sara Guna, RaktaPrasadaka* properties of *Rohithakatwak*. *Sara Guna* and *Kapha Pitta Shamaka* property of *Triphala, AvarodhaNashaka*, and *KaphaShamaka* properties of *Trikatu. Teekshanta*and *Ushnata, Pachana* properties of *Trimada*. *Rasayana, Chedana*, *AvarodhaNashaka* properties of *Loha* contributes in the protective property. Because of these properties it enters the *Srothas* and removes the obstruction, clears the channels, cures the disease and protects the liver.

CONCLUSION

Analysis of the biochemical and ponderal parameters did not contribute to the hepatoprotective efficacy of the test drug in an un-equivocal manner. Histopathological examination which provides true indication to the status of the organ clearly reveals marked protection in reference standard and in the therapeutic dose of *Rohithakadya Loha* showed moderate Hepato-protective activity in Paracetamol induced Liver toxicity in Albino Rats.

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Table 1: Effect of test drug on serum biochemical parameters

Parameters	Control group	Standard group	Test group
SGOT	NSI	NSI	NSD
SGPT	NSI	NSI	NSI
ALP	SD	SI	SI
Total protein	NSD	NSI	NSI
Albumin	NSI	NSI	NSI
Globulin	SI	NSD	SD
Total bilirubin	NSI	NSI	NSI
Direct bilirubin	NSI	NSI	SI
Creatinine	NSI	SI	SI

Table 2: Ponderal Changes

Parameters	PCM	Reference(sylimarin)	TED
Body weight change	SD	NSD	NSD
Liver weight	SI	SD	NSI

Histopathological Photographs

- **Paracetamol control group**

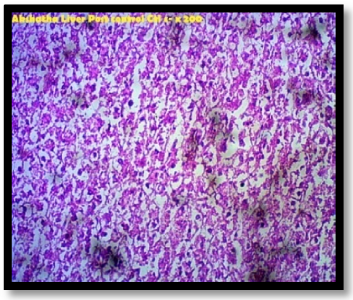


Fig No 1

Fig No 2

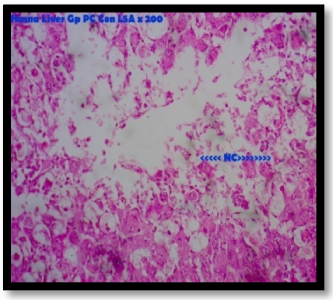
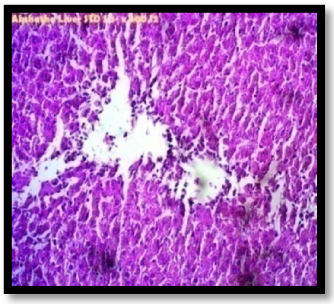


Fig No 3

- **Standard group**



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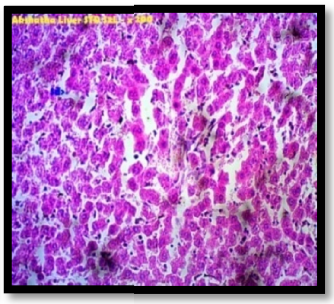

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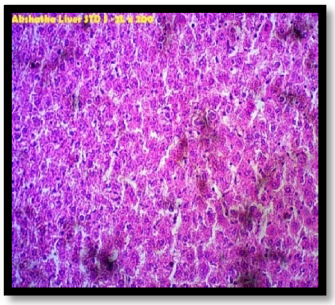
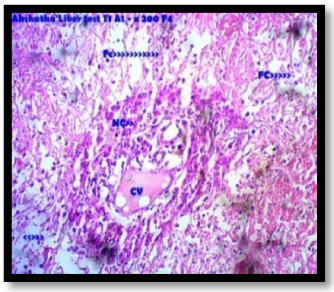


Fig No 1

- **Test group**



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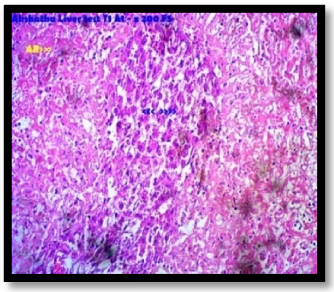

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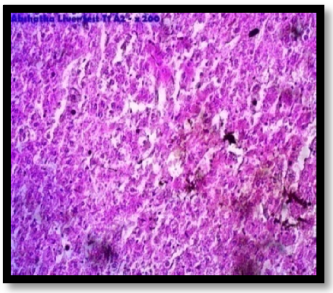

Fig No 9

Source of Support: Nil
Conflict Of Interest: None Declared

How to cite this URL: Akshatha Das A.V Et Al: Hepato-Protective Activity Of Rohithakadya Loha In- Vivo Animal Experiment. International Ayurvedic Medical Journal {online} 2017 {cited November, 2017} Available from: http://www.iamj.in/posts/images/upload/4083_4089.pdf