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HEPATOPROTECTIVE ACTIVITY OF *RHODIOLA ROSEA* ON RATS

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Abstract
Rhodiola rosea has been used as food and folk medicine to recover several diseases. In the present study were evaluated effect of ethanolic extract of *R.rosea* on Isoniazid and Rifampicin induced liver injury on rats. Isoniazid and Rifampicin was administered a 100 and 50 mg/kg bw dose to induced hepatotoxicity. *R.rosea* (400 and 600mg/kg, p.o) and Silymarin 100mg/kg, p.o. were administered once daily for 28 days. The liver injury was estimated by liver function function parameters ALP,AST,ALT,TP andTB. Oxidative stress markers GSH, MPO,MDA,SOD CAT as well histopathology of liver. Isoniazid and Rifampicin drugs significantly reduction in liver weight, increases in serum liver biochemical, decreses in Oxidative stress and changes in histopathology of liver. Treatment with *R.rosea* in a dose depended manner significantly changing in weight liver, liver function estimation , lipid peroxidation and histopathology of liver compare to control and Silymarin group. The present studies evidence that the ethanolic extract of *R.rosea* has shown hepatoprotective activity.

Key words: *R.rosea.* INH+RF,Liver injury and CAT.

INTRODUCTION

Tuberculosis (TB) a curable respiratory ailment instigated by *Mycobacterium tuberculosis*; mostly affecting the poor countries of Africa and Southeast Asia. According to World Health Organization (WHO), its prevalence recorded was 14 million, while 2.38 million deaths were estimated. Fixed dose combination followed by continuous treatment of Rifampicin and Isoniazid for 4–6 months [1]. However, this regimen causes hepatic injuries in clinical settings [2]. The clinical symptoms of anti-TB drug appear in nonspecific elevation of transaminases to fulminant of liver failure [3]. Hepatic injuries have been induced with isoniazid and rifampicin in experimental animals [4].

Rhodiola rosea common name ‘golden root’ L. of family (Crassulaceae) is a medicinal plant which grows throughout North Asia and the mountains of central Europe. For several centuries the plant has been documented as useful in the treatment of a extensive range of illnesses including Stress, stimulating the CNS system, decreasing depression, enhancing work performance, eliminating fatigue, preventing high altitude sickness, and wound healing, skin burns and contusions[5]. More recently *R. rosea* has been shown to have anti-hypoxic activity[6] , antioxidant activity[7], anti-prostate cancer and antibacterial activity[8] [4], anti-hepatic cancer activity[9][5] and the ability to

enhance learning and memory[10][6]. *R. rosea* extract is a valuable therapeutic as it does not exhibit detectable toxicity throughout a concentration range which exceeds concentrations responsible for its many biological activities [11][7].
However our present study was ethanolic extract of *R.rosea* on Isoniazid and Rifampicin induced liver damage on rats .

Materials and methods
Dried roots of *Rhdiola rosea* (*R. rosea*) (Golden root) family Crassulaceae were procured from local vendor, Seremban, Negerisembilan,Malaysia. Roots are authenticated by Dr. Long chiau Ming, Associated Professor, Deputy Dean faculty of Pharmacy, Quest international university Perak.(QUIP-RR/01/2019).

PREPARATION OF EXTRACTION
The roots material was powdered using mixer grinder and passed through sieve no 85. About The dried 150gm powder was subjected to soxhlets apparatus extraction using Ethanol solvent for 72 hrs. The extract were concentrated in rotary flash evaporators and stored in refrigerator

Preliminary phytochemical analysis: the extracts were then subjected to preliminary phytochemical analysis to assess the presence of various phytoconstituents .

Experimental animals procured
Adult wistar rats of male 9 to 11 week age, weighing 160–180gm were procured from Mahaveera enterprises, Hyderabad. Animals were housed in standard laboratory conditions at 25°c with 12 hr light-dark cycle with free access to chow and water *ad libitum*. The research protocol was approved by (HKES/COP/MTRIPS/IAEC/140/2022)

Evaluation of HepatoProtective Activity:
Hepatic injury: A dose of 50 mg/kg and 100 mg/kgb.w Isoniazid and Rifampicin respectively in Aqueous 1% CMC through oral for 28days.
The study design is divided into 5groups, six rats in each as mention in (table 1).

Group No	No. of Rats	Treatment	Dose
1	6	Control-Aqueous 1% CMC	10ml/kg b.w
2	6	Positive control – INH + RIF	50 mg/kg +100 mg/kg btw,
3	6	INH + RIF+ *M. oleifera*	50 mg/kg +100 mg/kg btw+400mg/kg
4	6	INH + RIF+ *M. oleifera*	50 mg/kg +100 mg/kg btw+600mg/kg
5	6	INH + RIF + Silymarin 100	50 mg/kg +100 mg/kg btw +5mlg/kg

Table1: Treatment of *R.rosea* on INH+RF induced liver injury

After 28 days treatment protocol animals were sacrificed, the following parameter are estimated such as weight of liver and liver profile for biochemical ,liver homogenate tissue the used for measurement of oxidative stress markers like Malondialdehyde (MDA), Reduced Glutathione (GSH), Superoxide dismutase (SOD) and Myeloperoxidase (MPO). Estimation of pro inflammatory cytokines are IL-6,IL-8, IL-1β and TNF- α level in homogenized liver and supernatant analysed with ELISA kit. liver tissue was fixed in 10% formaldehyde for histopathological evaluation using haematoxylin and eosin (H & E) stain.[12-16]

Statistical Analysis
The results were expressed as mean ± SEM, The data was analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test , *p* values <0.05, <0.01 and <0.001 were considered to be statistically significant, highly significant and very highly significant respectively.

Results

Preliminary Phytochemical Screening

The preliminary phytochemical studies of *R.rosea* contain primary and secondary metabolites such as alkaloids, flavonoids, steroids, Carbohydrate, aminoacids, tannins and poly phenolics, were present in extract.

Effect of ethanolic extract of *R.rosea* on liver weight in rats

Anti-TB drug INH+RF treated group significantly decrease the liver weight compared to control group. *R.rosea* treatment markedly ameliorated the effect of anti-TB drug on liver weight. Impact of *R.rosea* was comparable to the effect of Silymarin on hepatic weight.

Effect of *R.rosea* extract on liver function tests

The administration of INH+RF significantly increased the level of AST, ALT, ALP, TP, and TB in serum compared to the control group. The hepatotoxicity induced with INH+RF was ameliorated by the co-administration of *R.rosea* to INH+RF administered in rats. The protective effects of *R.rosea* on AST, ALT, ALP, TP, and TB were significantly decreases the liver serum in a dependent manner. The level of AST, ALT, ALP, TP, and TB in the liver serum Silymarin administered groups remained impervious compared to the control group as shown in (figure1)

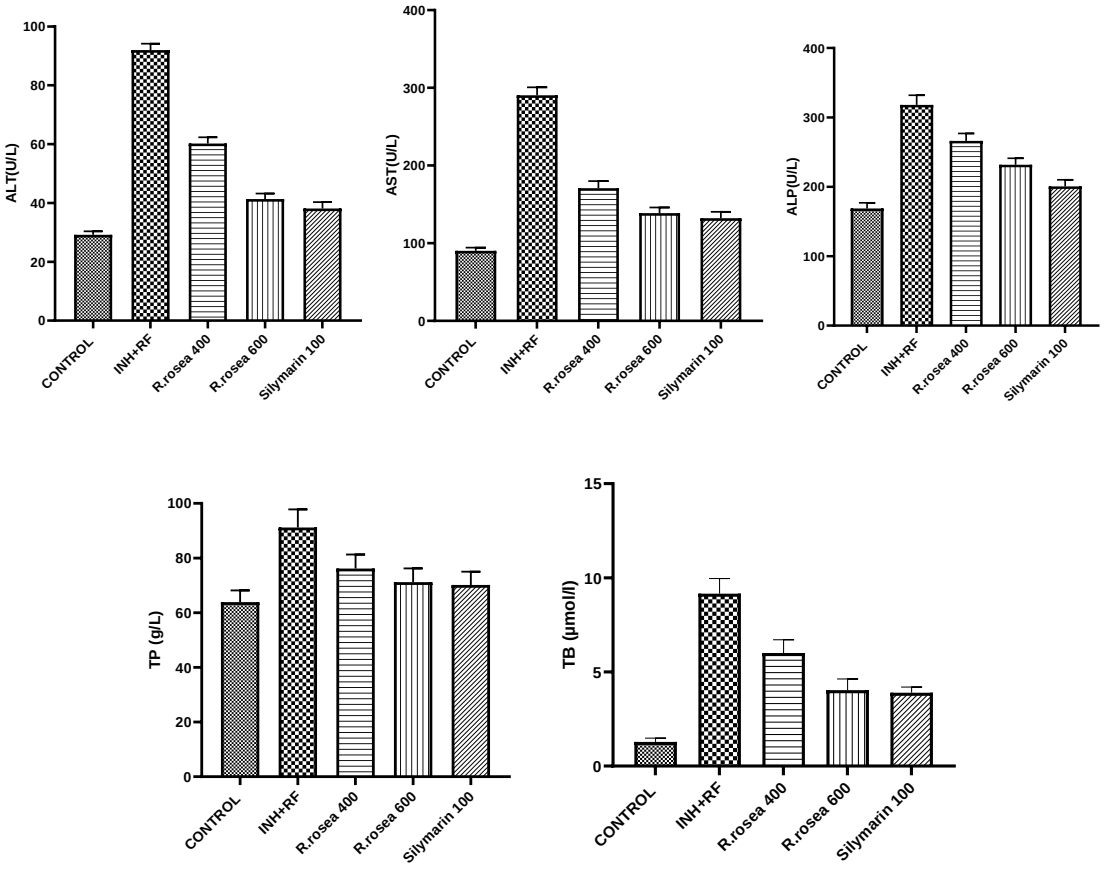

Figure: 1 Effect of *R.rosea* in serum liver function on INH+RF induced Liver injury in rats

The administration of INH+RF significantly increased the level lipid peroxidation MPO,MDA and significantly decreased GSH,SOD and CAT compared to the control group. The administration of *R.rosea* significantly decreased MPO, MDA levels and significantly increased GSH,SOD and CAT as shown in (figure 2)

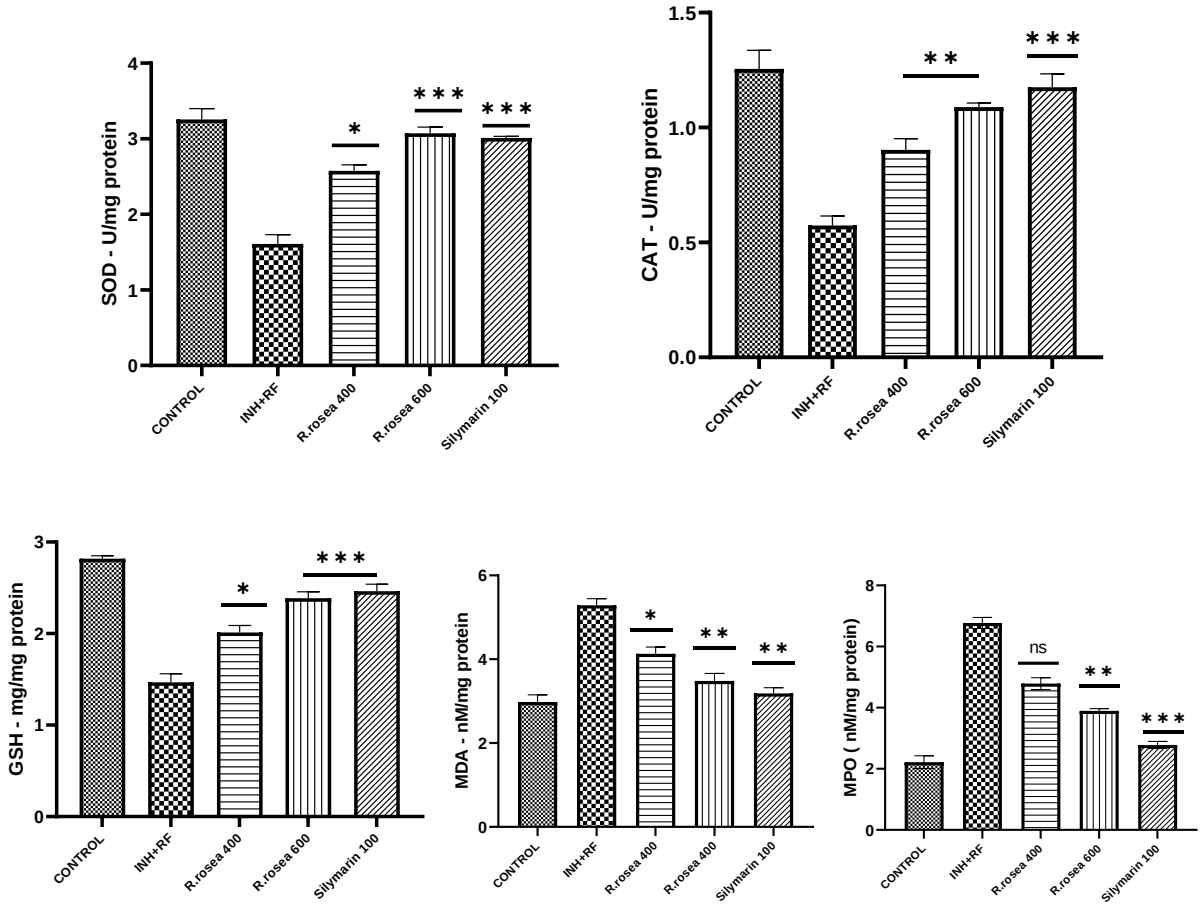

Figure: 2 Effect of *R.rosea* in oxidative Stress on INH+RF induced Liver injury in rats

The administration of INH+RF significantly increased in the inflammatory markers IL-6,IL-8 and TNF-α, treatment with *R.rosea* and Silymarin significantly decreased IL-6,IL-8 and TNF-α, levels as shown in (figure 3).

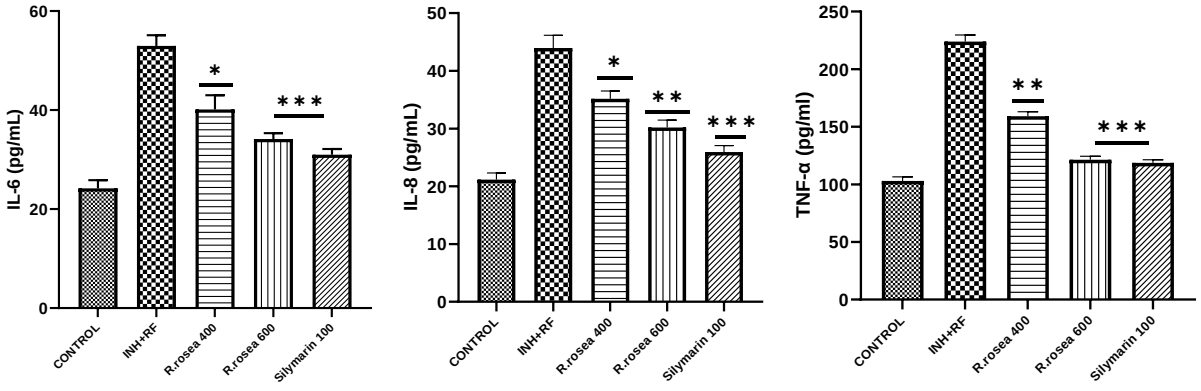

Figure: 3 Effect of *R.rosea* in Inflammatory cytokines on INH+RF induced Liver injury in rats

Histopathology of Liver:

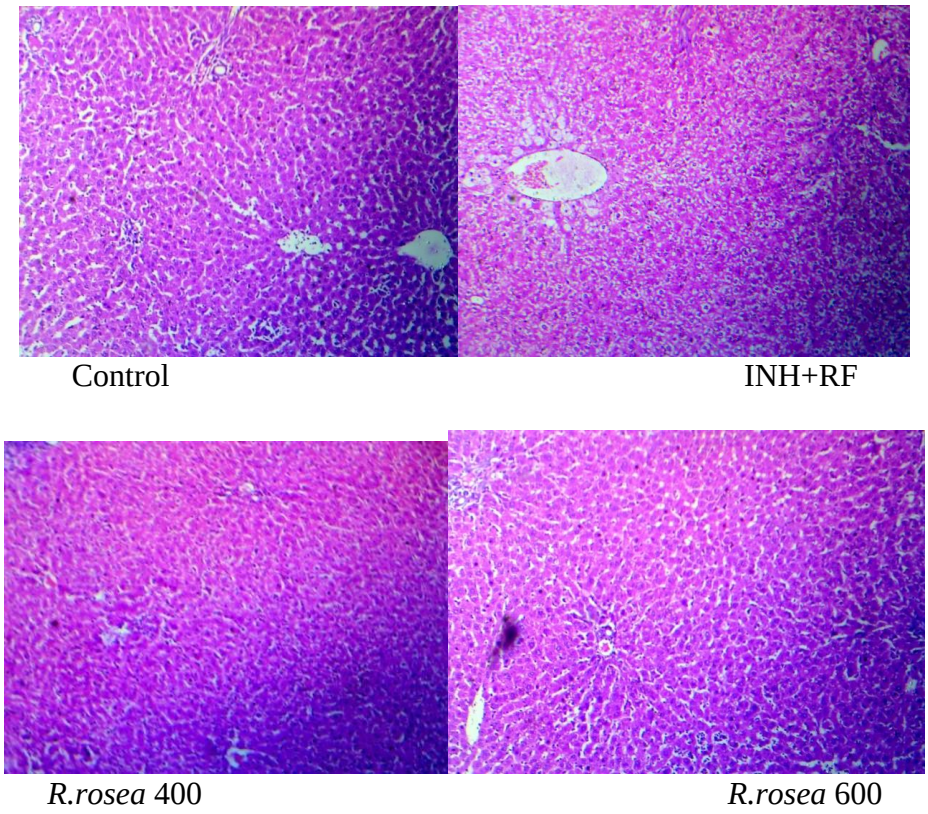

Figure 4: Histopathology of liver of *R.rosea* on INH+RF induced Liver injury in rats.

Effect of *R.rosea* on histopathology of liver

The histopathology of liver tissues stain with Hematoxylin and eosin are shown in (figure 4). The histological architecture of the control group showed the normal lobular structure of liver. In INH+RF treated rats, the histopathology of the liver was altered and fatty changes were prominent. The lobular structure was disrupted and there was congestion of blood vessels, a severe degree of hemorrhage, necrosis with fatty vacuolations. There were degenerative changes and the chromatin material showed clumped morphology. The cell membrane hepatocytes in some of the areas were not distinguished. Treatment of *R.rosea* 400 and 600 protected the liver from the toxicity of anti-tuberculosis drug and most of the changes induced with an anti-tuberculosis drug were absent from the histopathology. Silymarin treatment histopathological alterations induced with the INH+RF were recorded almost normal architecture of liver was apparent.

DISCUSSION:

Liver is the major organ which plays a key roles in metabolisms , biochemical ,physiological functions and Detoxification of endogen and exogenous compounds, such as drugs and xenobiotics, homeostasis, growth, energy and nutrient supply .[17-18] Liver diseases are known to be associated

with INH+RF damage hepatic metabolizing capacity and impaired activity of various hepatic enzymes[19].

It is suggested that isoniazid is metabolized into monoacetyl hydrazine as well as isonicotinic acid; the latter can be activated through metabolic oxidation of cytochrome P-450 to toxic species causing hepatic damages. Rifampicin aggravated the hepatotoxicity due to its high amidase activity and is involved in release of large concentrations of acetyl-hydrazine from isoniazid [20]. The reactive metabolites of an acetyl-hydrazine bind with hepatic proteins causing injuries [21]. Likewise the pyrazinamide is metabolized by the hepatic xanthine oxidase as well as microsomal amidase and the intermediaries; pyrazinoic acid and 5-hydroxy pyrazinoic acid are considered to be involved in hepatotoxicity [22]. Although the exact mechanism and contributing factors of hepatotoxicity induced with anti-tuberculosis drug are not clear; reactive oxygen species (ROS)-mediated oxidative damage is postulated to be the main factor of lipid peroxidation and consequently the hepatic injuries. Alterations in the enzymatic and non-enzymatic entities of the cellular defence mechanism have been reported with the use of anti-TB drug [23]. Hydrazine declines the level of cellular glutathione (GSH) and suggested to minimize the oxidative defence mechanism and consequently cause cellular injuries and death [24].

In the current research, the level of SOD, GSH, CAT and MPO content moved in the direction of control after treatment with *R.rosea*. This refurbishment may be accompanied with improvement of the antioxidant enzymes. The decrease of MDA,MPO , and increase of GSH, SOD and CAT in hepatic samples have been determined with the co-administration of *R.rosea* extract to INH+RF administered rats [25]. Our findings are relevant to other observations about hepatic tissue.
The plants provide a natural source to treat various aspects of diseases. It has been observed that most of the plant based drugs impart their therapeutic potential by exhibiting antioxidant activities. The plant extracts comprise a range of compounds including alkaloids, glycosides, flavonoids, fatty acids, saponins, sterols, and others. Polyphenolic compounds of plants are of remarkable importance because they confer such hydroxyl groups that show scavenging potential for free radicals [26]. On account of potent antioxidant properties; during current years many species of plants have been evaluated for the management and treatment of various ailments. For this reason, research work is being conducted to suggest an approach that involves certain agents tending to alleviate the anti-TB drug induced hepatotoxicity

CONCLUSION:

In conclusion, the result of the present study indicated that under the present experimental conditions. Etanolic extract of *R.rosea* possesses potent antioxidant activity, which may be due to presence of antioxidant component in the *R.rosea.*

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CONFLICT OF INTEREST

We have no conflict of interest to declare.

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