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HEPATOPROTECTIVE EFFECT OF AYURVEDIC FORMULATION IN ANTITUBERCULOSIS DRUGS INDUCED HEPATOTOXICITY IN WISTAR ALBINO RATS

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ABSTRACT

Introduction: Anti-tuberculosis drug induced hepatotoxicity is a major hindrance in the cure of tuberculosis. In conventional health science the therapeutic drug are withhold when evidence of liver damage is observed and is reintroduced after normalization of liver enzymes. Present article reports the hepato-protective effect of AYU-HP, an Ayurvedic formulation in ATT induced hepatotoxicity in experimental study. **Method:** The test drug was administered in Wistar rats concomitantly with the ATT (combination of H=100mg/kg, R= 300mg/kg and Z=700mg/kg) drugs for 21 days. The test drug was assessed in two dose level and compared their effect on hepatotoxicity with known hepatoprotective drug Sylimarin. The assessment parameters used were AST, ALT, ALP, Tot. Bilirubin and histological examination of liver. **Results:** Significant reduction in AST and ALT was observed in group treated with AYU-HP in both the dose levels as compared to ATT treated group which is comparable to standard hepatoprotective drug Sylimarin. In disease control group the swelling of hepatocytes, compressed & congested sinusoids, focal collection of lymphocytes & microphages near central vein was observed. In groups treated with AYU-HP & SY intact architecture of the liver tissue was noted. **Conclusion:** An herbal formulation AYU-HP has demonstrated hepatoprotective effect in Wistar albino rat model against anti-tuberculosis drug induced hepatotoxiicty.

KEYWORDS: Anti-tuberculosis drugs, Hepatotoxicity, Herbal formulation, Hepatoprotective drugs.

INTRODUCTION

Tuberculosis, caused due to tubercle bacillus has been recognized as a clinical entity since the ages of Hippocrates (460-370BC). About one third of the world's population has latent TB and roughly 9 million cases of active TB emerge annually resulting in 2-3 million deaths.[1] The figures exhibit the magnitude and importance of the problem, especially when most new cases emerge in populated nations like India and China.[2]

To prevent acquired resistance and a successful treatment; it is recommended to receive a combination chemotherapy containing Isoniazid (H), Rifampicin (R) and Pyrazinamide (Z) with or without ethambutol.[3] But drug induced hepatotoxicity is a potentially serious adverse effect associated with this regimen.[4] Studies showed that oxidative stress and idiosyncratic reactions play major role as a causative factor of hepato-toxicity due to ATT drugs.[5]

To manage the ATT induced hepato-toxicity it is recommended to withhold the therapeutic drug when observed an evidence of liver damage and reintroduce the same after normalization of liver enzymes.[6-7]

It has been seen that most of the patients suffering from tuberculosis face difficulty to complete the prescribed treatment course due to hepatotoxicity caused by this drugs regimen. Hence there is need of adjuvant therapy which could reduce the hepatotoxicity of these therapeutic drugs.

Concept of drug induced toxicity is considered in Ayurved under the branch of Agadtantra and therapeutic drug induced toxicity can be equated with *Kritrim Visha.* Numerous Ayurvedic formulations prescribed for the treatment of *Kritrim Visha* induced toxicity may be used for such illness.

Present article reports the hepato-protective effect of AYU-HP, an Ayurvedic formulation in ATT induced hepatotoxicity in experimental study.

MATERIALS AND METHOD

Part I- Preparation of the herbal formulation

The ingredients used for test drug- AYU-HP were *Bhumyamalki - Phyllanthus amarus* (Schmach & Thonn), *Guduchi- Tinospora cordifolia* (Willd), *Kalmegh-Andrographis paniculata* (Brum. F.), *Rohitaka- Tecomella undulate (*Sm.), *Kutaki- Picrorhiza kurrooa*

(Royle). The authentication of individual ingredients of AYU-HP was done in Aagharkar Research Institute, Pune. The physico-chemical analysis of raw material & finished product was done as per the *Ayurvedic* Pharmacopeia India norms and was found to be matched with the given standard values. Preparation of the herbal formulations in the *Kashaya* (decoction) form was done following all the steps as per guidelines laid by *Sharangadhar Samhita* and the Standard Operating Procedures.

Part II- Conduction of experimental study

Permission of Institutional Animals Ethics Committee (IAEC) was taken prior the conduction of experiment. The study was conducted at CPCSEA approved Central Animals House.

Antituberculosis drugs were used to induce hepatotoxicity in the dose of H- 100, R-300 and Z-700 mg/kg/d by oral route dissolved in distilled water.[8] *Sylimarin* a known hepatoprotective drug was purchased from Sigma Aldrich in pure powdered form and was given by oral route dissolving in distilled water in the dose of 100mg/kg/d.[9] The test drug was administered in two dose levels as X/2 = 0.70ml and X= 1.44ml/200gm/day. Liver enzyme activities ALT, AST, ALP and Total Bilirubin were measured using the bioassay kits, procured by Coral clinical system of Tulip group.

Experimental methodology

40 Wistar albino rats of either sex weighting 150-200 g. were equally allotted in five groups having eight animals in each group. The animals were housed in air-conditioned rooms [23-30^{0}C; Humidity 50-60%]. The animals were maintained at controlled environmental condition with natural light and dark cycle of 12 hours. The animals were maintained on a standard pellet diet and allowed to eat *ad libitum.*

The first group served as a control group and distilled water was given. In second group hepototoxicity was induced with antituberculosis drugs. In group three, four and five hepatotoxicity was induced and concomitantly sylimarin, test drug in two dose levels as X/2 and X was administered respectively. All the drugs were administered for 21 days. On 22nd day blood was collected by cardiac puncture under light ether anesthesia. First abdomen was opened by taking a midline incision. Then diaphragm was cut with precaution to expose beating heart. By using 24 gauge needles, blood was collected slowly from left ventricle for biochemical assay. Liver was dissected by cutting surrounding attachments and was sent for histopathological investigation.

Parameters of assessment

A. The following biochemical parameters were used.

- Serum alanine aminotransferase (ALT) assessed by Reitman & Frankels method[10]
- Serum aspartate aminotransferase (AST) assessed by Reitman & Frankels method[10]
- Alkaline phosphatase (ALP) assessed by Mod. Kind & King's method[11]
- Serum total bilirubin (TB) assessed by Frederick C. Peariman and Robert T. V. Lee method[12]

B. Histopathological parameters - Histopathological assessment of liver damage was done by using hematoxylin and eosin staining, paraffin block method.[13]

Statistical analysis: Data was expressed as Mean ± S.D. The parametric data was analyzed using One way ANOVA followed by Tuckey's post hoc-test, Non-parametric data was analyzed using chi square test. A value of $p<0.05$ was considered to be statistically significant. All statistical tests were carried out using Graphpad Instat software version 3.

RESULTS

The results obtained in the experiments have been shown in the following table.

Table no. 01: Results of test drugs on biochemical parameters of hepatic injury.

Groups	AST U/ml	ALT U/ml	ALP KA Units	Tol. Bil mg/dl
DW	149.08 ±40.14	74.3 ± 10.15	8.35 ± 5.82	0.28 ± 0.11
ATT	338.28 ± 77.46***	127.46 ± 26.48**	35.34 ± 25.27***	2.43 ± 1.92**
SY	171.78 ± 47.49##	75.09 ± 17.29##	15.56 ±3.07	1.68 ± 1.86
HP-1	212.22 ±29.77##	34.16 ± 7.81##	33.50 ± 21.33	1.67 ± 0.59
HP-2	248.77 ±38.79##	59.81 ± 18.06##	21.25 ±8.78	1.57 ± 0.83

*p<0.05, **p<0.01, ***p<0.001 as compared to DW

# p<0.05, ##p<0.01, ###p<0.001 as compared to ATT

Significant reduction in AST and ALT was observed in group treated with AYU-HP in both the dose levels as compared to ATT treated group which is comparable to standard hepatoprotective drug Sylimarin. No Significant reduction was noted in total bilirubin and alkaline phosphate in test drug and Sylimarin treated group.

In histopathological findings the disease control group demonstrated the swelling of hepatocytes, compressed & congested sinusoids, focal collection of lymphocytes &

microphages near central vein was observed. In groups treated with AYU-HP & SY intact architecture with no evidence of inflammation and congestion of the liver tissue was seen.

DISCUSSION

In Ayurvedic purview toxicity is a condition of '*Vishad'* and the drugs produces '*Vishad'* are *Visha*.[14] Hence all those drugs which produce delirious effect on body can be considered as '*Visha'*.

In conventional health science with advancement of science numerous therapeutic drugs have been evolved. These drugs increase the life span of individuals and provide an admirable protection against the bacterial infection. But drug induced liver injury is an associated adverse effect seen with many of the drugs and is the most common cause for a drug to be withdrawn from the market. First line anti-tuberculosis drugs (ATT) are one of them. Anti-tuberculosis drugs are effective to provide complete cure from *Mycobacterium tubercle* but drug induced hepatotoxicity is a one of the frequent and potentially serious adverse effect associated which can lead to therapy discontinuation. The Anti-tuberculosis treatment regimen must be discontinued once liver injury occurs, which may result in TB relapse, drug resistance and TB–related death. Considering the high incidence of TB and anti-tuberculosis drug-induced hepatotoxicity (ATDH) in Asian population especially Indian patients it is essential to find adjuvant formulation to prevent hepatotoxicity caused due to ATT drugs.

In the experiment it was noted that the liver injury biomarker ALT & AST showed significant reduction and the levels of ALP and total bilirubin demonstrated non-significant reduction in AYU-HP treated group. ALT & AST are bio-markers indicating hepatocyte injury. But ALT is more specific to liver architecture injury whereas elevation of AST may be seen in muscle injury also. ALP is used to detect blocked bile ducts. Total bilirubin levels are seen to be elevated in any condition that affects the processing and elimination of bilirubin or accelerates the breakdown of RBCs.

The rationale behind the above results may be explained by applying Ayurvedic concepts. The clinical manifestation of the toxicity induced by ATT drugs closely resemble to the symptoms of *Vidagdhājīrna*.[15] resulting from the *Uśan & Vidāhi dravya*. Thus it can be assumed that the ATT drugs possess *Uśan & Vidāhi* attributes and vitiate *pitta dosha.* As per the concepts of Ayurved the *Vidāhi* drugs are also known causative factors of *Raktavaha srotoduṣtī.*[16] *Rakta dhātū* and *Pitta Doṣa* shares *Āsray and Āsrayī bhāv* amongst them.[17]

The *Mūlasthāna* (Root) of *Raktavaha srotas* is quoted as '*Yakrit'*(Liver).[18] Hence any causative factor affecting *Rakta dhatu* will also affect its *mulasthan Yakrit* reflecting as hepatotoxicity. Besides one of the late manifestations of hepatotoxicity is Jaundice which is mentioned as *Kāmalā* in *Āyurvediya* terminology and is quoted as one of the *Raktapradoṣaja vyādhi*(blood disorders) by *Carakācārya.[19]*

The ingredients of AYU-HP, the test drug used in this experiment are *Bhumyamalki(Phyllanthus amarus* Schmach & Thonn), *Guduchi (Tinospora cordifolia* Wild), *Kalmegh (Andrographis paniculata* Brum. F), *Rohitaka (Tecomella undulate* Sm.*) Kutaki (Picrorhiza kurrooa* Royle.) The attribute of the individual ingredients of test drug was critically reviewed it was found that the dominant rasa of this formulation is *Tikta* which is *vishaghna* and *pitta shamak*. The possible *Samprapti bhanga* with the test drugs may be justified with the attributes of the test drugs.

Hetuviparit chikitsa' is a basic line of treatment adopted in Ayurved. Amongst the six rasa *Tikta rasa* (bitter) has been quoted as *Vishaghana*(antitoxic) in nature.[20]

Also in *Rasavaiśeṣika Sūtra*, chapter four *Tikta rasa* has been quoted to have opposite attribute to that of *Vidāhakar dravya* like ATT drugs. Hence *Tiktarasātmaka dravya* may correct the *Pitta Doṣa* and thereby *Raktaduṣtī* which may affect the *Mūlasthān -Yakrit* and ultimately may play role in the correction of *Yakritvikār* (Disorders of liver). Hence the *tiktarasatmaka* ingredients of the test drug having antitoxic action may have shown preventive effect in ATT induced toxicity.

The contemporary literature review revealed that the individual herbs of test drug have an ethano-medicinal status with high cultural index. Moreover, have been extensively researched for various clinical and experimental studies and demonstrated a variety of pharmacological activities especially hepatoprotective, antioxidant, antimicrobial. *Bhumyamalki* has exhibited hepatoprotective activity against carbon tetrachloride(CCl_4)[21] ethanol[22-23] and aflatoxin B[24] induced hepatotoxicity. *Guduchi* has shown prevention of hepatotoxicity in CCL_4[25-26] Lead,[27] antitubercular drug induced toxicity[28] Researcher has demonstrated the preventive effect of *Guduchi* on the Kuffer cells in experimental study[29] Pretreatment of *Kalmegha* extract prevented the hepatotoxicity induced by CCl_4 & t BHP[30-31] It was found to have hepatoprotective activity against galactosamine and paracetamol in an experimental model.[27,32,33] *Kalmegh* also showed hepatoprotection in ethanol toxicity[27] *Rohitak,*

(Tecomella undulate) exhibited hepatoprotective activity against thioacetamine and paracetamol[34-35] The hepatoprotective activity by the *Rohitak* is attributed to betulinic acid[36] Another ingredient of AYU-HP *Kutaki (Picrorhiza kurroa)* is also a hepatoprotective drug. Kutaki has shown the hepatoprotective activity in alcohol[37] galactamine[38] Paracetamol, [39-40] CCl4[37], and antitubercular drugs isoniazid and rifampicininduced hepatotoxicity[41] in various animal models. Hepatoprotective activity is also observed in fatty liver disease caused due to Aflatoxin[42] *Kutaki* has been found to be effective in viral hepatitis in clinical study.[35] Thus the reported studies supports the hepatoprotective finding of the test drugs on the basis of biochemical and histopathological findings.

CONCLUSION

Thus from the bio-chemical and histo-pathological findings it may be concluded that AYU-HP an herbal formulation is effective to prevent ATT induced hepatotoxicity in Wistar albino rats.

REFERENCES

1. Raviglione MC, Snide R, Kochi A. Global epidemiology of tuberculosis. Morbidity and mortality of a worldwide epidemic. JAMA, 1995; 273: 220-6.
2. World Health Organization. Global tuberculosis control: surveillance, planning, financing. WHO report 2002.Communicable Diseases published by WHO Press, Geneva, Switzerland, 2002; 237.
3. Chan ED, Iseman MD. Current medical treatment for tuberculosis. BMJ, 2002; 325: 1282-6.
4. Mahashur AA, Prabhudesai PP. Hepatitis and antitubercular therapy. J Assoc Physicians India, 1991; 39: 595-6.
5. Jussi J, Saukkonen, David LC, Robert M, Jasmer, Steven Schenker, *et.al*. on behalf of the ATS Hepatotoxicity of antituberculosis Therapy Subcommittee An official ATS Statement. Hepatotoxicity of Antituberculosis Therapy Am J Respir Crit Care Med, 2006; 174: 935-52.
6. Teleman MD, Chee CB, Earnest A, Wang YT. Hepatotoxicity of tuberculosis chemotherapy under general programme conditions in Singapore. Int J Tuberc Lung Dis, 2002; 6: 699-705.
7. Wada M. The adverse reactions of anti-tuberculosis drugs and its management. Nippon Rinsho, 1998; 56: 3091-5.

8. Kapgate SM, Patil AB. Standardization of Model of Induction of Hepatotoxicity with Antituberculosis Drugs in Wistar Albino Rats. Asian J Pharm Clin Res, 2017; 10: 150-3.
9. Vijaya P, Suja VV, Shyamala CS. Hepatoprotective Effect of Liv.52 on Antitubercular Drug-induced Hepatotoxicity in Rats. Fitoterapia, 1998; 6: 520-3.
10. Reitman S, Frankel S. Colorimetric method for determination of glutamate oxaloacetate and glutamic pyruvic transaminase. Am J Clin Pathol, 1957; 28: 56–62
11. P. R. N. Kind and E. J. King Estimation of Plasma Phosphatase by Determination of Hydrolysed Phenol with Amino-antipyrine J Clin Pathol, 1954; 4: 322–6.
12. Frederick C. Peariman, Robert T. V. Lee. Detection and Measurement of Total Bilirubinin Serum, with Use of Surlactants as Solubilizing Agents. CLIN. CHEM, 1974; 20: 447-53.
13. Gamble M. The hematoxylins and eosin. In: Bancroft JD, Gamble M, editors. Theory and Practice of Histological Techniques. London: Churchill Livingstone Elsevier, 2008; 6: 121-4.
14. Yadavji Trikamji, editor. Charak Samhita Chikitsasthan Varanashi: Chaukhambha Publishers, 2004; 570: 23-5.
15. Pt. Hari Sadashiva Shastri Paradkar editor. Ashtanghridaya Sutrasthan Varanashi: Chukhamba Surbharati Prakashan Publishers, 1935; 6: 153.
16. Yadavji Trikamji editor. Charak Samhita Vimansthan Varanashi: Chaukhambha Publishers, 2004; 6: 251.
17. Pt. Hari Sadashiva Shastri Paradkar editor. Ashtanghridaya Sutrasthan Varanashi: Chukhamba Surbharati Prakashan Publishers; 1935; 186(6): 11-25.
18. Yadavji Trikamji editor. Charak Samhita Vimansthan Varanashi: Chaukhambha Publishers, 2004; 250: 5-8.
19. Yadavji Trikamji editor. Charak Samhita Sutrasthan Varanashi: Chaukhambha Publishers, 2004; 179: 28, 11-13.
20. Pt. Hari Sadashiva Shastri Paradkar editor. Ashtanghridaya Sutrasthan Chukhamba Surbharati Prakashan Publishers, 1935; 176: 10, 14-16.
21. Yadav NP, Pal A, Shanker K, Bawankule DU, Gupta AK, Darokar MP *et al*. Synergistic effect of silymarin and standardized extract of Phyllanthus amarus against CCl_4-induced hepatotoxicity in Rattus norvegicus. Phytomedicine, 2008; 15: 1053–61.
22. Pornpen P, Chanon N, Somlak P, Chaiyo C. Hepatoprotective activity of Phyllanthus amarus extract in ethanol treated rats: In vitro and in vivo studies. Jour of Ethnopharmacology, 2007; 114: 169–73.

23. Adeneye AA, Benebo AS, Agbaje EO. Protective Effect of the Aqueous Leaf and Seed Extract of Phyllanthus amarus on Alcohol-Induced Hepatotoxicity in Rats. West African Journal of Pharmacology and Drug Research, 2007; 22(23): 42-50.
24. Naaz F, Javed S, Abdin MZ. Hepatoprotective effect of ethanolic extract of Phyllanthus amarus Schum. et Thonn. on aflatoxin B1-induced liver damage in mice. J Ethnopharmacol, 2007; 2: 503-9.
25. Biswadev B, Roychowdhury S, Ghosh S, Sengupta M. Hepatoprotective and immunomodulatory properties of Tinospora Cordifolia in CCl_4 intoxicated mature Albino Rats. Journal of Toxicological Sciences, 2002; 27: 139-46.
26. Kavitha BT, Shruthi SD, Rai PS, Ramachandra YL. Phytochemical analysis and hepatoprotective properties of Tinospora cordifolia against carbon tetrachloride-induced hepatic damage in rats. J Basic Clin Pharm, 2011; 2: 139-42.
27. Sharma V, Pandey D. Protective role of *Tinospora cordifolia* against lead-induced hepatotoxicity. Toxicol Int, 2010; 17: 12-7.
28. Panchabhai TS, Ambarkhane SV, Joshi AS, Samant BD, Rege NN. Protective effect of Tinospora cordifolia, Phyllanthus emblica and their combination against antitubercular drugs induced hepatic damage: an experimental study. Phytotherapy Reseach, 2008; 22: 646–50.
29. Nagarkatti DS, Rege NN, Desai NK, Dahanukar SA. Modulation of Kupffer cell activity by Tinospora cordifolia in liver damage. J Postgrad Med, 1994; 40: 65-7.
30. Kapil A, Koul IB, Banerjee SK, Gupta BD. Antihepatotoxic effects of major diterpenoid constituents of Andrographis paniculata. Biochem Pharmacol, 1993; 46: 182-5.
31. Tatiya AU, Surana SJ, Sutar MP, Gamit NH. Hepatoprotective effect of polyherbal formulation against various hepatotoxic agents in rats. Phcog Res, 2012; 4: 50-6.
32. Nagalekshmi R, Menon A, Chandrasekharan DK, Nair C K. Hepatoprotective activity of Andrographis Paniculata and Swertia Chirayita. Food Chem Toxicol, 2011; 49: 3367-73.
33. Handa SS, Sharma A. Hepatoprotective activity of andrographolide against galactosamine & paracetamol intoxication in rats. Indian J Med Res, 1990; 92: 284-92.
34. Khatri A, Garg A, Agrawal SS. Evaluation of hepatoprotective activity of aerial parts of Tephrosia purpurea L. and stem bark of Tecomella undulate. J Ethnopharmacol, 2009; 25: 1-5.

35. Patel KN, Gupta G, Goyal M, Nagori BP. Assessment of hepatoprotective effect of Tecomella undulata (Sm.) Seem., Bignoniaceae, on paracetamol-induced hepatotoxicity in rats. Rev. bras. Farmacogn, 2011; 21: 133-8.
36. Jain M, Kapadia R, Jadeja RN, Thounaojam MC, Devkar RV, Mishra SH. Hepatoprotective potential of Tecomella undulata stem bark is partially due to the presence of betulinic acid. J Ethnopharmacol, 2012; 143: 194-200.
37. Saraswat B, Visen PK, Patnaik GK, Dhawan BN. Ex vivo and in vivo investigations of picroliv from Picrorhiza kurroa in an alcohol intoxication model in rats. J Ethnopharmacol, 1999; 66: 263-9.
38. Vaidya AB, Antarkar DS, Doshi JC, Bhatt AD, Ramesh VV, Vora PV *et al*. Picrorhiza kurroa (Kutaki) Royle ex Benth as a hepatoprotective agent-experimental & clinical studies. J Postgrad Med, 1996; 42: 105.
39. Dwivedi Y, Rastogi R, Garg NK, Dhawan BN. Prevention of paracetamol-induced hepatic damage in rats by picroliv, the standardized active fraction from *Picrorhiza kurroa*. J Phytother Res, 1991; 5: 115-9.
40. Mogre K, Vora KK, Sheth UK. Effect of Picrorrhiza kurroa and Eclipta alba on Na+K+ATPase in hepatic injury by hepatotoxic agents. IJP, 1981; 13: 253-9.
41. Jeyakumar R, Rajesh R, Meena B, Rajaprabhu D, Ganesan B, Buddhan S *et al.* Antihepatotoxic effect of *Picrorhiza kurroa* on mitochondrial defense system in antitubercular drugs (isoniazid and rifampicin)-induced hepatitis in rats." J.Med. Plant. Res, 2008; 2: 17-9.
42. Shetty SN, Mengi S, Vaidya R, Vaidya AD. A study of standardized extracts of Picrorhiza kurroa Royle ex Benth in experimental nonalcoholic fatty liver disease. J Ayurveda and Integra Med, 2010; 1: 203-10.