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The effect of Argyrolobium roseum (Camb.) Jaub&Spach on some liver function biochemical parameters

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LIAQAT HUSSAIN[1], JAVARIA IKRAM[1], MUHAMMAD HANIF[2], FAISAL USMAN[2], MUHAMMAD ZIA-UL-HAQ[3], VICTOR VELTER[4], DIANA BADIU[5], TONY HANGAN[6]

[1]*College of Pharmacy, Government College University Faialabad, Pakistan*
[2]*Faculty of Pharmacy, Bahauddin Zakariya University, Multan-60800, Pakistan*
[3] *The Patent Office, Karachi, Pakistan*
[4]*Scientometrics Office, UESFISCDI, Bucharest- 010362,Romania*
[5]*Department of Biochemistry, Faculty of Medicine, Ovidius University of Constanta, Romania*
[6]*Department of Medical Informatics and Biostatistics, Faculty of Medicine, Ovidius Universityof Constanta,Romania*
**Address correspondence to: Department of Medical Informatics and Biostatistics, Faculty of Medicine, Ovidius University of Constanta (Romania)*, Email: toni@medcon.ro; tonyhangan@yahoo.com; *The Patent Office, Karachi, Pakistan, Email:* ahirzia@gmail.com

Abstract

Research on hepatoprotective effect from vegetables is increasingly focused on their potential on human health. In the present study, the effect of Argyrolobium roseum (Camb.) Jaub & Spach methanolic extract against hepatic toxicity induced by paracetamol (1g/kg) in blood samples of Swiss Albino mice was tested, by investigating the alanine aminotransferase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), total and direct bilirubin parameters. Standard drug silymarin (100mg/kg) was used as reference. Intra-peritoneal administration of the extract was achieved daily for 7 days at two different doses (i.e. 300 mg/kg and 600mg/kg) followed by paracetamol administration. Our results reveal the fact that methanolic extracts of Argyrolobium roseum (Camb.) Jaub&Spach protected the liver function from the hepatotoxic effect of paracetamol, as shown by the lower values of the five analyzed biochemical parameters in animals that received the methanolic extract as compared to the silymarin group, and merit considerations to be used as future components in a range of pharmaceutical formulations with hepatoprotective activity.

Keywords: *Argyrolobium roseum* (Camb.) Jaub&Spach, hepatoprotective, alanine aminotransferase, aspartate transaminase, alkaline phosphatase, bilirubin.

1. Introduction

In the recent years, there has been an increasing trend towards the exploration of safer and effective hepatoprotective potential from natural dietary sources like vegetables [1]. The antioxidant effect of vegetables, like *Argyrolobium roseum* (Camb.) Jaub&Spach (*Papilionaceae*), cultivated in northern areas like Kashmir, Peshawar, Haripur, Khan, Islamabad, Abbottabad, Jhelum, Murree, and Baluchistan, as a wild legume [2], is mainly due to the occurrence of flavonoids, glycosides, vitexin and D-pinitol [3]. However, few reports dealing with the obtaining of extracts and further determination of the compounds with hepatoprotective potential can be found mainly due to the low efficiency (in terms of quantities) in their extraction that did not encourage further investigations of these useful and promising extracts [3].

The extract of leaves is orally taken once a day before breakfast for treating liver and bladder inflammation including sexual disability [4]. The plant is also mentioned as a tonic, aphrodisiac [5, 6] and antidiabetic [7] in native medicine. Abbasi and contributors [8] showed that *Argyrolobium roseum* (Camb) Jaub&Spach can be used as a remedy for jaundice and hepatitis. Interestingly, Ahmed and colleagues [9] sustained the presence of natural antidiabetic and insulin secreting activity in *Argyrolobium roseum* (Camb.) Jaub&Spach. Other studies showed to have a role in the beta-cell neogenesis and hypoglycemic activity [10] being an immunosuppressive agent [10, 11]. However, how this extract can interfer into hepatoprotective process is still under debate.

In the present study, the influence of methanol extract from *Argyrolobium roseum* (Camb.) Jaub&Spach on alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), total and direct bilirubin biochemical liver parameters in blood samples using silymarin as reference was carried out.

2. Material and methods

2.1. Sample Preparation

The whole plant of *Argyrolobium roseum* (Camb.) Jaub&Spach was collected from the local supermarkets (about 5 kg), in the month of March, 2012 and immediately frozen and kept at -18^{0}C. Identification was carried out by the University of Karachi, Pakistan. The whole plant was washed under tap water, air dried. The dried material was then pulverized separately into fine powder by a mechanical grinder and stored in airtight bottles. About 2 kg of dried powder was soaked in 6 L methanol (95%) and shacked well. After 7 days soaking and shaking the extracts were separated by filtration and concentrated at 40^{0}C under reduced pressure by rotary evaporator. The weight of the dried extracts was then measured. The percentage yield of methanol extract was 9.3%. The extract was stored in air tight bottle at 4^{0}C.

2.2. Animals

The study was carried out on adult male Swiss Albino mice, weighing 250±10 g. All the animals were housed. The animals were individually housed in thermostated (22±2^{0}C) windowless plexiglass cages with constant humidity and controlled lighting conditions (12 h of light and 12 h of darkness per day) as well as with free access to tap water. They were feed under standard laboratory conditions.

The experiment was carried out in accordance with the Helsinki Declaration and guidelines of the Ethics Committee of the International Association for The Study of Pain. They were approved by the Animal Care Committee of the University of Karachi, Pakistan.

2.3. Paracetamol induced hepatotoxicity

Twenty five male Swiss Albino mouse were randomly divided into five groups, according to the treatment, in which the plant extract was given in two doses (300 (AR 300) and 600 (AR 600) mg/kg body weight), as follows:

Group I (n=5), served as control and received only the vehicle normal saline (1ml/kg/day) intraperitoneal (i.p.) once daily for 7 days.

Group II (n=5) received only paracetamol suspended in normal saline (1g/kg body weight), given daily by oral gavage (p.o.) for 7 days (GlaxoSmithKline Pakistan Ltd. Karachi) [12, 13].

Group III (n=5) received the standard drug silymarin (DiaSys Diagnostic systems, Germany) 300mg/kg i.p. daily for 7 days followed by paracetamol, in a single dose of 1g/kg body weight given p.o. on 7th day.

In group IV (n=5) the plant extract was administered (300mg/kg, i.p.) daily for 7 days followed by single dose of paracetamol 1g/kg body weight given p.o. on 7^{th} day (14).
In Group V (n=5) plant extract was administered in a higher dose (600mg/kg, i.p.) also daily for 7 days followed by single dose paracetamol 1g/kg body weight given p.o. on 7^{th} day.

The animals were sacrificed 24 h after the last dose using phenobarbitone anesthesia (35 mg/kg, i.p.) [15] and blood samples of each animal were taken from abdominal aorta. Blood samples were clotted for 45 min at room temperature and the serum was separated by centrifugation at 2500 rpm for 15 min (16). The collected sera were kept in Eppendorf tubes and stored at 4°C.

2.4. Biochemical studies

ALT, ALP, AST, total and direct bilirubin were determined using commercially available kits from Sigma-Aldrich (Germany) and assays were carried out using a spectrophotometer DU 800 (Beckmann, USA). ALT activity was determined by a coupled enzyme assay, which results in a colorimetric (570 nm)/fluorometric product, proportional to the pyruvate generated. One unit of ALT is defined as the amount of enzyme that generates 1.0 µmol of pyruvate per minute at 37^0C.

We further used the ALP diethanolamine detection kit in order to determine the presence of ALP activity by using p-nitrophenyl phosphate as the substrate.

In AST assay kit the transfer of an amino group from aspartate to α-ketoglutarate results in the generation of glutamate, and the production of a colorimetric product (450 nm) proportional to the AST enzymatic activity.

The bilirubin assay is based on the Jendrassik-Grof method, which utilizes the reaction of bilirubin with diazotized sulfanilic acid resulting in a colorimetric product measured at 530 nm, proportional to the bilirubin amount present in the sample. This assay kit measures both total and conjugated bilirubin.

2.5. Statistical analysis

All the aforementioned experiments were conducted in triplicate. Statistical comparisons were performed by one-way analysis of variance (ANOVA). In the case of the identification of statistical differences using ANOVA, the Student Newmal -Keuls test was used to compare the fractions using SPSS version 12.0 (SPSSS Inc., Chicago, IL, USA). Probability values < 0.05 were considered significant.

3. Results

Our results showed that *Argyrolobium* roseum (Camb.) Jaub&Spach extract efficiently decreased the values of the serum biochemical markers ALT, AST, ALP, direct and total bilirubin as compared to paracetamol group (Group II, figure 1).

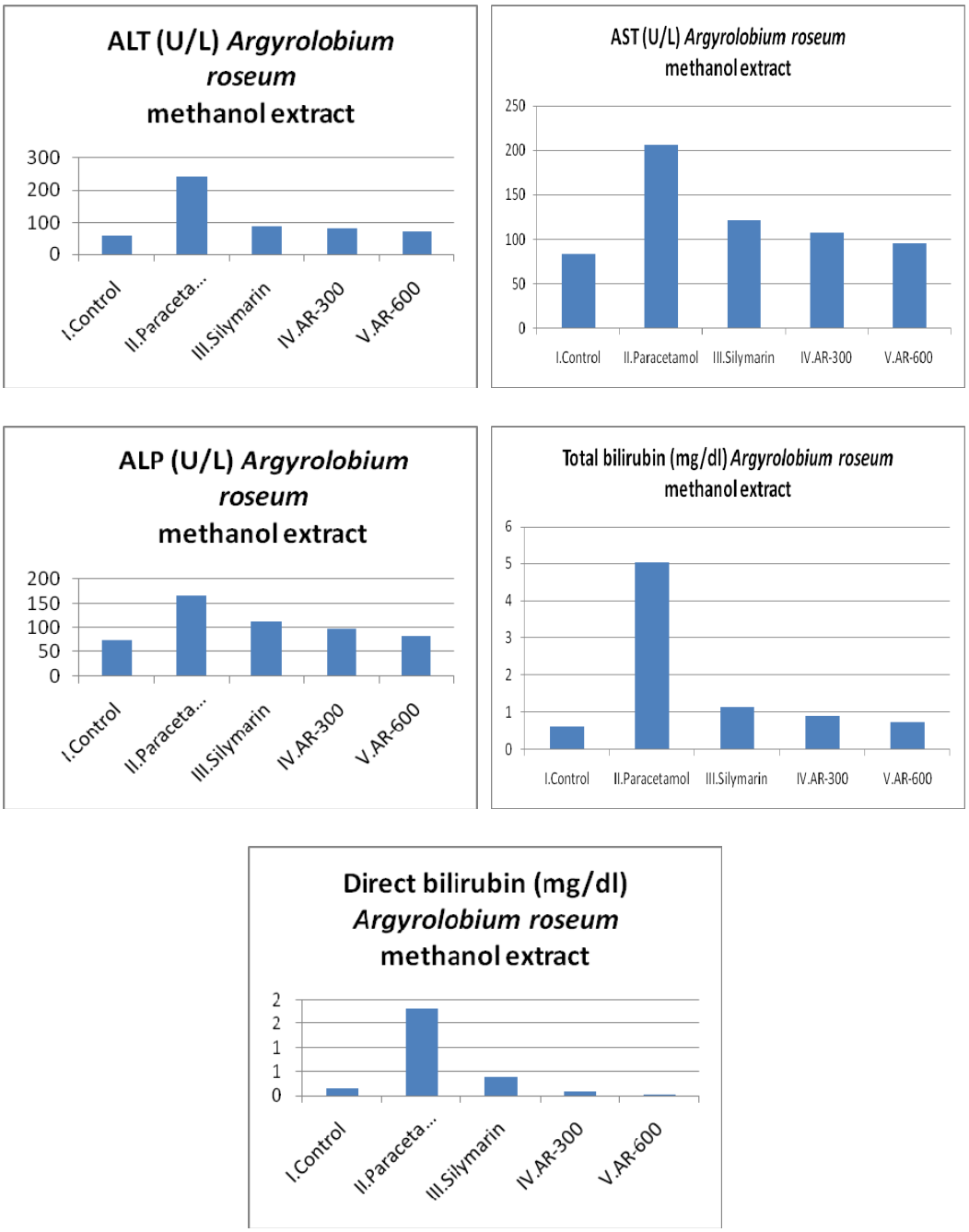

Figure 1. Biochemical parameters of ALT, AST, ALP, total and direct bilirubin in five groups.

The groups treated with 300mg/kg and 600mg/kg (Groups IV and V) plant extract showed values of 83.20 U/L and 72.82 U/L for ALT, 107.22 U/L and 95.18 U/L for AST, 97.52 U/L and 83.28 U/L for ALP, 0.91 mg/dl and 0.73 mg/dl for total bilirubin, 0.09 mg/dl and 0.03 mg/dl for direct bilirubin. However, the values from paracetamol treated group

(Group II), showed a sharp rise in ALT, AST, ALP, total and direct bilirubin (241.02 U/L, 205.96 U/L, 166.72 U/L, 5.02 mg/dl and 1.83 mg/dl) (table 1).

Table 1. Effect of *Argyrolobium roseum* methanol extract on ALT, AST, ALP, total and direct bilirubin of paracetamol induced hepatotoxic mice.

Group	Dose administered	Parameters				
		ALT (U/L)	AST (U/L)	ALP (U/L)	Total bilirubin (mg/dl)	Direct bilirubin (mg/dl)
I.Control	1 ml/kg i.p.	60.38±6.915	83.32±4.757	72.52±6.771	0.614±0.064	0.158±0.043
II.Paracetamol	1 g/kg p.o.	241.02±31.853*	205.96±33.208*	166.72±18.101*	5.022±0.804*	1.834±0.361*
III.Silymarin	100 mg/kg i.p.	90.82±11.092	121.04±10.878	113.64±9.149	1.152±0.295	0.394±0.134
IV.AR-300	300 mg/kg i.p.	83.20±19.735	107.22±3.891	97.52±11.252	0.91±0.624	0.09±0.314
V.AR-600	600 mg/kg i.p.	72.82±15.127	95.18±3.846	83.28±0.007	0.73±0.511	0.03±0.189

Values are expressed as mean ± SEM, n=5 mice in each group.
ALT= alanine aminotransferase; AST= aspartate aminotransferase; ALP= alkaline phosphatase; i.p.=intraperitoneal.
*p<0.0

Interestingly, the silymarin group (Group III) did not show a better improvement neither comparing with Group IV (AR-300) (90.82 vs. 83.20 U/L for ALT, 121.04 vs. 107.22 U/L for AST, 113.64 vs. 97.52 U/L for ALP, 1.152 vs. 0.91 mg/dl for total bilirubin and 0.394 vs. 0.09 mg/dl for direct bilirubin) nor with Group V (AR-600) (90.82 vs. 72.82 U/L for ALT, 121.04 vs. 95.18 U/L for AST, 113.64 vs. 83.28 U/L for ALP, 1.152 vs. 0.73 mg/dl for total bilirubin and 0.394 vs. 0.03 mg/dl for direct bilirubin).

4. Discussion

Recent studies revealed that paracetamol toxic dose intake may result in dependent hepatotoxicity of centrilobular region, due to the depletion of glutathione stores [17]. Such liver injury leads to release of serum biomarkers, which could indicate a mitochondrial damage [18].

Our findings show the effect of *Argyrolobium roseum* (Camb.) Jaub&Spach methanolic extract on five biochemical indicators for liver function (i.e. ALT, AST, ALP, total and direct bilirubin).

The methanolic extract of *Argyrolobium roseum* (Camb.) Jaub&Spach preserved ALT, AST, ALP, total and direct bilirubin at markedly reduced levels in mice treated with paracetamol, as compared to silymarin treated animals. The protective effect against paracetamol hepatotoxicity was more evident at higher dose (600 mg/kg) as compared to the lower dose (300 mg/kg).

Elegant work from Marwat and Khan [4] revealed that the *Argyrolobium roseum* plant possessed glycosides and flavonoids, which are known for their antioxidant, anti-inflammatory, antiulcer and hepatoprotective activity (4, 19). There is an extensive body of literature accumulated in the recent years suggesting the involvement of vitexin and d-pinitol found in high amounts in *Argyrolobium roseum* (Camb.) Jaub&Spach into liver function [10, 20].

Furthermore, vitexin had been previously showed to act as an antioxidant agent inhibiting almost 70% superoxide radicals [21] and d-pinitol had been mentioned to have a role in lipid lowering, decreasing cholesterol and hepatic lipid droplets [22].

5. Conclusions

Our results reveal the fact that methanolic extracts of *Argyrolobium roseum* (Camb.) Jaub&Spach protected the liver function from the hepatotoxic effect of paracetamol, as shown by the lower values of the five analyzed biochemical parameters in animals that received the methanolic extract as compared to the silymarin group, and merit considerations to be used as future components in a range of pharmaceutical formulations with hepatoprotective activity.

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