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Research article

PROTECTIVE ROLE OF *TYLOPHORA INDICA* ETHANOLIC EXTRACT ON ARTESUNATE INDUCED LIVER TOXICITY

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ABSTRACT

Objective: The hepatoprotective role of *Tylophora indica* ethanolic extract was studied on artesunate induced liver injury in wistar albino rats.

Methods: Liver toxicity was induced by administering artesunate110mg/kg orally for 14 days in wistar albino rats. Ethanolic (90%) extracts of *Tylophora indica* (EETI) was administered orally to the experimental animals for 14 days. The hepatoprotective activity of the extracts was assessed by analyzing the levels of various biochemical parameters like alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkalinephosphatase (ALP), γ-glutamyltransferase (GGT), bilirubin (BIL) and albumin (ALB) in serum. Meanwhile thelevels of antioxidant enzymes like superoxide dismutase (SOD) and catalase (CAT) were measured in rat liver homogenate.

Results: The results showed that on administration of artesunate for 14 days caused a significant increase ($p<0.001$) in the levels of ALT, AST, ALP and GGT in serum. The levels of SOD and CAT in liver homogenate were also decreased significantly ($p<0.01$) in artesunate administered animals. The levels of above biochemical parameters were significantly ($p< 0.001$) reversed in rats which received EETI.

Conclusions: The present study proves that the ethanolic extract of *Tylophora indica* has a significant protective action against artesunate induced hepatic injury.

Key words: Artesunate, Liver injury, Wistar albino rats, *Tylophora indica*, ethanolic extract, Hepatoprotection.

INTRODUCTION

Malaria an endemic disease continues to be a major health problem in many parts of the world (Shyamjith M et al, 2012).WHO reports millions of people in the world are at a risk of this parasitic infection (Col GS Saiprasad, 2003).Ours is a malaria endemic region.When a person complains of fever with chills, the most common differential diagnosis by a physician would be malaria (Govt of India, Malarai guidelines, 2009). Drug of choice in this condition are chloroquine, quinine, amodiaquine, proguanil, mefloquine and pyrimethamine. However emergence of drug resistance against the conventional drugs is a worry for health care professionals.Hence, in view of this, a newer drug like artesunate is used to treat complicated malaria. Artemisinin and its derivatives artesunate,artemether and arteether are used to treat malaria nowadays either in combination or singly.Artesunate based combinations therapy is the first line mode of treatment for the management of complicated malaria in most countries where this parasitic disease is endemic. This semisynthetic derivative of artemisinin is water soluble and can be given by injection (WHO guidelines, 2010; Sinclair D et al, 2009). Reports suggest that use of artesunate may cause hepatic dysfunction.Animal studies have reported the hepatotoxic effect of artesunate. It causes hepatocyte damage by free radicals. It causes a rise in the liver transaminases (Izunya et al, 2010; Arshad Ali Noorani et al, 2010). A large number of medicinal plants are tested and found to contain active constituents with protective properties against a variety of liver diseases. Liver protective plants contain a range of chemical constituents like phenols, coumarins, lignans, essential oil, terpenes, carotenoids, glycosides, flavonoids, organic acids, lipids, alkaloids and xanthenes. Recent facts have shown that plant drugs are comparatively non-toxic and safe (Sharma B, 2009). Hence, in view of this we selected *Tylophora indica* to see its role in artesunate induced hepatotoxicity. *Tylophora indica* (asclepiadaceae) is a branching climber found in many parts of India.

It was traditionally used in the treatment of various ailments like bronchial asthma.Several studies have reported its hepatoprotective, antitumour, antiinflammatory, antiasthmatic, analgesic, anticonvulsant, antirheumatic, antioxidant properties (Shyamjith M et al, 2013; Vipul G et al, 2007; Kumar Sunil et al,2012;Sabitha Rani et al,2012; HarmanjitKaur et al,2012).

MATERIALS AND METHODS

Drugs and Chemicals

Artesunate (Zydus Cadilla, Himachal Pradesh), Silymarin (Micro labs, Bangalore) were obtained from a pharmacy in Mangalore. All the chemicals used for biochemical analysis were of analytical grade and was procured from Himedia, India.

Instruments

Soxhlet apparatus, Autoanalyser, UV Spectrophotometer.

ANIMALS

Adult Wistar albino rats of either sex weighing 175-200 g were used in this study after obtaining Institutional Animal Ethical Committee Clearance (IAEC), Yenepoya University. The rats were maintained under standard conditions in the animal house (CPCSEA approved, Reg No: 347) under Department of Pharmacology, Yenepoya University, Mangalore. The rats were kept in polypropylene cages (U.N.Shah manufacturers, Mumbai) under standard housing conditions and maintained on standard pellet diet (Amrut Lab Animal Feed, Pranav Agro Industries Ltd, Sangli, Maharashtra), and water *ad libitum*. The rats were maintained on a 12:12 hour light-dark cycle.

Plant Materials and Extracts

The plants were cultivated during the month of June. The fresh leaves from *Tylophora indica* were collected in the month of September. They were authenticated by Dr.NoelineJ. Pinto, Head of Botany department, St.Agnes College, Mangalore, Karnataka, India.They were shade dried, and then grinded into coarse powder.

Ethanolic extract of *Tylophora indica* [EETI]

A weighed quantity (500 g) of the coarse powder was taken and extracted with ethanol (90 %) in a Soxhlet apparatus.The extract was concentrated on a water bath at a temperature not exceeding 60ºc. The percentage yield of the extractwas 10%. The ethanol extract was dissolved in distilled water.

Experimental protocol

The rats were divided into 4 groups with 12 animals in each group. Group I received normal saline orally and served as a normal control. Group II received artesunate110mg/kg body weight for 14days intraperitonially in normal saline. This group served as disease control. The dose of artesunate was extrapolated from the human dose using the conversion table (Ghosh MN, 1984). Group III received EETI 300mg/kg bodyweight (VipulGujrathi et al, 2007; Shyamjith M et al, 2013) in distilled water orally along with artesunate administration. Group IV received silymarin, a known hepatoprotective agent, 100mg/kg body weight (Sethuraman MG, 2003) in distilled water orally along with artesunate administration. This group served as standard control.On 15th day blood samples of the animals were taken by cardiac puncture under ketamine anesthesia (150mg/kgbodyweight, I.P). Once the blood was withdrawn, the animals were sacrificed by high dose of ketamine (300mg/kgbodyweight I.P). The liver was dissected out for preparing homogenate.

Assessment of hepatoprotective activity

The blood which was drawn under ketamine anesthesia was allowed to clot and the serum was separated at 3000 rpmfor 10minutes. The serum was used for the assay of ALT, AST, ALP, GGT, BIL and ALB.

Assessment of antioxidant activity

The liver which was dissected out was washed immediately with distilled water to remove blood. It was used forpreparing homogenate for the estimation of SOD and CAT.

All the biochemical procedures were carried out based on the earlier published methods (Shyamjith et al, 2013).

Statistical Analysis

Statistical Analysis of data for significant variation within the groups was performed using the Prism statistical software.One way analysis of variance (ANOVA) and multiple group comparisons were made using Tukey Kramer test using Graph pad Prism software. The values were expressed as mean ± S.D for 12 samples in each group.*P* value < 0.05 was considered as significant.

RESULTS

Effect on LFT

The results (Table:1) showed that there is a significant increase ($p<0.01$) inthe levels of ALT, AST, ALP, and GGT in serum of artesunate administered rats (Group II) on comparing with the normal rats (Group I). At the same time, the serum levels of BIL and ALB were not significantly ($p>0.01$) altered in artesunate administered rats (Group II) on comparing with the normal rats (Group I). The levels of ALT, AST, ALP, and GGT were significantly ($p< 0.01$) reversed in rats which received EETI and Silymarin (Group III, IV).

Effect on Antioxidant enzymes in Artesunate induced hepatotoxicity

The results (Table: 2) showed that administration of artesunate for 14 days caused a significant decrease in the levels of SOD and CAT in liver homogenate (Group II) on comparing with the normal rats (Group I). The levels of above biochemical parameters were significantly ($p< 0.01$) reversed in rats which received EETI and Silymarin (Group III, IV).

Table: 1 Effect on LFT

GROUP	AST	ALT	ALP	GGT	BIL	ALB
I. NORMAL	244.37±12.68	61.62±17.08	160.78±7.49	3.10±6.75	0.37±0.04	3.3±0.094
II. ARTESUNATE	1490.63±26.20[a]	480.21± 5.7[a]	615.42±11.5[a]	19.34±2.17[a]	0.40±0.025[c]	3.2±0.23[c]
III. ARTESUNATE+ EETI	470.95±11.42[b]	51.02± 4.2[b]	162.27±3.23[b]	4.20±1.15[b]	0.32±0.048c	3.3±0.75
IV. ARTESUNATE+ SILYMARIN	450.1± 6.67[b]	50.88±1.49[b]	195.50±10.2[b]	3.29±0.02[b]	0.31±0.00[c]	3.03±0.01

Values are expressed as Mean ± Standard Deviation; n=12
a : $p<0.001$ on comparing artesunate (Group II) with normal group (Group I); considered extremely significant
b : $p<0.001$ on comparing Tylophora and silymarin groups (Group III, IV) with artesunate group (Group II); extremely significant
c : $p>0.05$; not significant on comparing artesunate (Group II) with normal group (Group I); considered not significant.

Table: 2 Effect on antioxidant enzymes

GROUP	SOD	CAT
I. NORMAL	534.31 ± 11.95	17.28±1.24
II. ARTESUNATE	49.3 ± 3.20[a]	0.652±0.01[a]
III. ARTESUNATE+ EETI	380.27±19.6[b]	8.82± 0.97[b]
IV. ARTESUNATE + SILYMARIN	411.07 ± 6.90[b]	8.21 ± 0.01[b]

Values are expressed as Mean ± Standard Deviation; n=12
a : $p<0.001$ on comparing artesunate (Group II) with normal group (Group I); considered extremely significant
b : $p<0.001$ on comparing Tylophora and silymarin groups (Group III, IV) with artesunate group (Group II); extremely significant

DISCUSSION

Malaria is an important tropical mosquito-borne communicable disease. It is a leading cause of mortality and morbidity in developing areas of the world. The treatment of multi-drug resistant plasmodium falciparum malaria has stood a great test to medicine. Artemisinin and its derivatives (artesunate, artemether, arteether, &hydroartemisinin), obtained from *Artemisia annua* is used against multidrug- resistant strains of P.falciparum with good tolerability and absence of noteworthy adverse effects. Artesunate, one of the most widely used artemisinin compounds, is a water soluble hemisuccinate derivative given parenterally either by intravenous or intramuscular injection (Sanjana et al, 2012).

Recently some animal studies have reported the hepatic toxic potential of artesunate (Izunya et al, 2010; Arshad Ali Noorani et al, 2010). In this study the ethanolic extract of *Tylophora indica* was used to evaluate its hepatoprotective role in artesunate induced liver toxicity. The mechanism of artesunate induced liver injury is not known properly. It is believed to be due to the generation of free radicals (Arshad Ali Noorani et al, 2010). Ironically this free radical generation is the mechanism behind its antimalarial activity. Another possible mechanism of its liver toxic nature can be attributed to the involvement of its metabolite like Artenimol.

In the present study assessment of liver toxicity was done by measuring the levels of ALT, AST, ALP, GGT, BIL andALB in serum. An elevated level of AST, ALT, ALP and GGT in serum is an indication of hepatocellular disruption. When there is liver injury these enzymes leak into blood stream from the damaged tissues and show an elevated level in serum (Thapa BR et al, 2007; Vinayak Dnyandev Sapakal et al, 2011). In this study, the elevated levels of these markers were seen after artesunate administration, thus confirming its hepatotoxic potential (Group II). Co-administration of EETI with artesunate (Group III) reversed the levels of these markers almost to normal. This shows that EETI has a membrane stabilizing activity, thereby preventing the disruption of hepatocytes by artesunate.

In this study, liver homogenate of the rats administered with artesunate showed a decreased activity of SOD & CAT. This gives a clear indication about the role of oxidative stress induced by artesunate.Co-administration of EETI with artesunate (Group III) has increased the SOD and CATactivity. This shows that both EETI has anantioxidant activity, thereby preventing the cellular damage by reactive oxygen species. The antioxidant capacity of this indigenous plant was reported in the earlier studies.

From the above findings and facts, it can be concluded that *Tylophora indica* has prevented the artesunate induced liver injury by virtue of its membrane stabilizing property, and by antioxidant property.

Previous studies have reported the hepatoprotective property of *Tylophora indica.* This property of this indigenous medicinal plant is due to the presence of various phytoconstituents. The active constituent of Tylophora indica is an alkaloid tylophorine (VipulGujrathi et al, 2007; Shyamjith M et al, 2013). Further studies are being carried out to establish the role of tylophorine in drug induced liver injury.

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