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Hepatoprotective effect on methanolic extracts of *Tagetes erecta* leaves and *Tridax procumbens* against drug induced hepatic injury

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ABSTRACT

The current study was undertaken to evaluate the preclinical efficacy of methanolic extract of *Tagetes erecta* (METE) and methanolic extract of *Tridax procumbens* (METP) against Isoniazid and Rifampicin (INH-RIF) induced hepatic injury. Animals were randomly divided into seven groups, vehicle (control) or INH-RIF (50 mg/kg, i.p.), METE (200 and 400mg/kg, p.o.), METP (200 and 400mg/kg, p.o.) and standard silymarin for 28 days. INH-RIF intoxicated rats displayed significant ($p<0.05$) elevation in serum hepatic markers, lipid peroxidation and decrease in antioxidants like SOD, CAT, Gpx and GSH in liver tissue. Treatment with METE & METP (200 and 400 mg/kg, p.o.) restored the altered biochemical level to normalcy. Thus, the outcome of the study reveals that METE & METP showed promising hepatoprotective activity in INH-RIF induced hepatic damage mediated by its membrane stabilizing and antioxidant effect. All the methanolic plant extracts produce significant hepatoprotective activity against drug induced hepatic injury.

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INTRODUCTION

Tuberculosis (TB) is a worldwide health problem with the highest prevalence rate in India and also imposes a huge economic burden (Kumar and Khulbe, 2016; Sotgiu *et al.*, 2017). The pharmacotherapy for TB is generally known as "directly observed treatment, short-course" (DOTS), which encompasses a combination of drugs rifampicin (RIF), isoniazid (INH) and pyrazinamide and has an achievement rate of more than 80% approach (Darvin *et al.*, 2018). However, the main draw-back in DOTS therapy is, upon chronic administration, it causes hepatotoxicity and commonly referred to as drug mediated hepatotoxicity (Abbara *et al.*, 2017). Previous studies indicate that RIF and INH alone or in combination, elicits hepatotoxicity in patients undergoing treatment (Kumar and Khulbe, 2016). The mechanism of hepatotoxicity provoked by INH-RIF is still obscure, but studies indicate that oxidative stress might be the prime mechanism in INH-RIF (Mitchell *et al.*, 1975). Further reports are highlighting that Hydrazine (HYZ), a metabolite of INH, is converted to the toxic compound by CYP450, which leads to hepatotoxicity. RIF aggravates hepatotoxicity by inducing CYP450, as a result, more toxic metabolites are generated from hydrazine (Tostmann *et al.*, 2008). In addition, HYZ depletes the reserved glutathione (GSH) level in the liver, precluding oxidative damage and cell toxicity (Sarich *et al.*, 1998; Huang *et al.*, 2003). Mounting herbal plants are recommended for the management of drug induced liver injury and they

exhibit significant protection to a wide range of hepatotoxins (Singh *et al.*, 2016).

Tagetes erecta is a weed of the family Asteraceae, present in many parts of India and thrives in moist places (Pandey and Tripathi, 2014). Previous reports show the preventive effect of *Tagetes erecta* on CCl_4 provoked hepatotoxicity (Priyanka *et al.*, 2013). In this scenario, the current study was conducted to delineate the hepatoprotective of methanolic extract of *Tagetes erecta* on antitubercular drug isoniazid and Rifampicin (INH-RIF) combination mediated liver damage.

Tridax procumbens Linn. (Asteraceae) is a potent herb and possess an array of biological properties. Commonly (Rajaram and Ashvin, 2013). A recent study has displayed the cardioprotective property of *Tridax procumbens* in isoproterenol induced myocardial infarction (Shanmugapriya and Maneemegalai, 2018). In this backdrop, the present study was undertaken to evaluate the methanolic extract of Tridax procumbens on DOX induced oxidative cardiotoxicity.

MATERIALS AND METHODS

Drugs and Chemicals

Isoniazid, Rifampicin, Doxorubicin and Silymarin were procured from Sigma, USA. The other required reagents were of the highest purity and analytical grade.

Plant material

The whole plant of *Tagetes erecta* and *Tridax procumbens* was procured separately from the various gardens and nurseries of Palvancha, Bhadradri district, Telangana, India. The collected plant was authenticated by Dr. K.Madhava Chetty, Assistant Professor, Sri Venkateswara University, Chitoor district, Andhra Pradesh. Then, the plant materials were placed under the shade for drying and powdered using a mill and stored in an airtight container.

Preparation of extract

Powdered plant material weighed about 250 g of *Tagetes erecta* was subjected to extraction using 1000 ml of methanol by simple maceration technique for 72 hours. Distillation was carried to obtain the concentrated extract,$1/4^{th}$ of its original volume. The final yield obtained was 12% w/w (METE).

Powdered plant material weighed about 250 g of *Tridax procumbens* was subjected to extraction using 1000 ml of methanol by simple maceration technique for 72 hours. Distillation was carried to obtain the concentrated extract,$1/4^{th}$ of its original volume. The final yield obtained was 15% w/w (METP).

Preliminary Phytochemical screening

The phytochemical analysis of methanol extract of *Tagetes erecta* and *Tridax procumbens* ware reveals triterpenoids, flavonoids, steroids, tannins, saponins and alkaloids (Bhagat and Kondawar, 2019; Satish *et al.*, 2012).

Animals

All animal studies were conducted as per the protocol of CPCSEA and the Institutional Animal Ethical Committee (IAEC). CPCSEA Reg. No: 1641/PO/E/S/14/CPCSEA. The standard experimental protocols and procedures adopted in this biological evaluation were described below.

Acute toxicity studies

The acute toxicity studies were performed as per the OECD guideline No. 425 by using albino mice.

Study Design

The study was conducted on male Wistar rats (150 $\pm$ 10 g). Animals were obtained from the Animal House, Browns College of Pharmacy. Animals were fed with commercially available standard rat pellet feed (M/s Pranav Agro Industries Ltd., India) under the trade name Amrut rat/mice feed and water was provided ad libitum. The animals were deprived of food for 24 hours before the experiment but allowed free access to water. The rats were housed under the conditions of controlled temperature ($25 \pm 2\,^{\circ}C$) and were acclimatized to 12 hours in light: 12 hours in dark cycles.

Isoniazid/ Rifampicin induced hepatotoxicity

The rats were divided into seven groups, n=6,

Group 1

Normal control rats received vehicle 2% gum acacia suspension for 14 days. (1 ml/ kg b.wt.), p.o for 28 days

Group 2

Rats received INH and co-administered RIF (50 mg/kg; b.wt) p.o daily for 28 days

Group 3

Rats treated with silymarin (100mg/kg; b.wt) p.o for 28 days

Group 4

Rats received 200mg of methanolic extract of *Tagetes erecta* (METE) using a vehicle 2% gum acacia p.o for 28 days.

Group 5

Rats received 400mg of methanolic extract of *Tagetes erecta* (METE) using a vehicle 2% gum acacia p.o for 28 days.

Group 6

Rats received 200mg of methanolic extract of *Tridax procumbens* (METP) using a vehicle 2% gum acacia p.o for 28 days.

Group 7

Rats received 400mg of methanolic extract of *Tridax procumbens* (METP) using a vehicle 2% gum acacia p.o for 28 days.

Meanwhile, group 3-7 rats were treated with INH and co-administered RIF (50 mg/kg b.wt) p.o daily for 28 days, one hour after the drug treatment.

After the final doses of extract and INH-RIF, the access to food was restricted overnight and the animals were anaesthetized using phenobarbital sodium (35mg/kg) intraperitoneally and sacrificed by cervical decapitation. The blood was withdrawn from the jugular vein in heparanized tubes and the serum was separated for the measurement of hepatic marker enzymes. The liver tissue was excised, cleaned from adherent tissues, washed in ice cold saline and dried. Then a 100 mg weighed tissue was homogenized in cold Tris-HCl buffer (10% w/v) and used for the analysis of various biochemical markers in INH-RIF induced hepatic damage.

Analysis of hepatic markers

Serum biochemical parameters like alanine transaminases (ALT), asparate transaminases (AST), total protein (TP), albumin (ALB), total bilirubin (TB), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and gamma glutamyl transferase (GGT) were assayed by biochemical kits supplied by Span Diagnostics Ltd, Gujarat, India.

Estimation of lipid peroxidation

The lipid peroxidation (LPO) marker, malondialdehyde (MDA), was measured according to the instructions provided in the kit procured from Span Diagnostics Ltd, Gujarat, India.

Estimation of antioxidants

The hepatic level of antioxidants catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione (GSH) were estimated as per the instructions provided in the kit obtained from Span Diagnostics Ltd, Gujarat, India.

Statistical analysis

The data were represented as mean ± Standard error mean (SEM). The data were analysed by ANOVA, followed by Tukey's comparison using SPSS version 18.0. $p < 0.05$ was taken as statistically significant.

RESULTS

Acute oral toxicity of *Tagetes ericta* and *Tridax procumbens*

Upon administration of extracts, there was no mortality, unwanted clinical reactions, a marked reduction in body weight or gross pathological changes seen in rats. LD_{50} of METE and METP was higher than 2000 mg/kg.

Effect of METE and METP administration on hepatic markers

In the present study, significant elevation of ($P \leq 0.05$) serum AST, ALT, ALP and LDH was observed in animals treated with INH-RIF. Supplementation of METE (200 and 400 mg/kg) to the rats brought down to increase in serum transaminases, ALP and LDH near normal levels. Increased levels of TB ($P \leq 0.05$) were observed in INH-RIF. However, treatment with METE and METP at the dose of 200 and 400 mg/kg showed a significant reduction of serum TB levels. TP and ALB are used to assay liver function. Significant decrease of serum TP ($P \leq 0.05$) and ALB ($P \leq 0.05$) were noticed in the rats in group II. Treatment with METE (200 and 400 mg/kg) enhanced the concentration of proteins and ALB. Serum GGT is one of the highly sensitive markers for liver function. Significant increase in serum GGT ($P \leq 0.05$) is observed in group II animals. Treatment with METE and METP (200 and 400 mg/kg) showed a significant decrease in serum GGT. The results were displayed in Tables 1 and 2 respectively.

Effect of METEand METP on hepatic lipid peroxidation and antioxidants

In the current study, there was a significant decrease of hepatic SOD, CST, GPx and GSH with a concaminant increase of MDA in rats intoxicated with INH-RIF ($p<0.05$). However, treatment with METE and METP (200 and 400 mg/kg) completely brings back the normal levels of these hepatic antioxidants and significantly reduced the hepatic MDA formation (Table 3).

DISCUSSION

The current study was carried to delineate the hepatoprotective potential of *Tagetes erecta* methanolic extract on oxidative assault provoked by antitubercular drugs isoniazid and Rifampicin (INH-RIF) in a murine model. Oxidative damage is the main cause of hepatocellular injury elicited by INH-

Table 1: Effect of METE and METP with INH-RIF on serum hepatic marker enzymes

Groups	AST (U/L)	ALT (U/L)	GGT (U/L)	ALP (U/L)	LDH (U/L)
Control	37.74±3.89	31.48±3.06	4.94±0.73	260.60±13.15	122.50±9.25
INH-RIF	145.0±2.64*	77.85±4.8*	7.00±0.35*	569.30±32.55*	331.1±28.34*
Silymarin (100mg/kg)+ INH-RIF	68.48±5.18#	38.76±2.52#	5.08±0.33#	294.70±20.74#	178.0±16.19#
METE (200mg/kg)+ INH-RIF	101.0±10.24#	50.36±0.12#	5.87±0.19#	430.4±30.04*#	230.76±12.64#
METE (400mg/kg)+ INH-RIF	72.45±10.12#	41.87±1.32#	5.12±0.12#	345.54±18.12#	172.54±14.45#
METP (200mg/kg)+ INH-RIF	105.05±12.45#	50.56±0.76#	5.47±0.25#	435.24±30.12*,#	230.45±11.45#
METP (400mg/kg)+ INH-RIF	75.45±9.25#	43.54±1.44#	5.76±0.25#	360.67±18.12#	172.92±13.70

Table 2: Effect of METE and METP with INH-RIF on serum hepatic marker enzymes

Groups	Total Protein	Albumin	Total Bilirubin
Control	6.21±0.22	2.48±0.13	0.36±0.082
INH-RIF	4.48±0.42*	1.75±0.18*	0.85±0.10*
Silymarin(100mg/kg)+INH-RIF	6.24±0.17#	2.48±0.10#	0.31±0.06#
METE (200mg/kg)+ INH-RIF	5.55±0.18	2.34±0.17#	0.42±0.07#
METE (400mg/kg)+ INH-RIF	6.98±0.16#	2.40±0.10#	0.31±0.06#
METP (200mg/kg)+ INH-RIF	5.54±0.14	2.36±0.15#	0.44±0.07#
METP (400mg/kg)+ INH-RIF	6.76±0.17#	2.32±0.08#	0.34±0.05#

Table 3: Effect of METE and METP with INH-RIF on hepatic lipid peroxidation and antioxidants

Groups	SOD	CAT	GPx	GSH	MDA
Control	3.46 ± 0.03	61.08 ± 0.62	16.30 ± 0.27	3.65 ± 0.03	14.00 ± 0.07
INH-RIF	1.83± 0.04*	45.63 ±0.25*	10.57 ±0.16*	1.75 ± 0.03*	23.15 ±0.22*
Silymarin (100mg/kg)+ INH-RIF	3.31± 0.03#	57.65± 0.31#	15.77 ± 0.17#	3.24 ± 0.02#	14.62±0.183*
METE (200mg/kg)+ INH-RIF	2.65±0.02#	50.15±0.42#	12.83±0.19#	2.60±0.04*	20.63±0.27*
METE (400mg/kg)+ INH-RIF	3.01±0.03#	57.05±0.25#	13.33±0.29#	2.80±0.04*	18.40±0.30*
METP (200mg/kg)+ INH-RIF	2.48 ± 0.02#	52.45 ± 0.42#	12.03 ± 0.19#	2.70 ± 0.04*	19.83 ± 0.27*
METP (400mg/kg)+ INH-RIF	3.21± 0.03#	56.75 ± 0.25#	14.83 ± 0.29#	2.60 ± 0.04*	16.40 ± 0.30*

RIF. The biotransformation of INH leads to the generation of acetyl onium ion, which is highly reactive, ketene and acetyl radical and these radicals covalently bind with macromolecules present in the liver leading to hepatic damage (Ramappa and Aithal, 2013). Further, it has been shown that Rifampicin actively induces many enzymes involved in the metabolism like cytochrome P450 (CYP3A4) through PXR located in the hepatocytes. Thus CYP3A4 activation preludes to rampant metabolism of isoniazid and releases noxious metabolites, which accelerates the metabolism of Rifampicin and causes hepatotoxicity (Nannelli *et al.*, 2008). During hepatic damage, membrane integrity of the hepatic cytoplasmic membrane is damaged as a result of lipid peroxidation generated by free radical from INH-RIF metabolism (Jadhav *et al.*, 2010). Due to the distortion of the hepatocytes membrane, the hepatic markers enzymes present inside are released into the bloodstream, which indicates hepatic damage (Baniasadi *et al.*, 2010). In this study, INH-RIF intoxicated rats displayed a significant increase in the serum level of AST, ALT, ALP, GGT and LDH. Treatment with METE significantly restored the altered liver marker enzymes to normal and thus prevented the hepatic membrane damage, which is lined with a previous report (Priyanka *et al.*, 2013; Khulbe, 2015). Total bilirubin is a vital marker for the diagnosis of hepatic injury. Elevated serum bilirubin levels in the event of hepatotoxicity might be due to reduced hepatic clearance of bile, which leads to hepatitis (Ramaiah, 2007). In our study, METE treatment significantly reduced the total bilirubin to normal, which is in corroboration with earlier reports (Priyanka *et al.*, 2013; Khulbe, 2015).

During hepatic damage, protein like albumin level is decreased due to the inability of the liver to synthesize these biomolecules. In this study, INH-RIF intoxicated rats displayed a reduced level of serum protein and albumin, which is in corroboration with earlier reports (Marasani, 2014). Meanwhile, treatment with METE significantly increased the total bilirubin to normal, which is in corroboration with earlier reports (Khulbe, 2015).

The INH-RIF provoked hepatic injury leads to a reduction in antioxidant protective mechanism due to the generation of highly reactive toxic metabolites, which preludes to lipid peroxidation and depletion of glutathione stores. In our study, the MDA, a prominent index of lipid peroxidation, is effectively increased in hepatic tissue of rats intoxicated with INH-RIF. However, treatment with METE effectively reduced the MDA by inhibition the chain termination of the lipid peroxidation process. Studies have shown that the LPO process further decreases the GSH level. Hydrazine, a prime toxic metabolite INH, decreases the GSH level by binding to its sulfhydryl group and thus generates noxious free radicals (Khulbe, 2015). In our study, INH-RIF intoxicated rats displayed decreased GSH level as a result of lipid peroxidation induced by anti-tubercular drugs. METE treatment significantly increased the GSH and thus restored the antioxidant defence system (Priyanka *et al.*, 2013; Khulbe, 2015). Further, the antioxidant enzymes SOD, CAT, and GPx are significantly decreased in rats insulted with INH-RIF and METE treatment effectively increased the antioxidant levels to normal. Thus, the hepatoprotective activity rendered by METE in the present study might be due to the presence of various phytoconstituents like wedelolactone, Eclalbasaponins, α and β-amyrin, Oleanolic and ursolic acids, which possess significant antioxidant and free radical scavenging properties (Gopi and Jayasri, 2012).

Although Dox has a broad chemotherapeutic range, its clinical utility is minified due to dose mediated cumulative cardiotoxicity. The cardiotoxicity mechanism of Dox is mainly due to the formation of an iron-anthracycline complex that releases free radicals and elicits distortion of the plasma membrane and cytoskeleton of cardiomyocytes (Zhang *et al.*, 2012). Thus, the present study was designed to evaluate the cardioprotective potential of *T.procumbens* extracts against DOX induced cardiotoxicity.

In the present study, DOX intoxicated rats displayed significant elevation of CK-MB, LDH and cTn-T. Treatment with METE and METP at the dose of 200 and 400mg/kg significantly decreases the serum level of cardiac markers to normal due to its cardiac membrane stabilizing action (Osman *et al.*, 1993).

In the present study, DOX treated rats displayed marked elevation of lipid peroxidation and protein carbonyl content (PCC). However, treatment with METE and METP DOX intoxicated rats significantly reduced the lipid peroxidation and protein carbonylation and it might be due to the presence of flavanoids, steroids and triterpenoids (Sanghavi *et al.*, 2014).

Our body encompasses a series of antioxidant to encounter free radicals. In the present study, DOX intoxicated rats displayed a decreased level of antioxidants like GSH, SOD, CAT and Gpx and treatment with METE and METP at 200 and 400mg/kg significantly increases the antioxidants level to normal.

Previous studies report that centaureidin and

procumbenetin are the flavanoids identified in T. procumbens (Jachak *et al.*, 2011; Ali *et al.*, 2001). Thus, the cardioprotective potential of *T. procumbens* might be due to the presence of flavanoids.

CONCLUSIONS

On the basis of our findings, methanolic extract of *Tagetes erecta* and *Tridax procumbens* may improve the protective INH-RIF induced hepatotoxicity and the DOX induced cardiotoxicity, respectively, by regulating the marker enzymes, inhibition of lipid peroxidation, improving the status of antioxidants.

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Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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