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Full Length Research Paper

Assessment of Lycium Barbarum for detoxification of Patulin toxin and their effects on rat serum globulin

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The aim of this study was to show the efficiency of Goji extract on rat serum globulins which treated with patulin mycotoxin. 50 adult albino male rats were divided into 5 groups. Control group was injected subcutaneously daily with distilled water; groups I and II were injected subcutaneously daily with Patulin (0.2 mg/kg/day) for one and two weeks respectively. Group III was treated with an equal dose of patulin for two weeks then injected subcutaneously with Goji extract (2ml/kg /day) for two weeks. Group IV was treated by Goji for two weeks then they were treated with an equal dose of patulin for two weeks. The mean values of albumin level in treated groups with patulin showed highly significant decrease compared with control group. But the mean values of α_1, α_2, β and γ globulins showed highly significant increase in GI and G II compared with control group. However there is no significant change in the mean value of albumin, α_1, α_2, β and γ globulins in G III and G IV compared with the control.

Keywords: Patulin, Goji extract, Scopoletin, electrophoresis

INTRODUCTION

Patulin (PAT, 4-hydroxy-4*H*-furo [3,2c] pyran-2 (6*H*)-one; (Figure 1), a mycotoxin of *Penicillium*, *Aspergillus* and *Byssochlamys* molds, is believed to exert its toxic, chromosome damaging, and carcinogenic activity by covalent binding to cellular nucleophiles, in particular to the thiol groups of proteins and glutathione (GSH). Ralph and Manfred provide clear evidence that PAT is capable of cross linking proteins, and that not only thiol but also amino groups are involved in these crosslinks. Moreover, a marked influence of the protein tertiary and quarternary structure on PAT-induced crosslink formation could be demonstrated for microtubule proteins as an example of proteins with experimentally controllable superstructure (Ralph and Manfred.1999).

Goji berry, (Figure 2) also known as wolfberry (*Lycium barbarum* and *Lycium chinense*), belongs to the Solanaceae family. In recent years, interest in Goji berries has increased dramatically, due to their high potential nutrient value, health benefits and longevity. These properties have made Goji berries a favorite among healthy lifestyle consumers and individuals wishing to benefit from their claimed weight loss properties (Monzón et al., 2011). goji berries act as a crucial role in biological activities, including effects on ageing, neuroprotection, increased metabolism, glucose control in diabetics, glaucoma, anti-oxidant properties, immune modulation and antitumor activity (Amagase and Farnsworth, 2011). Where, these healthy contributions have been proved and associated with the presence of

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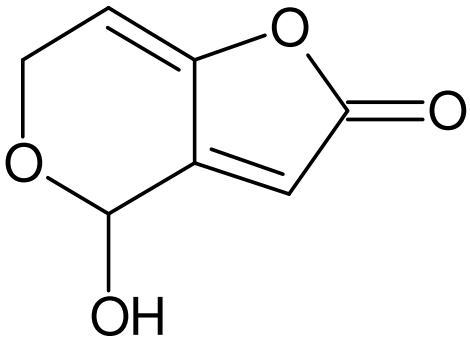


Figure 1. Patulin mycotoxin

Figure 2. The fruit of Lycium Barbarum (Goji) (Monzón.et al.2011)

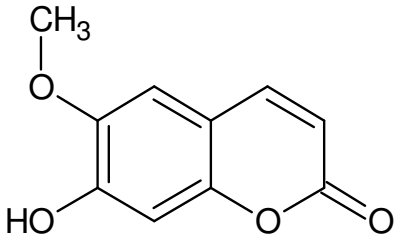


Figure 3. Scopoletin

various functional components including Polysaccharides, carotenoids, fatty acids, Flavonoids and scopoletin.

Scopoletin Figure 3 (6-methoxy-7-hydroxycoumarin) was isolated from traditional Chinese medicine that has been used for the treatment of various rheumatoid diseases for a long history. It has been shown to exert several biological activities, such as anticholinesterasic, antithyroid, antioxidant, antihyperglycemic, hypouricemic, antitumoral, and anti-inflammatory activities. However, little information is available on the analgesic and anti-inflammatory effects of scopoletin *in vivo* (Tien et al., 2012) .

Proteins are large molecules composed of covalently linked amino acids. Depending on electron distributions resulting from covalent or ionic bonding of structural subgroups, proteins have different electrical charges at a given pH. Proteins were fractioned into five classical fractions: albumin, alpha 1, alpha 2, beta and gamma globulins. Electrophoresis is the process of migration of charged molecules through solutions in an applied electric field. It is often classified according to the presence or absence of a solid supporting medium or matrix through which the charged molecules move in the electrophoretic system. Most practical application of electrophoresis in biochemistry employs some forms of zonal electrophoresis, in which the aqueous ionic solution is carried in a solid support and samples are applied as spots of material is cellulose acetate electrophoresis (Robert and Garrity.,1999).

MATERIAL AND METHODS

Chemicals

Patulin (4-hydroxy-4H-furo[3, 2-c]pyran-2 (6H)- one), Sodium Dihydrogen Phosphate, Di Sodium Hydrogen Phosphate, Ponceau S, Trichloracetic acid and Unhydrous acetic acid were purchased from Sigma Chemical Company.

Extraction of Goji

Goji berry (5 g), 25 mL alcohol and 75 ml distilled water were added into Erlenmeyer flask (the ratio of goji berry and alcohol is referred to the ratio of making commercial goji wine). The Erlenmeyer flask was sealed with parafilm and tinfoil and put in refrigerator for 5 days. The solid and liquid phases were separated by filtration. after finishing steeping process, solvent is removed from filtrate by rotatory evaporator to obtain crude goji extract (Yang and Baojun, 2013).

GC–MS for Goji extract

The chemical constituents of the extract were analyzed using gas chromatography coupled with mass spectrometry (GC–MS). GC–MS was performed on Agilent 5975 GC/MSD system. A DB-5 MS UI stainless steel capillary column 30 m_0.25 mm (1.0 µm film thickness), the column temperature was initially held at 50 °C for 0.0 min. and then programmed to 130°C at a rate of 10.0 °C per minute, then programmed to 230°C at a rate of 4.0 °C per minute and hold time for 7.0 minutes.

Mass unit conditions were as follows: Ion source 230 °C, ionization energy 70 eV and electron current 1455 mA.

Helium was used as the carrier gas at 1 ml per minute.

The injection temperature was 280 °C; the injection mode is pulsed splitless with 50:1 ratio at 1 min.

2.4 Theoretical Calculations

The calculations were performed on PC computers using MOPAC2000 program package (Stewart,1999) with Win Mopac 2.0 (Win Mopac, 1997) as a graphic interface to obtain Geometry Optimization of the suggested structures found in Goji Berry extract by AM1 method.

2.5 Experimental design and animals

Fifty adult albino male rats each weighs (240-300 g) were conditioned at room temperature, commercial balanced diet and tap water were provided in the experiment. Animals were divided randomly into five groups (10 rats each in two cages) and were subjected to the following schedule of treatments:

Control group: was injected subcutaneously daily with distilled water;

G I and G II: were injected subcutaneously daily with Patulin (0.2 mg/kg b. w.) for one and two weeks respectively.

G III: was treated by Patulin for two weeks then injected subcutaneously with Goji extract (2 ml / kg b.w.) for two weeks.

G IV: was treated by Goji for two weeks then injected subcutaneously with the same toxin for two weeks. Animals were anesthetized under light diethyl ether and blood samples were collected from the heart into plain tubes and stored at -20°C.

Determination of serum proteins

Blood samples from all groups were collected and the serum was separated by electrophoresis (SEAC, 2001) on cellulose acetate strips at pH=7.4 using phosphate buffer and stained with ponceau s dye for quantitative analysis of electrophoretically separated fractions. A densitometer (code 53871210, SEAC) was used according to Jeppsson et al. (1979). Total proteins and albumin were determined according to Peters et al. (1982)

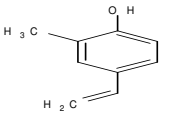
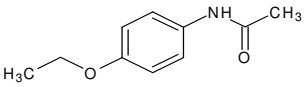
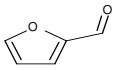
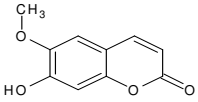
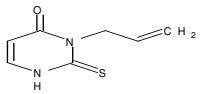
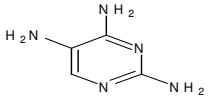
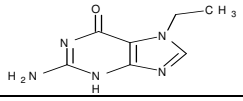
Statistical analysis

Statistics was performed using the statistical graph pad prism 5. One way analysis of variables (ANOVA) was used posted by Newman-keuls test. All results are expressed as mean ± SE and the level of significance between groups were *$p<0.05$, ** $p<0.01$, *** $p<0.0001$.

RESULTS AND DISCUSSION

Qualitative analyses of target antioxidant compounds found in Goji extract were achieved using a GC-MS method resulting in many constituents that are listed in Table 1. The optimized molecular structure of the scopoletin, using semi empirical gas phase AM1 (Austin Model 1) methods using Win MOPAC package, is shown in Figure 4. According to the frontier molecular orbital theory, the formation of a transition state is due to an interaction between HOMO and LUMO orbitals of reacting species (Fukui, 1975). Thus, the treatment of the frontier orbitals separately from the other orbitals is based on the general principles governing the nature of chemical reaction. HOMO is often associated with the electron donating ability of a molecule. High E_{HOMO} values indicate that the molecule has a tendency to donate electrons to appropriate acceptor molecules with low energy empty molecular orbital (Arslan.et al., 2009). E_{LUMO} indicates the ability of the molecules to accept electrons. The lower values of E_{LUMO}, the more probable, the molecules to accept electrons. Increasing values of the E_{HOMO} facilitate adsorption and therefore inhibition by influencing the transported process through the adsorbed layer (Dewar, et al.1985). Low absolute values of the energy gap (ΔE) of scopoletin gives good inhibition

Table 1. Quantum chemical indices of heat of formation, dipole moment (μ), E_{LUMO}, E_{HOMO} and the ΔE energy gap of Goji Berry extract

Compound	Heat of formation (kcal/mol)	Dipole moment (debye)	E LUMO	E HOMO	Energy difference (ev)
	-12.46812	0.964	0.0285	-8.5	8.6218
	-58.27323	4.359	0.361	-8.4	8.7185
	-26.04864	2.910	-0.46	-9.73	9.2778
	-75.71076	2.732	-3.69	-9.33	5.6408
	-13.952	2.247	-0.477	-8.94	8.4631
	28.45	3.623	-0.713	-9.015	8.3018
	48.463	3.129	0.124	-8.81	8.949
	53.587	3.199	-0.12	-8.87	8.7524

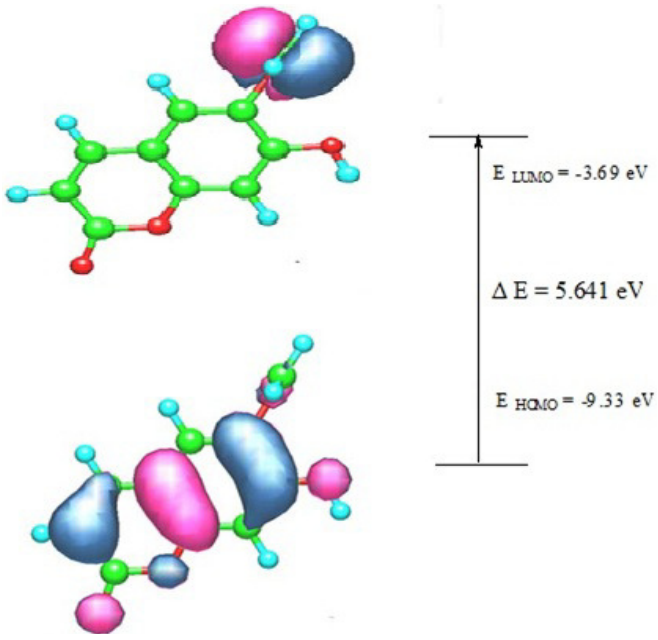


Figure 4. Highest Occupied Molecular Orbital (HOMO) and Lowest Unoccupied Molecular Orbital (LUMO) for scopoletin

efficiencies because the energy required to remove an electron from the last occupied orbital will be low (Domenicano and Hargittai, 1992), which facilitate oxidation inhibition process.

Table 2. Effect of patulin on serum protein and albumin in control rats , rats treated with patulin (0.2 mg/kg b.w) for one and two weeks respectively and rats treated with goji extract (2 ml/kg b.w)

Serum proteins	Control	G I	G II	G III	G IV
Albumin	41.26±0.3	7.96±0.17***	8.48±0.17***	59.25±0.08 n.s	70.43±1.5 n.s
$_1\alpha$	5.07±0.09	14.52±0.02***	12.06±0.17**	8.23±0.009 n.s	9.01±0.4 n.s
$_2\alpha$	13.16±0.2	26.83±0.3***	24.31±0.07***	9.28±0.1 n.s	18.68±0.06 n.s
β	8.88±0.4	26.05±0.27***	26.91±0.02***	7.47±0.016 n.s	8.9±0.37 n.s
γ	6.69±0.19	55.78±0.009***	53.18±0.33***	5.02±0.04 n.s	5.024±0.04 n.s

Data are expressed as mean ± SE. Number of sample in each group is 10.
G I, G II = Treated group with Patulin.
Significant change in comparison between groups:
**p<0.01; ***p<0.001
N.S Non significant (P > 0.05)

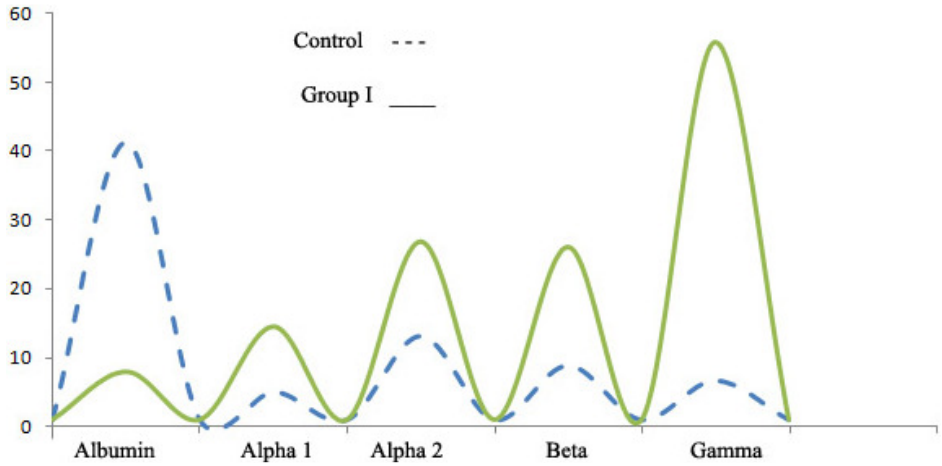


Figure 5. Serum protein fractions in control and treated rats after one week patulin

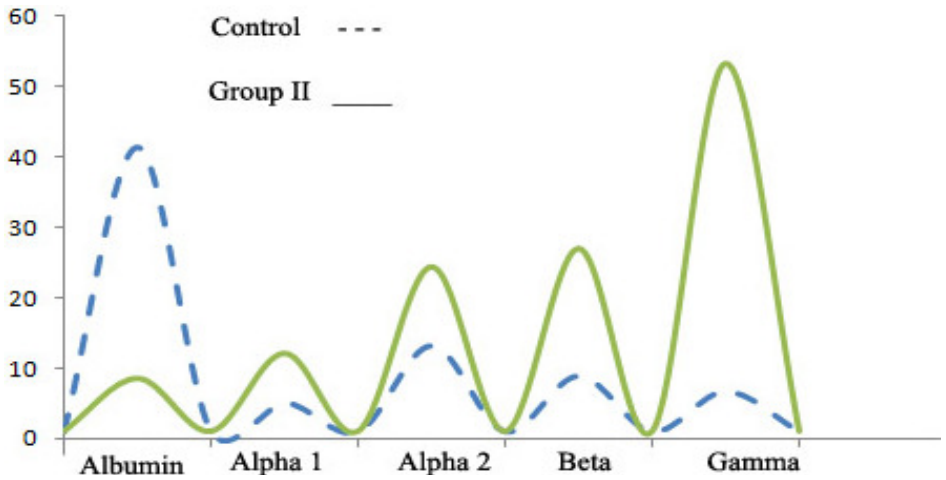


Figure 6. Serum protein fractions in control and treated rats after two weeks patulin

Electrophoretic pattern of serum protein

The serum protein electrophoretic patterns are shown in table (2) and figures (5 – 8). The percentage of albumin in group I and group II showed highly significant decrease ($P < 0.001$) and significant increase ($P < 0.001$) of α_1, α_2, β and γ in the mean values compared with control. However there is no significant change ($P > 0.05$) in the mean value of albumin, α_1, α_2, β and γ globulins in G III and G IV compared with control group.

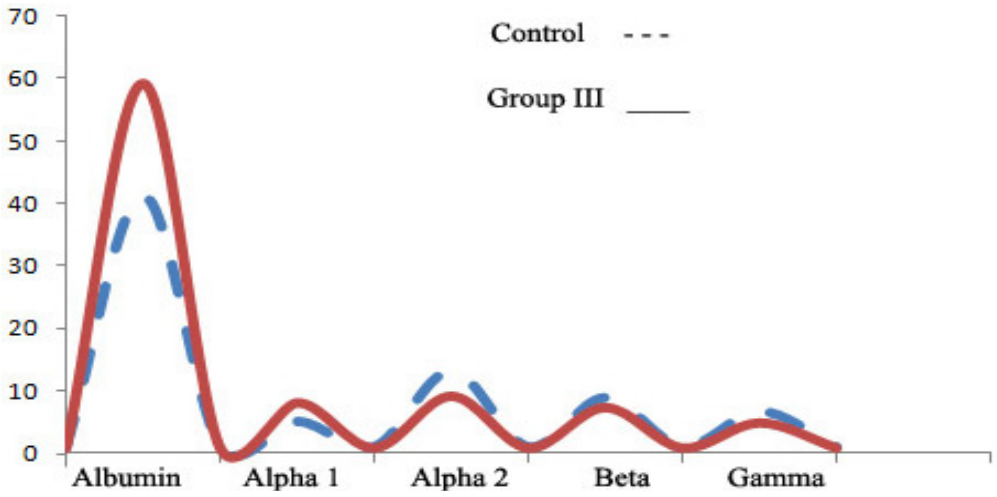


Figure 7. Serum protein fractions in control and treated rats of Goji extract after two weeks patulin

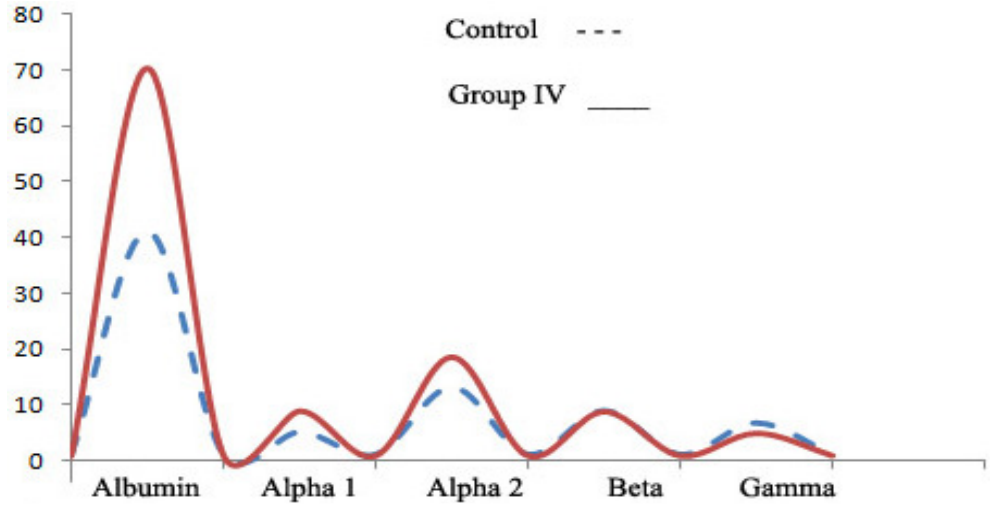


Figure 8. Serum protein fractions in control and treated rats of Goji extract before two weeks patulin

The objective of electrophoresis is to demonstrate the presence of constituents which have different mobilities and to measure the relative concentrations of these constituents. The amount of albumin synthesis in the liver of normal rats was found to be about 14% of the total protein synthesis (Peters and Peters, 1972; Keller and Taylor, 1976). In the present study, the percentage of albumin in group I and group II showed highly significant decrease ($P < 0.001$) in the mean values compared with control. Where, J. R. Marrack and H. Hoch state that, the albumin concentration falls in most conditions in which protein concentrations are abnormal and the rise in relative concentration of the globulin components may reflect this reduction of albumin. This is could be due to: response to infection or injury; effects of deficiency of protein; changes due to excess of lipid in the serum; changes associated with disease of the liver; changes in the serum of patients with myelomatosis. Also, reduced serum albumin (SA) levels might be due to several diseases, including cancer, kidney diseases, human immunodeficiency, chronic liver diseases and autoimmune diseases (F S Al-Joudi.2005).

It is of interest that, Globulins include alpha-1, alpha-2, beta and gamma globulins (immunoglobulins). The first three are made in the liver, and the gamma globulins being made by plasma cells in the bone marrow and tissues. alpha-1 globulins, consist mainly of alpha-1 antitrypsin. The alpha-2 components are mainly alpha-2 macroglobulin and haptoglobin. Beta globulins are composed mainly of transferring, which may be increased in iron deficiency but not by inflammation. In the current study, the percentage of $\alpha 1$, α_2, β and γ in group I and group II showed highly significant increase ($P < 0.001$) in the mean values compared with control. (Michael et al.2014) postulated that Elevated globulins, usually resulting from increases in immunoglobulins

and/ or acute phase reactants, suggests the possibility of multiple myeloma, monoclonal gammopathy, malignancy or inflammation associated with a rheumatic condition or infection.

Furthermore, there is no significant change ($P > 0.05$) in the mean value of albumin, α_1, α_2, β and γ globulins in Therapeutic and Prophylactic group with goji compared with control group. Where, Goji berry (*Lycium barbarum*) is type of fruit which is rich in antioxidant content and is valued in Chinese culture for its benefits to anti-aging, vision, kidney and liver. Furthermore, Recent studies showed that extracts from *L. barbarum* possess biological activities including anti-aging, anti-tumor, immune stimulatory and cytoprotection. Most of these studies confirmed that the protective function of *L.barbarum* is due to its anti-oxidative effects (Iluni, 2011).

CONCLUSION

The present study indicated that, Biochemical and theoretical studies of goji berry extract can be useful to protect human and animal against adverse health effects of patulin and also, can improve the changes in free radicals due to the high antioxidant activity of goji components.

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