ORIGINAL ARTICLE

Evaluation of effect of *Argyrolobiumroseum A*queous Extract on Carbon Tetrachloride induced liver injury in rabbits

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ABSTRACT

Liver diseases are a matter of serious concern all over the world. They may lead to serious consequences such as cirrhosis and hepatocellular carcinoma. Many efforts are being made to discover new hpatoprotective agents. Herbal medicines are usually considered as safe and effective for various ailments. The therapeutic value of *Argyrolobiumroseum* has been recognized in traditional medicine. Hepatoprotective effect of aqueous extract *Argyrolobiumroseum* whole plant against CCl_4 induced hepatotoxicity was evaluated. In this study thirty five male rabbits were used. Animals were divided into five groups, containing seven animals in each group. Liver injury was induced by carbon tetra chloride 0.4ml/kg/day (1:1 in olive oil) in twenty eight rabbits. Hepatoprotective effect of aqueous extract of *Argyrolobiumroseum* with doses of 200mg/kg/day, 400mg/kg/day and 600 mg/kg/day was evaluated. Serum AST, ALP, ALT and total bilirubin were evaluated by using Human CE kits in semi-automatic clinical chemistry analyzer, backman coulter CX5. There was a significant elevation of Alanine transaminase (ALT), alkaline phosphatase (ALP), Aspartate transaminase (AST) and total bilirubin levels indicating development of hepatotoxicity. The level of ALP, ALT, AST and total bilirubin were significantly reduced(pvalue 0.001) in *Argyrolobiumroseum* (400mg/kg) treated rabbits showing the hepatoprotective effect of *Argyrolobiumroseum*.

Keywords: Hepatoprotective, *Argyrolobiumroseum*, carbon tetrachloride, hepatotoxicity, rabbits, serum transaminases

INTRODUCTION

Hepatotoxicity is liver injury leading to impaired liver function resulting from exposure to drugs or other noninfectious agents [1]. Liver is susceptible to injuries induced by drugs and xenobiotics because of its unique function and anatomical position. It is main site of adverse drug reaction as drug biotransformation mainly takes place in the liver[2]. Major goal in the treatment of liver diseases is to enhance liver detoxification process and to prevent further damage[3]. In the absence of significant and safe hepatoprotective agents in modern therapeutics more attention is being paid towards the development of plant based hepatoprotective agents. Natural remedies are considered to be a safe and effective alternative for treatment of hepatotoxicity[4].

Argyrolobiumroseum is a rare herb belonging to family pappilonacea. It grows in tropical and temperate regions of Pakistan, India, Bangladesh, Nepal, Madgascar, South Africa and Arabia[5]. It has a weak stem, ascending branches and trifoliate leaves[6]. *Argyrolobiumroseum* is traditionally used for the treatment of jaundice and hepatitis, to relieve stomach and bladder inflammation andf or various skin diseases such as boils and scabies[7,8,9,10]. Scientifically antihyperglycemic and immunosuppressant effect of *Argyrolobiumroseum* is documented[11,12].

Carbon tetra chlorideis commonly used toxin to induce hepatotoxicity in experimental animals. CCl_4 is metabolized by CYP2E1 resulting in generation of reactive metabolites trichlormethyle (.ccl3) and peroxytrichlormethyle (.ooccl3) radicals. These radicals bind to lipids, proteins and nucleic acid by covalent bonds.[13]The level of molandialdehyde indicates lipid peroxidation whereas leakage of aspartate transaminase, alanine transaminase and lactate dehydrogenase are important index of hepatotoxicity[14].

MATERIAL AND METHODS

Fresh plants of Argyrolobiumroseum were procured from Burkot valley of Khyber Pakhtunkhawah proper identification of plant was done by authorized personnel from PCSIR. Fresh plants were shade dried for 2 weeks and then pulverized by using an electric blender. Every sixty grams of powdered plant material blended in one liter of cold distilled water maintained on mechanical shaker and filtered using a Buchner funnel and what man no 1 filter paper. The filtrate was quickly frozen at – 48^{O}Celsius and dried for 48 hours using a freeze dryer .[15]The dried extract

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(372g) obtained was kept in tightly closed bottle protected from light in refrigerator at 2-8^{o} Celsius to be used throughout the experiment. Total yield was about 4.1%. The aqueous extract of *Argyrolobiumroseum* was preferred to use for experimental process as most of the constituents of Argyrolobiumroseum were present in aqueous extract.[16]The calculated dose for individual rabbit i.e. 200mg/kg, 400mg/kg and 600mg/kg was dissolved in 5 ml distilled water. Doses were administered through nasogastric tubes number 6.

Adult healthy rabbits *(Oryctolaguscuniculus)* of male gender weighing 1-1.507 kilogram were purchased from local market. Animals were numbered and kept in iron cages in individual groups, under hygienic conditions, at room temperature, under natural light and dark cycles to maintain biological clock in animal house of UVAS. Food and water was provided ad libitum.CCl_4 and olive oil were taken in a ratio of 1:1 and a total dose of 0.4ml/kg body weight was used to induce hepatotoxicity. CCl4 (1:1 olive oil) was administered by intraperitoneal route using 1ml disposable syringes to induce toxicity[17].

Grouping of animals: Rabbits were divided into five experimental groups, containing seven rabbits in each group randomly. Group 1 was normal control group and received 0.2 ml/kg/day olive oil intraperitoneally and distilled water 5 ml orally. Group 2 was positive control group and received 0.4 ml/kg/day carbon tetra chloride (1:1 in olive oil) intraperitoneally and distilled water 5 ml orally. Group 3, 4 and 5 received 0.4 ml/kg/day carbon tetra chloride (1:1 in olive oil) intraperitoneally and *Argyrolobiumroseum* aqueous extract orally in a dose of 20, 400, 600 mg/kg/day. All the treatment was provided daily for 14 days.

Two to three milliliters blood was collected from the ear marginal vein through 5 ml disposable syringes on day 0, 7 and 15. The collected blood sample was transferred to labeled gel tubes and allowed to clot at room temperature for 30 minutes, then centrifuged at 3000 rev/min for 15 minutes[18]. The clear serum obtained was separated and shifted in serum cups and preserved at -20 ^{o}C till serum analysis was started. Lab tests were performed for analysis of Alkaline phosphatase (U/L) Alanine transaminase (U/L) Aspartate transaminase (U/L) Total bilirubin (mg/dl) .All the tests were performed on semiautomatic clinical chemistry analyzer, Beckman coulter CX5. Methods were followed as provided by the manufacturer.

Data analysis: All data was entered on graph pad prism version 5 for statistical analysis.The data was evaluated by one way analysis of variance followed by Turkey's multiple comparison tests. P value less than 0.05 was considered significant

RESULTS AND DISCUSSION

All parameters including ALT, AST, ALP, and total bilirubin were significantly raised in positive control group(group 2). However *Argyrolobiumroseum* aqueous extract significantly decreased the level of ALT, AST, ALP and total bilirubin (pvalue 0.001). Maximum effect was observed in 400 mg/kg/day dose. Mathematically significant difference was observed among group 4 and group 5 but not statistically significant.

Table 1:Comparison of mean ALT level (u/l) among groups 1, 2, 3, 4, and 5 (ANOVA).

Alanine transaminase (U/L)	**Group 1** **Mean±S.D**	**Group 2** **Mean±S.D**	**Group 3** **Mean±S.D**	**Group 4** **Mean±S.D**	**Group 5** **Mean±S.D**	**P.value**
Day 0	55.43±11.5 (n=7)	62±7.87 (n=7)	61±8.75 (n=7)	58.86±10.88 (n=7)	54.85±9.22(n=7)	0.558
Day 7	53.0±13.35 (n=7)	284.2±60.33 (n=6)	280.7±21.65 (n=7)	162±20.62 (n=7)	187.7±77 (n=7)	0.001***
Day 15	58.0±9.29 (n=7)	349.6±95.19 (n=5)	205.2±104.2 (n=5)	159.7±24.98 (n=7)	158.3±13.9 (n=7)	0.001***

*** p-value ≤ 0.001, ** p-value ≤ 0.01,* p-value 0.05

Table 2:Comparison of mean aspartate transaminase (u/l) of group 1, 2, 3, 4, and 5 at day 0, 7 and 15.

Aspartate transaminas (U/L)	**Group 1** **Mean±S.D**	**Group 2** **Mean±S.D**	**Group 3** **Mean±S.D**	**Group 4** **Mean±S.D**	**Group 5** **Mean±S.D**	**P.value**
Day 0	39.43±8.10 (n=7)	47.14±10.38 (n=7)	52.71±8.67 (n=7)	49.29±10.98 (n=7)	49.14±11.25 (n=7)	0.886
Day 7	36.71±7.2 (n=7)	231.3±13.74 (n=6)	288±39.21 (n=7)	160.7±35.34 (n=7)	126.9±11.85 (n=7)	0.001***
Day 15	41.86±7.75 (n=7)	333.8±180.7 (n=5)	211.6±106.6 (n=5)	164.3±110.1 (n=7)	142.1±16.91 (n=7)	0.001***

*** p-value ≤ 0.001, ** p-value ≤ 0.01,* p-value- 0.05

Tab 3**:** Mean alkaline phosphatase of group 1, 2, 3, 4, and 5 at day 0, 7 and 15.

Alkaline phosphatase(U/L)	**Group 1**	**Group 2**	**Group 3**	**Group 4**	**Group 5**	**P.value**
	Mean±S.D	**Mean±S.D**	**Mean±S.D**	**Mean±S.D**	**Mean±S.D**	
Day 0	63.0±24.0 (n=7)	61.86±28.79 (n=7)	58.57±22.8 (n=7)	62.0±21.76 (n=7)	61±19.93 (n=7)	0.983
Day 7	63.0±29.46 (n=7)	313.01±129.6 (n=7)	252.4±140.6 (n=7)	153.3±43.03 (n=7)	119.6±9.7 (n=7)	0.0001***
Day 15	66.14±20.72 (n=7)	268.4±122.1 (n=7)	146.4±76.35 (n=7)	140.1±60.13 (n=7)	142.1±24.09 (n=7)	0.0001***

Table 4: Mean total bilirubin of group 1, 2, 3, 4, and 5 at day 0, 7 and 15.

Total bilirubin(mg/kg)	**Group 1**	**Group 2**	**Group 3**	**Group 4**	**Group 5**	**p.Value**
	Mean±S.D	**Mean±S.D**	**Mean±S.D**	**Mean±S.D**	**Mean±S.D**	
Day 0	0.27±0.13 (n=7)	0.3±0.14 (n=7)	0.38±0.13 (n=7)	0.37±0.15 (n=7)	0.38±0.15 (n=7)	0.44
Day 7	0.31± 0.15 (n=7)	0.66±0.27 (n=6)	0.48±0.13 (n=7)	0.24±0.12 (n=7)	0.44±0.20 (n=7)	0.001***
Day 15	0.23±0.15 (n=7)	2.16±0.89 (n=5)	0.4±0.19 (n=5)	0.78±0.6 (n=7)	0.4±0.13 (n=7)	0.001***

Fig 1:Comparison of mean alanine transaminase level of group 1, 2, 3, 4, and 5 at day 0, 7 and 15

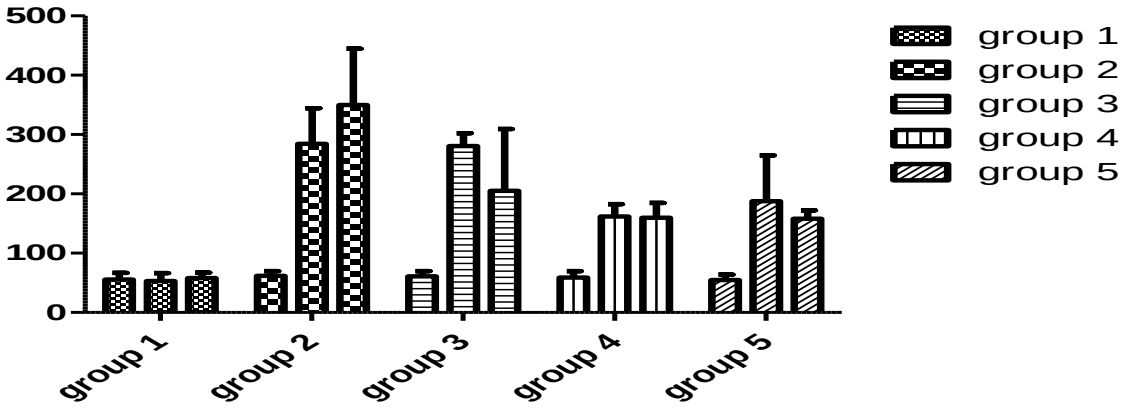

Fig 2: Mean aspartate transaminase of group 1, 2, 3, 4, and 5 at day 0, 7 and 15.

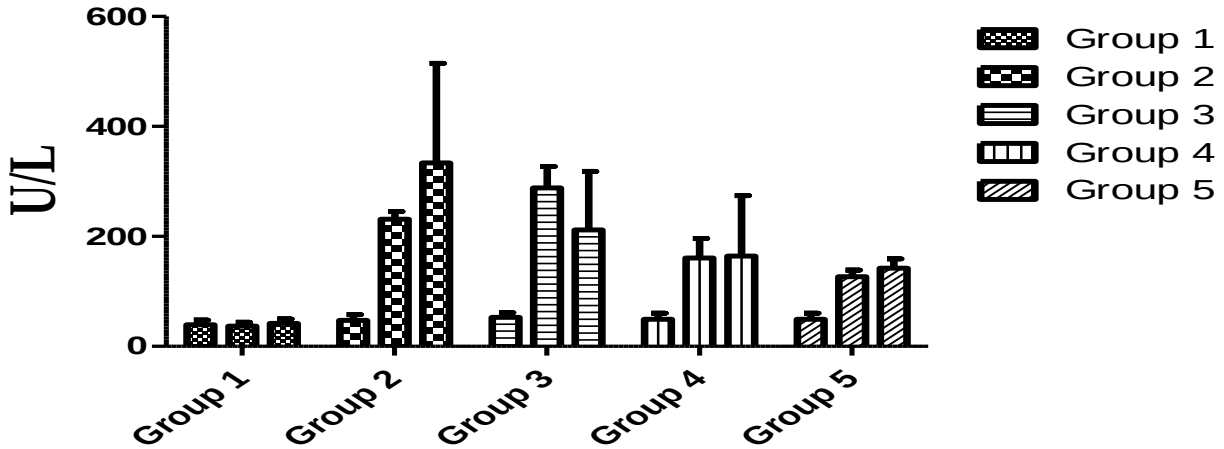

Fig. 3: Mean alkaline phosphatase of group 1, 2, 3, 4, and 5 at day 0, 7 and 15.

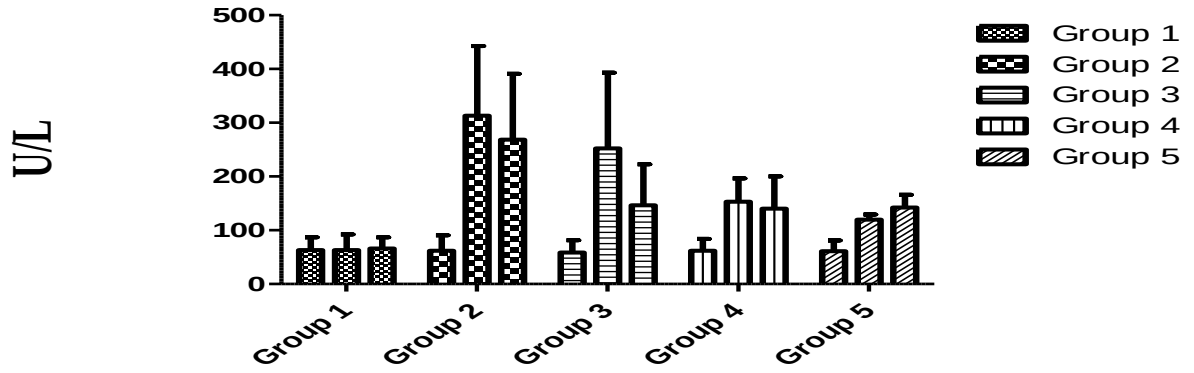

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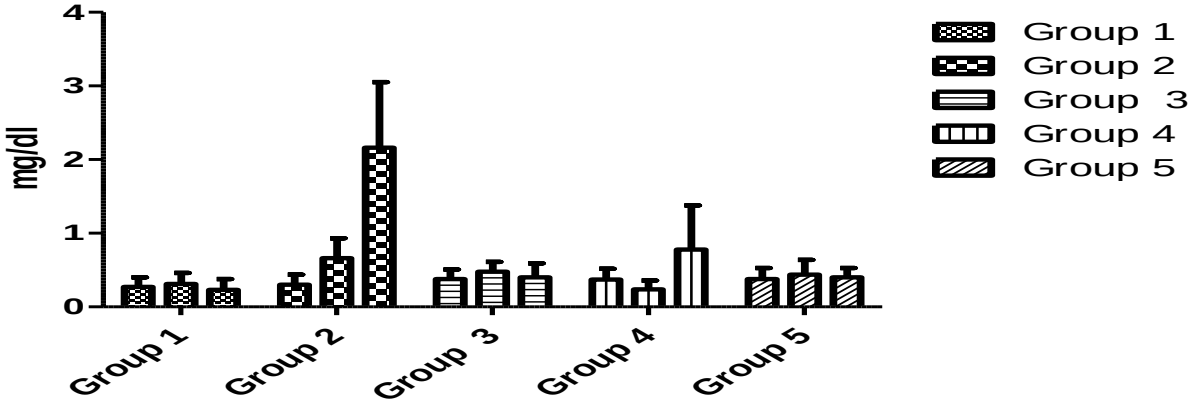
Fig 4; Mean total bilirubin of group 1, 2, 3, 4, and 5 at day 0, 7 and 15.



CCl_4 induces lipid peroxidation of hepatocyte cell membrane leading to loss of structural and functional integrity of cell membrane and loss of cellular contents. ALT is specifically high in liver diseases[19]. These results were in consistence with previous study by Hussain et al who studied the effect of methanolic extract of *Argyrolobiumroseum* on paracetamol induced hepatotoxicity[20]. Zhou Yusi et al, demonstrated the hepatoprotective effect of d.pinitol against galatosamine induced hepatotoxicity. They also explained that d.pinitol possessed antioxidant effect. D.pinitolelevated level of glutathione reductas, catalase and superoxide dismutase, hepatic cytochrome p450 2E1, hepatic glutathione levels and prevented lipid peroxidation[21].

AST along with ALT is an important indicator of hepatocyte damage. AST is relatively nonspecific and raised levels are also in hepatic cardiac and muscle injuries. Treatment with CCl_4 alone (group 2) caused significant increase in level of serum aspartate transaminase as compared to normal control group (group 1) p<0.001***. Serum AST level was significantly reduced in *Argyrlolobiumroseum* aqueous extract treated group as compared to positive control group (p<0.001***). These results were in consistence with previous study by Majielse who studied the effect D. pinitol against chemical induced hepatotoxicity [22].

ALP is relatively more related to functional integrity of hepatocyte. During cellular damage this enzyme leaks into circulation**.** Therefore CCl_4 toxicity also elevates ALP level due to hepatocyte and bile duct injury[17]. Treatment with CCl_4 alone (group 2) caused significant elevation in level of alkaline phosphataseas compared to normal control group(group 1) p.value 0.001***. Serum ALP level was significantly reduced in *Argyrlolobiumroseum* aqueous extract treated group as compared to positive control group. This effect may be due to the ability of D. pinitol to modulate cell surface glycoproteins and to protect membrane of both lysosome and mitochondria because of its antioxidant nature[23].

Bilirubin is break down product of RBC'S. A major function of liver is to converts bilirubin into water soluble form of bile pigment, bilirubin diglucronide. This conjugated and unconjugated bilirubin passes from hepatocytes to bile canaliculi through ATP-dependent canalicular multidrug resistant protein[24]. Treatment with CCl_4 alone (group

2) caused significant elevation in level of serum bilirubin level as compared to normal control group (group 1) p<0.001***. Serum bilirubin was significantly reduced in *Argyrlolobiumroseum* aqueous extract treated group as compared to positive control group (p<0.001***). The decrease in serum bilirubin level may indicate functional stability of hepatocytes. This effect may be due to the antioxidant nature of constituents of *Argyrolobium roseum*[21].

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