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HEPATOPROTECTIVE ACTIVITY OF CHASSALIA CURVIFLORA (WALL.)THWAITES ROOTS AGAINST PARACETAMOL INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT

Chassalia curviflora(Wall.)Thwaites are mainly used in folklore practice. It is mainly used in jaundice by the Kani tribes of Wayanad. The objective of the study was to evaluate its hepatoprotective action in animal model. **Methods:**Test drug: *Chassalia curviflora*(Wall.)Thwaites root aqueous extract. Toxicity induction: Paracetamol 3g/kg. Standard: Sylimarin 100mg/kg.TED: 200mg/kg, TED x 2:400mg/kg .Animal used: Wistar albino ratsParameters used-Serum biochemical parameters and histology of liver. Statistical analysis: Analysis of variance (ANOVA) followed by Dunnett's 't' test. P value <0.05 was considered statistically significant.**Result:** Both TED and TEDX2 showed reversal of the altered parameters but statistically not significant. Test drugs at therapeutic doses show action almost similar to that of silymarin. Histopathological changes indicated significant hepatic injury in the paracetamol control group. This toxicant-induced injury was found to be reversed moderately at the TED dose and significantly at TED x 2 doses. **Conclusion:** The internal administration of aqueous extract of *Chassaliacurviflora*(Wall.)Thwaites roots is having hepatoprotective action almost similar to that of silymarin in an animal model.

Keywords: *Chassalia curviflora*, hepatoprotective,animal experiment

INTRODUCTION

Chassalia curviflora(Wall.)Thwaites commonly called small curved flower woody Chassalia is a shrub or tree up to 2m tall of the Rubiaceae family. It is mainly distributed in the eastern Himalayas from Sikkim eastwards to Assam, at an altitude of 600 – 1300 m, the Andaman islands, the Western peninsula, and the western ghats.[1] It is mainly used in jaundice by the Kani tribes of Wayanad.[2] The juice of leaves boiled with oil is used for ear and eye diseases, ulcers, and sore throats.[3] Decoction of the root is given as a remedy for phlegm, rheumatism, and pneumonia.[4] It is proven for its antibacterial,[5] antihypertensive,[6] antioxidant[7] and acaricidal[8] activity. Ethanolic extract of leaf showed significant hepatoprotective action in carbon tetra chloride-induced hepatotoxicity.[9]The liver is very much important in carrying out different functions related to metabolic, haemopoietic, and immunological functions of the body. In the present era, changing lifestyles lead to hepato toxicity. Current treatments are expensive, with side effects,but are not very useful to cure disease and for the overall health of the patient. Most of the drugs in *Āyurvēda* for liver disorders are not easy to cultivation and propagation. So finding new medicines is a need. It is a widely grown plant that does not need much care, is easilypropagated, and is cost-effective. The objective of the study was to evaluate hepatoprotective action in animal models.

Materials and Method

Test Drug:

The test drug *Chassalia curviflora*(Wall.)Thwaites were collected from VPSV ayurveda college, Kottakkal campus and authenticated by the department of dravyagunaVijnana. Roots cleaned and dried in shade and coarsely powdered. The aqueous extract was prepared in the phytochemistry laboratory at SDM Centre for research.

Chemicals

The reference standard drug Silymarin was purchased from the market with the trade name Silybon -70 mg, Mfg. Lic NO: MNB/09/592, Mfd- Jan 2017, Exp-feb 2020, Manufactured by micro labs limited, HB – 211, village katha, P.O. Boddi, tehsil, Nalagarhdist, Solan – 173205 (H.P.).Toxicant Paracetamol–Dolo 650, Mfg. Lic NO: M/600/2012, Mfd- Sep 2017, Exp-Aug 2020, Manufactured by micro labs limited, Mamring, Namthang road, Sikkim 737132. The biochemical and enzymatic kits for biochemical investigations were obtained from ERBA Diagnostic Mannheim, Transasia Biochemicals Ltd., Daman.

Animals

Wistar strain albino rats of either sex of body weight ranging from 150 - 270 g were selected for the present study. They were obtained from an animal house attached to S.D.M Centre for Research in Ayurveda and Allied Sciences, Udupi, Karnataka. They were maintained on the feed of "Sai Durga feed and food, Bangalore" and tap water was given *ad-libitum*. The temperature and humidity were kept at optimum, and animals were exposed to natural day and night cycles. The experiments were carried out in conformity with the Institutional Animal Ethics Committee (IAEC) and after obtaining its permission (Approval SDMCRA/IAEC/CPCSEA/17-18/KT- 02)

Treatment protocol

Rats were divided into five groups of six animals in each group (n = 6).From the acute toxicity study, LD50 was found to be more than 2000 mg/kg. Group I(control)received tap water. Group II received paracetamol (3g/kg, p.o),0.5 % gum acacia, and distilled water.Group III(Standard)received Silymarin(100 mg/Kg, p.o). Group IV received *Chassalia curviflora*(Wall.)Thwaites TED(200 mg/kg p.o).Group V received *Chassalia curviflora*(Wall.)ThwaitesTED × 02 (400 mg/ kg p.o).

The Test drug aqueous extract of *Chassalia curviflora*(Wall.)Thwaites and reference drugs were administered orally for 10 consecutive days and one dose of the toxicant (paracetamol) was administered orally to each group, except the water control group, on the 6th and 8th day 1h after test drug administration. After 48 hours of the toxicant Paracetamol, the blood was collected in the tubes and sent to the biochemistry laboratory for biochemical investigations. All the animals were sacrificed by cervical dislocation. The liver was dissected, cleaned to remove extraneous tissues, blotted to remove blood stains, and weighed. A piece of

liver tissue was preserved in 10% formalin for histopathological processing.

Serum was separated and serum level of biochemical parameters namely SGOT, SGPT, glucose, total protein, albumin, globulin, total bilirubin, direct bilirubin, urea, creatinine, cholesterol, and triglyceride were estimated as per the standard procedure prescribed by the manufacturer (AGAPPE diagnostics Ltd., Kerala, India)whereas serum level of ALP was estimated as per the standard procedure described by the manufacturer (Span diagnostics Ltd., Surat, India) of diagnostic kit.

Statistical analysis: The data obtained were 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.

Results

The effect of the test drug, paracetamol, and sylimarin on biochemical parameters are recorded in Tables 1 and 2. Non-significant but moderate elevation of SGOT was observed in the paracetamol control group. This SGOT activity elevation was found to be reduced by test drugs in therapeutic dose & double dose and reference standard- silymarin. And when considering percentage change, the double dose showed a reversal of hepatotoxicity than the standard drug sylimarin and therapeutic dose. The paracetamol control group showed a significant elevation of SGPT activity in comparison to the normal control group. This elevation was reversed by groups given silymarin, test drugs in therapeutic dose & double dose which indicates hepatoprotection of the drugs. A statistically non-significant increase in alkaline phosphatase activity was observed after paracetamol injection. This elevation may be due to moderate cholestasis in the biliary tract leading to liver injury. This elevation was reversed in a non-significant manner by reference standard and therapeutic dose indicating the presence of hepatoprotection in test formulation.

Serum blood sugar level was found to be significantly increased by paracetamol administration in toxic doses. The non-significant reversal was found in the test drug in the therapeutic dose, and reference standard group. Reversal of hyperglycemia, even though non-significant, can be considered an indicator of mild to moderate hepatoprotection. In advanced liver disease, the albumin level is decreased. A mild and statistically non-significant decrease in serum albumin was observed after the administration of paracetamol and the non-significant decrease was found in the therapeutic dose group and standard group. In advanced liver diseases, there will be an increase in globulin levels. Paracetamol produced mild and statistically non-significant increases. In the standard group therapeutic dose group, a non-significant decrease in globulin level was observed which shows hepatoprotective activity while in the double dose group, a non-significant increase was seen. Total bilirubin was significantly elevated in paracetamol control indicating hepatocellular damage. This elevation was not significantly decreased by TED1 and the reference standard group. This may be indicative of the presence of hepatoprotective activity in TED1. But was not significantly increased by the drug in double doses. Direct bilirubin level was non significantly increased in the paracetamol control group. Eventhough the reversal is statistically non-significant in TED1, the test drug was found to decrease direct bilirubin which may be indicative of the hepatoprotective activity of the test drug.

Elevation of blood urea can be considered a good index of paracetamol-induced liver injury – its reversal as an index of hepatoprotection by the test drug. Thus significant reversal in therapeutic dose can be considered as an index of hepatoprotection of the drug. The toxicant paracetamol increased the creatinine level significantly in the serum. The involvement of impaired kidney function in liver disorders reveals the impact of impaired lipid metabolism on kidney function leading to the elevation of serum creatinine levels. In the present study, significant reversal was observed in the therapeutic and double dose and non-significant reversal in the standard group.Significant elevation in serum cholesterol level was observed which may be indicative of toxicant-induced cholestasis. However, this elevation in serum total cholesterol level was reversed by groups given the standard drug sylimarin, test drug in the therapeutic dose, and double dose.

Table. 1 Serum biochemical parameters

GROUP	SUGAR	SGOT	SGPT	ALP	TOTAL PROTEIN	ALBUMIN	GLOBULIN
Normal control	102.33±7.43	135.83±6.12	63.66±2.24	451.66±97.98	7.01±0.13	3.63±0.14	3.38±0.22
Paracetamol control	148.83±12.85*	197.14±41.10	158.57±38.27*	563.00±61.74	7.28±0.17	3.32±0.16	3.84±0.16
Standard	125.0±7.62	193.71±14.80	104.85±13.01	432.75±14.19	6.30±0.22**	2.87±0.14	3.42±0.24
TED	127.42±9.85	180.0±4.86	136.2±18.27	511.33±124.16	6.50±0.19*	2.95±0.22	3.55±0.14
TED*2	211.2±15.24**	150.8±39.11	92.6±25.43	735.00±52.12	8.26±0.15*	4.20±0.14**	4.06±0.26

Data : MEAN±SEM, **P<0.01, * P<0.05

Table. 2 Serum biochemical parameters

GROUP	TOTAL BILIRUBIN	DIRECT BILIRUBIN	UREA	CREATININE	CHOLESTEROL	TRIGLYCERIDE
Normal control	0.17±0.10	0.09±0.01	34.66±1.30	0.36±0.02	47.33±2.65	94.33±8.96
Paracetamol control	0.33±0.04*	0.18±0.00	40.4±3.77	0.94±0.04**	118.6±9.94**	67.8±14.00
Standard	0.22±0.02	0.10±0.01	26.6±1.03**	0.72±0.05	65.71±6.33	93.00±10.49
TED	0.26±0.03	0.12±0.02	25.0±2.20**	0.54±0.12**	67.6±9.57	88.4±12.92
TED*2	0.35±0.04	0.24±0.11	34.2±4.10	0.40±0.04**	67.8±4.42	196.6±54.99**

Data : MEAN±SEM, **P<0.01, * P<0.05

Histopathological study of the normal control group showed normal cyto architecture with hepatic cells, Kupffer cells, central vein, portal tracts, etc(figure 1A). The Paracetamol control group showed severe and extensive necrotic changes, fatty degenerative changes, cell infiltration, central vein dilatation, haemorrhage streaks, and infarcts indicating moderate to severe (3/4- grade) hepatic injury(figure 1B). The standard group showed moderate to a significant reduction in hepatotoxicant-induced degenerative changes. The sections looked almost normal in some rats(figure 1C). TED group showed micro fatty degenerative changes, central vein dilatation, haemorrhage streaks, and cell depletion of moderate intensity in sections from two rats; mild degenerative changes were observed in sections from one rat, and in the remaining two rats almost normal cytoarchitecture was observed. Mild to moderate overall changes (2/4)were observed. The overall inference is moderate to good protection in the majority of the rats(figure 1D). TEDX2 group showed mild to moderate micro fatty changes in sections from one rat, mild degenerative changes in the form of central vein dilatation, periportal cell infiltration was observed in sections from two rats, normal profile in sections from one rat; in the sixth rat, moderate necrotic changes and central vein dilatation was observed. The severity was much less in comparison to the positive control group. Overall severity is much less in comparison to the positive control- 1/4

grade(figure 1E).

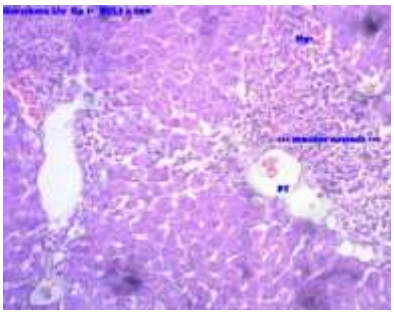
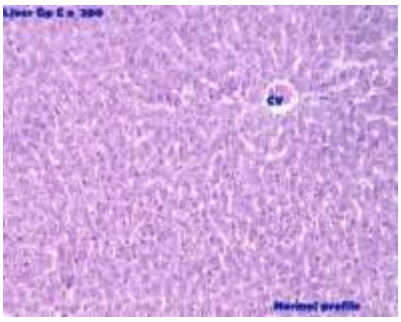


figure 1A



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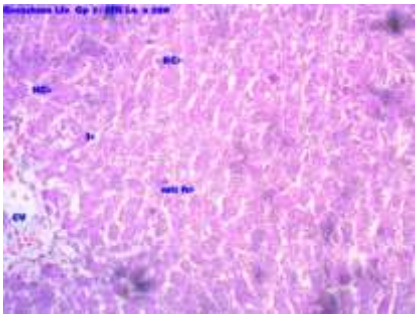

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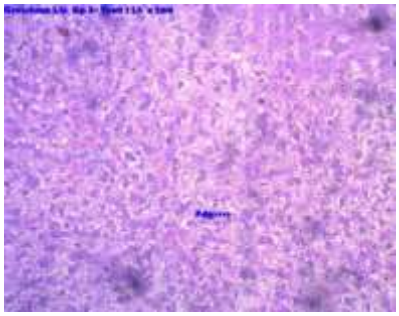

figure 1D

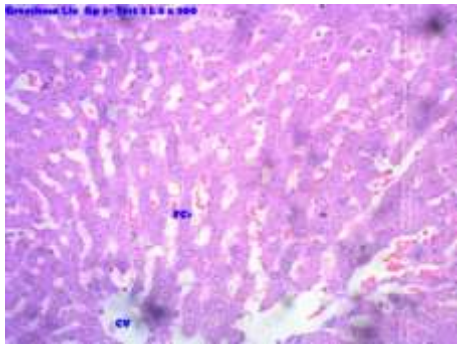

Figure 1E

CV – central vein NC- necrosis PT – portal tract HC – hepatic cell FC – fatty changes Fdg - fatty degenerative change S – sinusoides

Fig 1A: normal control showing normal cytoarchitecture **Fig 1B**: paracetamol control group showing massive necrosis and fatty changes **Fig 1C**: standard group showing moderate to a significant reduction in the hepatotoxicant induced degenerative changes **Fig 1D**: TED group showing mild to moderate reduction in degenerative changes **Fig 1E**: TEDX2 group showing significant reduction in degenerative changes.

DISCUSSION

The present study was undertaken to assess the hepatoprotective activity of the drug in paracetamol-induced toxicity.Even though paracetamol is considered safe at therapeutic doses, at higher doses, it produces centrilobular hepatic necrosis that can be fatal. Hepatotoxicity is initiated by the formation of a reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI), which depletes cellular glutathione and forms protein adducts on mitochondrial proteins. This leads to mitochondrial oxidative and nitrosative stress, which eventually leads to loss of the ability of the mitochondria to synthesize ATP which leads to

necrosis.[10] During hepatic damage, cellular enzymes like AST, ALT, and ALP present in the liver cells leak into the serum, resulting in increased concentrations. The oral administration of the test drug resulted in the control of these hepatic parameters. Pharmacological studies on the drug showed the presence of alkaloids, flavonoids, tannins, saponins, steroids, and gylcosides.[11] Many flavonoids are reported for their hepato protective activities. Functional hydroxyl groups in flavonoids mediate their antioxidant effects by scavenging free radicals or by chelating metal ions.[12] Studies have illustrated the beneficial effects of saponins on blood cholesterol levels, cancer, bone health, and the stimulation of the immunesystem[13]. Tannins are strong antioxidants, with anti-inflammatory and antiviral activities. Hence the hepatoprotective activity of *Chassalia curviflora*(Wall.)Thwaitesmay be due to the presence of these flavanoids and tannins.

CONCLUSION

Based on the biochemical parameters, the study of the drug at therapeutic dose shows hepatoprotective action almost similar to that of silymarin. Based on the histopathology report toxicant-induced injury was found to be reversed moderately at the TED dose and significantly at TED x 2 doses.Hence it can be concluded that the internal administration of the aqueous extract of *Chassalia curviflora*(Wall.)Thwaites is having hepatoprotective action almost similar to that of silymarin in an animal model.

REFERENCES

1. Hooker J D. Flora of British India. New Delhi: periodical expert book agency;1997. p. 178; vol3
2. Mahajan B, Ramadevi R. folk medicine for Yakritvikara (liver disorder) – a scientific review. IAMJ[internet].2015 June[cited on 2017 jan27]; 3(6):33.
Available from: http://iamj.in/posts/images/upload/1828_1847.pdf
3. Gupta A K, Sharma M, editors. Review on Indian Medicinal plants. New Delhi: Medicinal plants unit, Indian council of medical research;2008. p. 11; vol 6.
4. Nair C K N, Mohanan N. medicinal plants of India with special reference to ayurveda. Delhi: NAG publishers; 1998. p. 113.
5. Raja RD, Jeeva S, Prakash JW et al. Antibacterial activity of selected ethno medicinal plants from south India. Asian Pac J Trop Med. 2011 may; 4(5): 378-8. doi: 1o.1016/S 1995-7645(11)60107- 7.Epub 2011 jan22.
6. Hazirah WN, Omar NA. Antihypertensive activity and phytochemical analysis of *Chassaliacurviflora* (Wall.)Thwaites extracts. IJIMS[Internet]. 2014[cited 2017 jan25]; 2(1): 163-174. Available from http://imsear.li.mahidol.ac.th/bitstream/123456789/176314/1/ijims2014v2n1p163.pdf
7. Krishnamoorthy k, Senguttavan J, Krishnaswamy T. Evaluation of phytochemicals and invitro antioxidant activities of some selected Indian medicinal fruits from kannur city, Kerala.WJPPS[internet] .2013 sep[Cited 2017 Jan 25];2(5):4121-4138.Available from file:///C:/Users/USER/Downloads/article_wjpps_1382972979.pdf
8. Krishna Ajeesh TP, Krishna adarsh JP, Juliet Sanis, et al. Pharmaco-chemical characterization and acaricidal activity of ethanolic extract of Chassaliacurviflora (Wall ex Kurz.)Thwaites. Phcog J.2016;8(3):215-19. doi 10.5530/pj.2016.3.6
9. Gopal G R, Anuja G I, Latha P G et al. Anti-Hepatotoxic Property of Roots and Leaves of *Chassaliacurviflora* (Wall.) Thwaites against CCl4 Induced Liver Damage in Rats. IJPSDR[Internet]. 2016[cited 2017 jan27];8(5):269-74. Available from http://www.ijpsdr.com/pdf/vol8-issue5/6.pdf
10. Jack A. Hinson, Dean W. Roberts, and Laura P. James, Mechanisms of Acetaminophen- Induced Liver Necrosis,HandExp Pharmacol.2010;(196):369
11. Martins D, Nunez C V. Secondary Metabolites from Rubiaceae Species.Molecules 2015;*20*: 13422-13495. doi:10.3390/molecules200713422
12. Shashank Kumar et al, Chemistry and Biological Activities of Flavonoids: An Overview the Scientific World Journal Volume 2013 (2013), Article ID 162750, 16 pages http://dx.doi.org/10.1155/2013/162750)
13. Neeraj Varma etal, Phytoconstituents and Their Mode of Extractions: An Overview Res J. Chem. Environ. Sci. Vol 4 [2] April 2016: 08-15 Online ISSN 2321-1040 CODEN: RJCEA2 [USA] ©Academy for Environment and Life Sciences, INDIA Website: www.aelsindia.com/rjces.html)

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