Pharmacological Screening Of *Ixora Coccinea* For Hepatoprotective Activity

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Abstract
Liver is the major organ which plays a key roles in metabolisms , biochemical ,physiological functions. *Ixora coccinea* used Indian traditional Ayurvedic system of medicine for a variety of ailments. In the present study was evaluated effect of ethanolic extract of *I. coccinea* on Isoniazid and Rifampicin induced liver damage on rats. Isoniazid and Rifampicin was administered a 100 and 50 mg/kg bw dose to induced hepatotoxicity, *I. coccinea* 300 and 500mg/kg, p.o and Silymarin 100mg/kg, p.o. were administered once daily for 28 days. The liver injury was estimated by liver profile parameters ALP,AST,ALT,TP and TB. Oxidative stress markers GSH, MPO,MDA,SOD and CAT as well histopathology of liver. Inflammatory cytokines IL-6,IL-8 and TNF-α Isoniazid and Rifampicin drugs significantly reduction in liver weight, increases in serum liver biochemical, decreses in Oxidative stress, increases in Inflammatory cytokines levels and changes in histopathology of liver. Treatment with *I. coccinea*in a dose depended manner significantly changing in weight liver, liver function estimation , lipid peroxidation, Inflammatory cytokines levels and histopathology of liver compare to control and Silymarin group. The present studies evidence that the ethanolic extract of *I. coccinea* has shown hepatoprotective effect.

Keywords: *I. coccinea*, INH+RF,Hepato, 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].
Ixora coccinea of family (Rubiaceae), commonly known as flame of wood, jungle geranium, and Vethi, is a beautiful shrub with several medicinal properties, *I. coccinea* consists of tropical evergreen trees that are native to the tropical regions of Asia [4] comprising about 500 different species with its centre of diversity in Tropical Asia [5]. The word "Ixora" was coined from a Portuguese version of Iswari, which is the name of the Goddess "Parvati" to which *Ixora coccinea* flowers are offered, while "*coccinea*" is a Latin word that means scarlet coloured. [6] plants used in Indian traditional Ayurvedic system of medicine for a variety of ailments. Leaves are used to treat diarrhoea; cough, fever, sore, chronic ulcers, and skin diseases; antioxidant, anti-nociceptive [7], anti-mitotic, anti-inflammatory, cardio protective, anti-ulcer, anthelmintic, anti asthematic, hypo lipidemic and hypoglycaemic activities while flowers in catarrhal bronchitis and dysentery[8]. Phytochemical investigation of *I. coccinea* revealed important phytochemicals such as ursolic acid, lupeol, oleanolic acid, rutin, sitosterol, lecocyanadin, anthocyanins, proanthocyanidins, quercetin, and kaempferol glycosides [9].
The leaves of *I. coccinea* yielded kaemferol, flavonoids, quercetin, anthrocyanidins, ferulic acids and other phenolic acids [10]. However our present study is finding the effect of ethanolic extract of *I. coccinea* on Isoniazid and Rifampicin induced liver damage on rats .

Materials and methods

Fresh leaf of *Ixora coccinea* of family (Rubiaceae) were procured from local nursery vendor Hyderabad, Telangana. Leaf are authenticated by Dr.Vijaya Bhasker Reddy, Assistant Professor, Department of Botany , Osmania university, Hyderabad. A voucher specimen (No.OUAS-164).

PREPARATION OF EXTRACTION

The fresh leaf around 2kg shade dried for 15 days; fruit material was powdered using mixer grinder and passed through sieve no 85. Weight About 150gm of dried fruit powder was subjected to soxhlet's 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+ *I. coccinea*	50 mg/kg +100 mg/kg btw+300mg/kg
4	6	INH + RIF+ *I. coccinea*	50 mg/kg +100 mg/kg btw+500mg/kg
5	6	INH + RIF + Silymarin 100	50 mg/kg +100 mg/kg btw +5mlg/kg

Table1: Treatment of *I. coccinea* 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.[11-18]

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

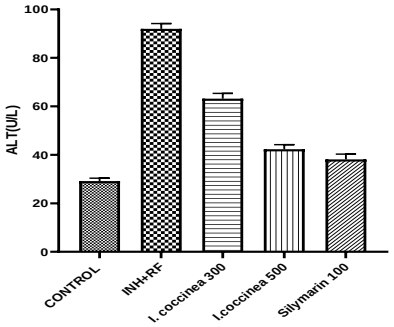
The preliminary phytochemical studies of *I. coccinea* contain primary and secondary metabolites such as alkaloids, flavonoids, steroids, Carbohydrate, aminoacids, tannins and poly phenolics, were present in extract.

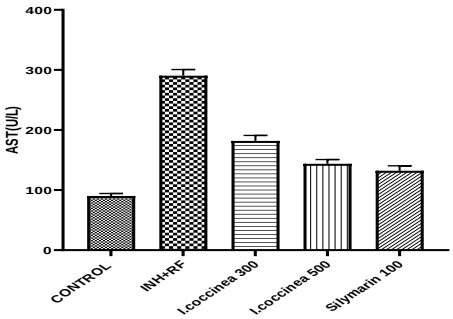
Effect of ethanolic extract of *I. coccinea* on liver weight in rats

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

Effect of *I. coccinea*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 *I. coccinea* to INH+RF administered in rats. The protective effects of *I. coccinea* 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)





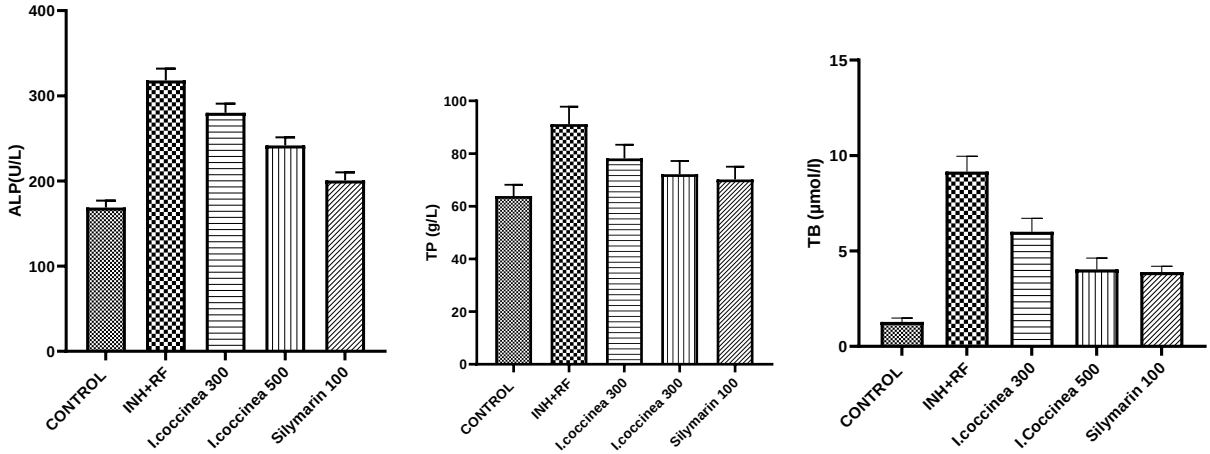


Figure: 1 Effect of *I. coccinea* in serum liver function on INH+RF induced Liver injury in rats

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 *I. coccinea*significantly decreased MPO, MDA levels and significantly increased GSH,SOD and CAT as shown in (figure 2)

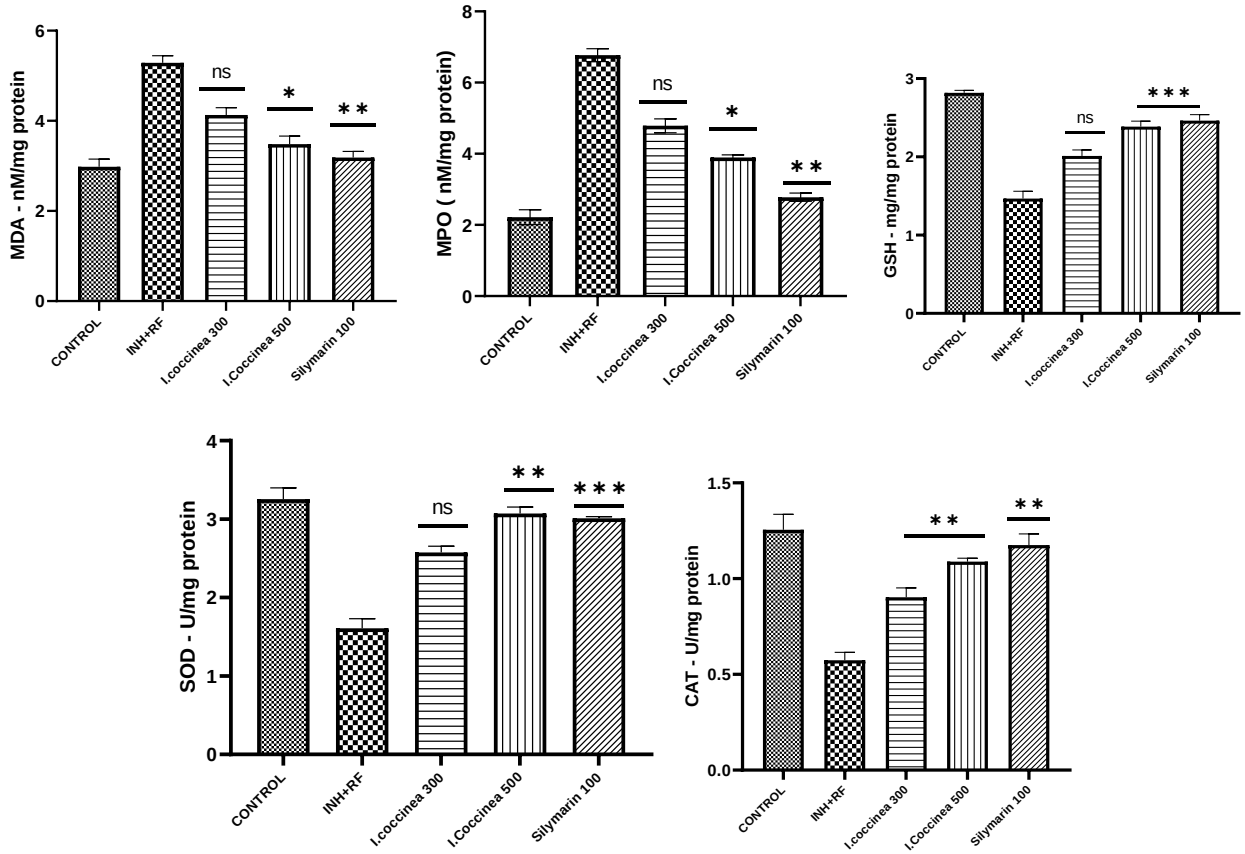


Figure: 2 Effect of *I. coccinea* in oxidative Stress on INH+RF induced Liver injury in rats

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

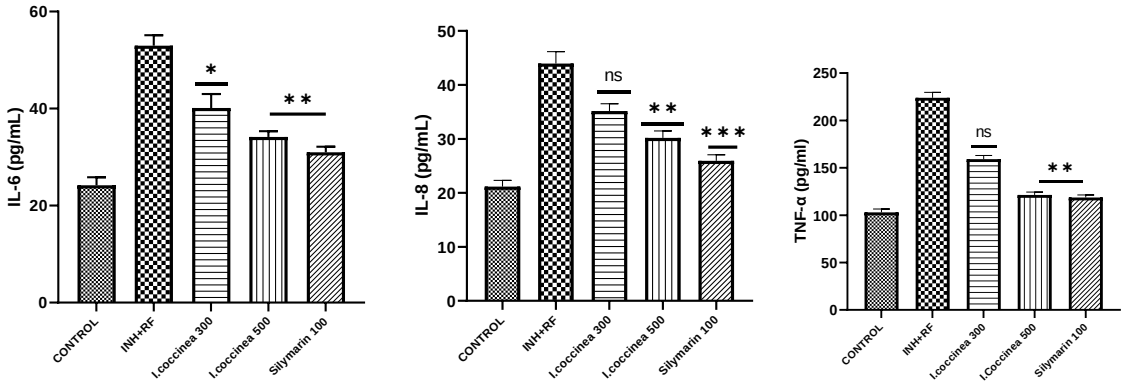
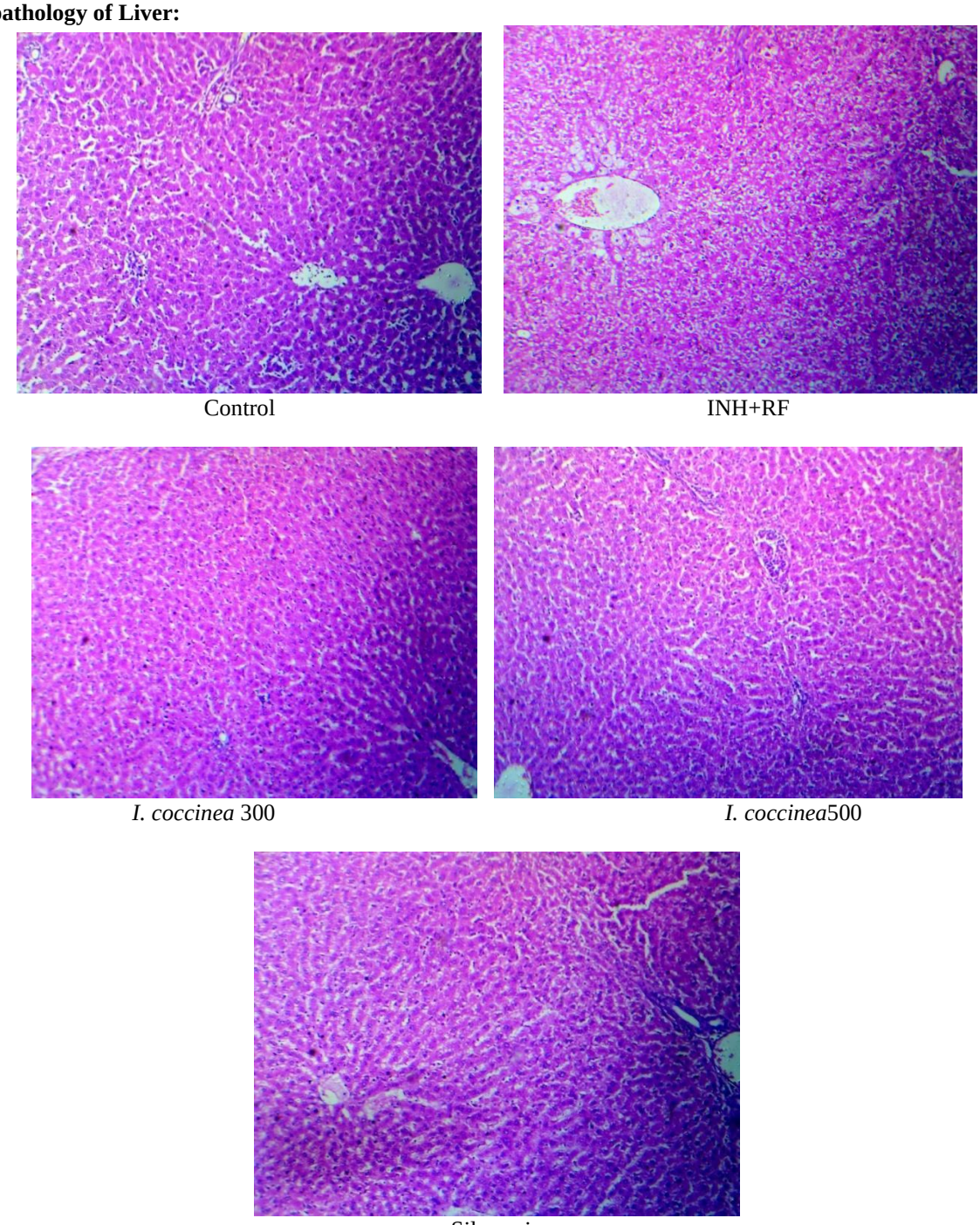


Figure: 3 Effect of *I. coccinea* in Inflammatory cytokines on INH+RF induced Liver injury in rats

Histopathology of Liver:



Control INH+RF

I. coccinea 300 *I. coccinea*500

Silymarin

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

Effect of *I. coccinea* 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 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 *I. coccinea* 300 and 500 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:

Induction of hepatic injuries with the INH+RF drug is multifaceted, but the major mechanism seems to be oxidative stress induced generation of free radicles. In this investigation, the INH+RF drug to rats shifts the dynamic equilibrium of metabolism towards the oxidative stress as observed by an increase of hepatic TBARS and H_2O_2 while a decrease in hepatic GSH and the antioxidant enzymes. Insufficient hepatic level of CAT, SOD, and MPO was unable to scavenge the excessive generation of lipid hydroperoxides. These reactive intermediate metabolites play a crucial role in developing oxidative stress and consequently cause hepatic damages [19]. Amongst the cell based antioxidants GSH, CAT, MDA, MPO and SOD are extensively explored for their significant role in defense mechanism. Superoxidase is an exceptionally active antioxidant enzyme that catalyzes the dismutation reaction of superoxides to H_2O_2 and O_2 whereas CAT is a ubiquitous enzyme but mainly rich in the liver and is engaged in a breakdown of H_2O_2 to water. In the GSH reaction system, GSH is oxidized to GSSG by the help of GPx which transformed back to GSH by the reducing power of GSR. GSH also works as a cofactor for GST that is present equally in the cytosol and endoplasmic reticulum, essentially engage in catalyzing the production of GSH electrophile conjugate therefore, detoxifying xenobiotics to generate irreversible compounds. It is detected that lipids peroxidation can induce a genetic increase of fibrogenic cytokines by commencing the generation of collagen and stimulating liver stellate cells [20]. Enhanced Complement system activation is known for tissue injuries and free radicals activates complement system. More complement activation causes more C3b generation and that can lead to tissue injuries [21]. In the current research, the level of GSH, CAT, MDA, MPO and SOD content moved in the direction of control after treatment with MEM. This refurbishment may be accompanied with improvement of the antioxidant enzymes. The decrease of TBARS and increase of GSH in hepatic samples have been determined with the co-administration of *I. coccinea* extract to INH+RF drug administered rats [22-23]. Our findings are relevant to other observations about hepatic tissue . Antioxidants reduce the damaging effects by donating electron or hydrogen atom to free radicals, hence protect cellular integrity and health. These compounds can also repair the damage caused by ROS. Natural antioxidants mainly come from plants in the form of phenols, flavonoids, ascorbic acids, vitamins and carotenoids. These compounds act as free radical scavengers, reducing agents, complexers of pro-oxidants and quenchers of singlet oxygen. [24]

CONCLUSION:

In conclusion, the result of the present study indicated that under the present experimental conditions. Etanolic extract of *I. coccinea* possesses potent antioxidant activity, which may be due to presence of Natural antioxidants mainly come from plants in the form of phenols, flavonoids, ascorbic acids, vitamins and carotenoids.

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CONFLICT OF INTEREST

We have no conflict of interest to declare.

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