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**Amelioration of isoniazid and rifampicin-induced liver toxicity by *Amaranthus graecizans* subsp. *silvestris* in rat**

## Amelioration of isoniazid and rifampicin-induced liver toxicity by *Amaranthus graecizans* subsp. *silvestris* in rat

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### Abstract

*Amaranthus graecizans* subsp. *silvestris*, a folk medicine for the treatment of inflammation, was used to evaluate its hepatoprotective potential against rifampicin and isoniazid-induced liver damage. Wistar albino rats were divided into four groups: Group I served as control (distilled water treated), Group II served as hepatotoxic group (isoniazid 50 mg/kg and rifampicin 100 mg/kg, treated), Group III served as positive control (silymarin 100 mg/kg, treated) while Group IV served as *A. graecizans* subsp. *silvestris* extract (400 mg/kg) treated group. The results suggest that the liver markers (alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and total bilirubin) were significantly increased in the animals of Group II. The methanolic extract showed a significant decrease in the raised liver enzymes of Group IV and encountered the liver damage caused by isoniazid and rifampicin. Histopathological examination of liver also revealed the improved architecture in the extract-treated group. Thus, the methanolic extract has potential liver protective action due to its phytochemicals.

### Introduction

Directly observed treatment short course (TB-DOTS) with isoniazid, rifampicin and pyrazinamide are highly effective treatment for the management of tuberculosis. Both isoniazid and rifampicin are well documented for their hepatotoxicity. Isoniazid produces liver injury by producing reactive toxic metabolites (isonicotinic acid, and hydrazine and acetylhydrazine free radical) that interacts with the liver proteins and results into anomaly of the liver anatomy. On the other hand, rifampicin when given in combination with isoniazid causes cytochrome P<sub>450</sub> enzyme-induction and results in the increased metabolism of isoniazid (Gangadharam, 1986; Wang et al., 2016; Pandit et al., 2012).

The animal model of hepatotoxicity is usually produced by either paracetamol or carbon tetrachloride. Then, different plants are examined to see their hepatoprotective

effects (Qadir and Ahmad, 2017). However, antitubercular drugs-induced hepatotoxicity and the effect of the plant to overcome it is observed only in a few plants such as *Monothea buxifolia* (Ullah et al., 2016), *Moringa oleifera* (Pari and Kumar, 2004).

*Amaranthus graecizans* subsp. *silvestris* (Vill.) Brenan, also known as "Mediterranean amaranth", belongs to the family Amaranthaceae, occurs in the warmer parts of Europe to the cooler regions of Western Asia and North Western India, also found in tropical Africa (Flora of Pakistan, 1972). It is considered as a wild food plant, semi-cultivated in Africa and consumed as a vegetable (Tabuti, 2007). It is also used as a folk medicine. The young leaves and shoot of the plant either boiled or steamed are employed to treat sore throat, as an immune system booster and to relieve joint pain by the natives in Uganda. The adverse effect associated with its use is the throat irritation (Nabatanzi



and Nakalembe, 2016). In Pakistan, it is called 'Phulari' by the natives and it is predominantly northerly distributed. The leaves of the plant are used to treat inflammation, piles and gonorrhoea (Hussain et al., 2010; Arshad et al., 2011; Nisar et al., 2011). The plant possesses remarkable anti-oxidant properties that make it a suitable candidate to counter oxidative stress (Ishtiaq et al., 2014). The methanolic extract of the herb is also reported to have significant analgesic and anti-inflammatory action which ensures the promising anti-oxidant abilities. The plant has also shown anti-cholinesterase and anti-protease activity (Ishtiaq et al., 2017).

Oxidative stress is one of the main reason behind multiple degenerative diseases including liver damage. Parenchymal cells are primary cells subjected to oxidative stress-induced injury in the liver (Li et al., 2015). As, it plays a core role in liver aberrations and their progression, the utilization of herbal drugs with anti-oxidant potentials have been suggested as healing agents, as well as adjuncts, to neutralize liver damage (Casas-Grajales and Muriel, 2015). With this view, the present project was designed to evaluate hepatoprotective potential of *Amaranthus graecizans* subsp. *silvestris*, against isoniazid and rifampicin-induced liver toxicity in Wistar rats.

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## Materials and Methods

### Chemicals

Methanol, isoniazid and rifampicin were procured from the Pacific Pharmaceuticals Ltd. Lahore. Diagnostic kits of transaminases, alkaline phosphatase and bilirubin were obtained from the Global (UK). Silymarin was acquired from the Wilson's Healthcare, Pakistan. All the chemicals were of analytical grade and reagents were freshly prepared in the laboratory.

### Plant material

Fresh plant parts were collected from the Chakwal and authenticated by Dr. Muhammad Ajaib, Department of Botany, Mirpur University of Sciences and Technology, Pakistan against voucher specimen of GC. Herb. 2329. The collected material was cleaned, dried and then powdered and preserved in amber colored bottles.

### Animal

Wistar rats of either sex were purchased from the University of Veterinary and Animal Sciences, Lahore Pakistan. The animals were kept at room temperature 25°C under 12 hours of dark and light cycles. The animals were fed with the standard pelleted diet with water *ad libitum*.

### Preparation of methanolic extract

The powder (500 g) was macerated in 700 mL of 90% methanol for seven days. The macerated material was

shaken periodically to facilitate extraction. After seven days, the sample solution was first filtered through Whatman No. 1 filter paper. The extract was evaporated using the rotary evaporator at a temperature lower than 40°C. The extract was collected and stored in an airtight container which was placed in the refrigerator for further investigations (Miliauskas et al., 2004).

### Phytochemical screening

The phytochemical analysis was carried out according to the standard procedures (Akbar et al., 2014).

### Study design

The study was conducted for 21 days. The weight of the rat was between 150-200 g. Twenty animals were randomly divided into four groups (n=5). Group I was given with distilled water as a control. Group II was administered with isoniazid (50 mg/kg) and rifampicin (100 mg/kg). Group III was treated with silymarin (100 mg/kg), isoniazid (50 mg/kg) and rifampicin (100 mg/kg). Group IV was treated with *A. graecizans* subsp. *silvestris* (400 mg/kg), INH (50 mg/kg) and RMP (100 mg/kg).

All the treatment groups were administered orally in distilled water (10 mL/kg) for 21 days. At the end of the treatment, animals were anesthetized by i.p. administration of 5 mL/kg of a solution of 1% chloralose in 25% urethane (w/v). Blood samples of animals were collected by cardiac puncture in sterile heparinized tubes and allowed to clot for 30 min. Serum was separated and used for the assay of serum marker enzymes (Salama et al., 2013).

### Histopathological examination

Fresh liver tissues (previously trimmed to 7 µm thick) were placed in plastic cassettes and immersed in neutral buffered formalin for 24 hours. Fixed tissues were processed routinely and then fixed in paraffin, sectioned, deparaffinized and rehydrated. The tissues were then stained with eosin and hematoxylin. The extent of combinational necrosis of isoniazid and rifampicin was evaluated by structural changes in liver sections stained with hematoxylin and eosin (Habbu et al., 2008).

### Statistical analysis

The values are expressed as mean ± SEM. SPSS 21 was used for the statistical analysis. Statistical significance of the differences between control and treated groups was calculated using One-way ANOVA with post-hoc Student's t-test. p<0.05 was considered to be significant.

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## Results

### Phytochemical analysis

Phytochemical screening revealed the presence of alkaloids, carbohydrates, saponins, flavonoids, phenols,



Table I

## Effects of extract on the liver markers

Group		Alanine transaminase ( $\mu\text{L}$ )	Aspartate transaminase ( $\mu\text{L}$ )	Alkaline phosphatase ( $\mu\text{L}$ )	Total bilirubin (mg/dL)
I	Control	23.8 $\pm$ 2.1	20.6 $\pm$ 1.2	50.9 $\pm$ 0.6	2.1 $\pm$ 0.5
II	Isoniazid (50 mg/kg) plus rifampicin 100 mg/kg)	49.1 $\pm$ 4.4 <sup>a</sup>	45.2 $\pm$ 4.8 <sup>a</sup>	124.6 $\pm$ 19.5 <sup>a</sup>	4.4 $\pm$ 0.1 <sup>a</sup>
III	Silymarin (100 mg/kg)	36.3 $\pm$ 1.7 <sup>b</sup>	40.6 $\pm$ 3.5	56.7 $\pm$ 0.8 <sup>b</sup>	1.3 $\pm$ 0.2 <sup>b</sup>
IV	Extract (400 mg/kg)	24.7 $\pm$ 2.9 <sup>b</sup>	26.4 $\pm$ 1.7 <sup>b</sup>	48.1 $\pm$ 0.9 <sup>b</sup>	1.9 $\pm$ 0.1 <sup>b</sup>

All groups are compared with Group II using SPSS by applying one way ANOVA. <sup>a</sup>represents significance level of  $p < 0.05$ . <sup>b</sup>significant p value comparison of Group II with Group III and Group IV

Table II

## Histopathological examinations of livers of different treatment groups

Groups	Portal area	Cell ballooning degeneration	Cytoplasm	Canaliculi cholestasis	Necrosis	Vascular injury	Steatosis	Interface hepatitis fibrosis
I	Normal	-	Normal	-	-	-	-	-
II	Portal area expansion (moderate)	Mild	Cytoplasmic degeneration (Mild)	Mild	Mild necrotic tissue	-	-	-
III	Bile duct proliferation (Mild)	-	Cytoplasmic ballooning (mild)	-	-	-	-	-
IV	Normal	-	Normal	Mild	-	-	-	-

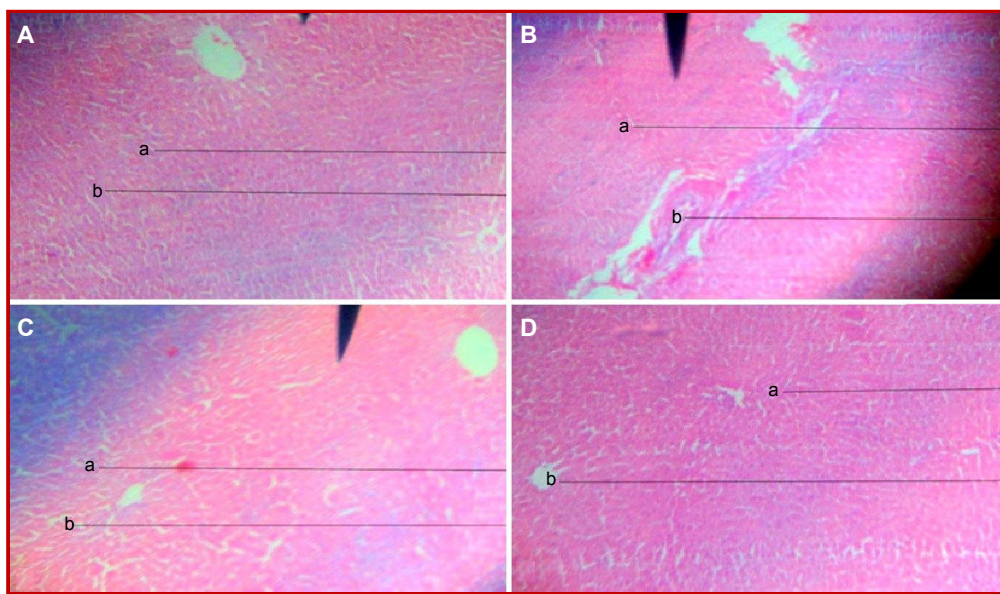


Figure 1: A) Control group a: Normal hepatocytes b: Normal sinusoids, B) Toxic group a: Cytoplasmic degeneration b: Portal vein expansion, C) Standard group a: Normal hepatocytes b: Normal sinusoids, D) Experimental group a: Normal hepatocytes b: Normal portal vein

steroids and glycosides in the extract whereas proteins and triterpenoids were absent.

#### Hepatoprotective activity

The hepatoprotective activity of the extract showed

significant  $p < 0.05$  reduction in aminotransferases, alkaline phosphatases and total bilirubin levels. Significant results were also obtained with silymarin but the more pronounced hepatoprotective effect was observed with

the methanolic extract (Table I).

### Histopathological examination

The histopathological examination also showed less damage to the hepatocytes and liver architecture in methanolic extract and silymarin treated groups when compared with isoniazid (50 mg/kg and rifampicin (100 mg/kg) treated group (Figure 1). The detailed histopathological observations were tabulated (Table II).

### Discussion

Methanolic extract of *A. graecizans* subsp. *silvestris* was evaluated for its hepatoprotective potentials against the combination of isoniazid and rifampicin. It was observed that the methanolic extract of *A. graecizans* subsp. *silvestris*, significantly ( $p < 0.05$ ) reduced the liver profile parameters (ALT, AST, ALP and total bilirubin) and showed the protection of hepatocytes against isoniazid- and rifampicin-induced liver injury when compared with the hepatotoxic group. The histopathological assessment also revealed the regeneration of hepatocytes against the destruction caused by these anti-tubercular agents.

The hepatoprotective activity of the plant extract could be due to the presence of the alkaloids, saponins, phenolic compounds, tannins, flavonoids and steroids (Agbafor et al., 2014).

The basic phytochemical group screening of the *A. graecizans* subsp. *silvestris* also unveiled the occurrence of alkaloids, carbohydrates, saponins, flavonoids, phenol, steroids, and glycosides in the sample whereas proteins and triterpenoids were absent.

This variety of phytochemicals in the extract may produce their hepatoprotective effect either by preventing or scavenging the free radicals generated as it holds appreciable anti-oxidant activity (Ishtiaq et al., 2014) or through an enhanced protein synthesis as observed in histopathological sections of the liver. The extract may also increase the excretion of isoniazid and rifampicin.

A number of plants, containing these phytochemicals demonstrated hepatoprotective effects in various researches when evaluated. Various plants *i.e.* *Celosia cristata* and *Bupleurum scorzonrifolium* are the rich source of saponins, has shown to exhibit hepatoprotective effect (Wang et al., 2010; Matsuda et al., 1997). Similarly, the plants enrich with flavonoids and phenolic acids *i.e.* *Equisetum arvense* and *Laggera alata*, also possess hepatoprotectant potentials (Wu et al., 2006; Oh et al., 2004). Quercetin is a flavonoid, when it's given to hepatocompromized rats, it results in the lowering of elevated hepatic enzyme levels (Janbaz et

al., 2004). Certain plants *i.e.* *Hypericum erectum*, *Sarcandra glabra* etc. with hepatoprotective glycosides are also reported in the literature (An et al., 2009; Li et al., 2006).

### Conclusion

*A. graecizans* subsp. *silvestris* showed the presence of alkaloids, carbohydrates, saponins, flavonoids, phenol, steroids, and glycosides. The methanolic extract showed hepatoprotective action in rat model against isoniazid plus rifampicin-induced hepatotoxicity.

### Ethical Issue

The protocols followed to carry out the project was approved by the Animal Ethical Committee of Punjab University College of Pharmacy, prepared by National Institute of Health (AEC/PUCP/1041/4313).

### Conflict of Interest

We declare that we have no conflict of interest.

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