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Biochemical Investigation To Evaluate Chronic Effects Of "Arq-E-Gulab" On Liver Function Test

Muhammad Osama[*1,2], Rahila Ikram[3], Calvin R. Wei[4], Raheela Saleem[5], Ghulam Rasool Bhurgri[6], Fahad Jibran Siyal[7], Waseem Abbas[8]

[1*]Department of Pharmacology, Faculty of Pharmacy, University of Karachi, Karachi, Pakistan.
[2]Department of Pharmacy Practice, Faculty of Pharmacy, University of Karachi, Karachi, Pakistan.
[3]Faculty of Pharmacy, Salim Habib University, Karachi, Pakistan.
[4]Department of Research and Development, Shing Huei Group, Taipei, Taiwan.
[5]College of Pharmacy, Liaquat University of Medical and Health Sciences, Jamshoro, Sindh, Pakistan
[6]Department of Pharmacology & Therapeutics, Indus Medical College, University of Modern Science, Tando Muhammad Khan, Sindh, Pakistan.
[7]Department of Pharmacology, Chandka Medical College, Shaheed Mohtarma Benazir Bhutto Medical University, Larkana, Sindh, Pakistan.
[8]Institute of Pharmacy, Shaheed Mohtarma Benazir Bhutto Medical University, Larkana, Sindh, Pakistan.

***Corresponding Author:** Muhammad Osama
*Department of Pharmacology, Faculty of Pharmacy, University of Karachi, Karachi, Pakistan.
Email:- osama_hum@hotmail.com

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Abstract
Natural remedies plays a pivotal role in global health. Natural remedies are often a popular choice in primary health care as they are considered to be low-cost, safe, and easy to access while having a wide variety of medicinal and biological activities. The tradition of consuming natural remedies is very common all over the globe especially in various regions of Pakistan, India, China, Sri Lanka, Thailand, Japan etc. Majority of the population prefers these natural remedies over conventional allopathic drugs to treat minor ailments and disorders. However, these natural products are not 100% free from adverse effects. Usually awareness and reporting culture of adverse events with natural products is very uncommon all over the world. Arq-e-Gulab (AG) is a famous Unani herbal preparation which is commonly known as Rose Water prepared by water-distillation of rose petals of Rosa damascena Mill. flower. It is traditionally used in various ophthalmic preparations to treat eye related allergies and inflammatory states. It is also used in cooking as flavoring agent in preparation of various sweets and meat recipes especially in Asian region. This study aims to investigate the impact of chronic dosing of AG on liver enzymes to evaluate its effect on liver. This study was carried out on albino rabbits. AG was given at two different doses for 60 days for evaluation of biochemical effects. Our study reveals that AG does not possess any hepato-toxic effects.

Keywords:- Hepatoprotective, Hepatotoxic, Rosa damascena Mill., Liver function test, liver enzymes

INTRODUCTION

The interest of mankind towards natural remedies mainly originated while fulfilling their need for food and shelter [1]. Prehistorically, human beings greatly relied on natural substances of mineral, plant and animal origins in order to achieve or maintain good physical and mental health, to ease sufferings and pain or to obtain therapeutic benefits in different ailments [2]. The most primitive type of health care known are plants [3]. The utilization of plant based products is very old, probably as ancient as existence of human civilizations

[4]. Numerous evidences on use of plants as remedy have been reported 60,000 year ago [5]. In different literatures including Roman, Greek, Egyptian, Chinese, Indian and Syrian books, these evidences of health care remedies are 5000 years old [6]. The trend of consuming natural products has been increased greatly all over the globe. Sometimes when conventional remedies fails to treat any chronic ailments effectively, people often opt for unconventional remedies including the herbal preparations [7]. The main reason of approaching complementary or traditional medicine system is to get relief from symptoms of chronic ailments, to treat chronic disease condition or management of side effects of conventional therapies [8]. Also there is a general perception that these plant based preparations are safe, with fewer adverse effects, are economical and locally available [9].

Rosaceae family plants are considered as one of the world's most renowned ornamental plants mainly due to their fragrances and visual beauty among the flowering plants. Up till now almost 100 species of rose have been discovered and identified, among which Rosa damascena Mill. possesses great significance because of its unique flavor, distinctive aroma and natural beauty [10]. Rosa damascena Mill. is also known as Gul-e-Muhammadi and Damask rose in native languages [11].

Arq-e-Gulab (AG); a famous unani herbal product is also known as Rose Water is water distillate of Rosa damascena Mill. flower prepared by hydro-distillation process. Numerous studies have reported the chemical composition of AG claiming that it is a tremendous source of polyphenols, flavonoids, terpenes and other volatile components. AG is widely used as a flavoring agent in making sweet and meat recipes and has a coolant effect [12]. Traditionally in Unani traditional system, it is used as an anti-septic agent effective in eye washing and for mouth disinfecting purpose. It is also used in the preparation of herbal mouthwashes to treat and heal stomatitis and mouth ulcers. It is used along with other medicinal herbs in ophthalmic preparations used to treat dry eyes and conjunctivitis. It also exhibits anti-pyretic action. It possesses anti-spasmodic activity and is effective in relieving abdominal pain, chest and bronchial congestion [13]. It also possesses mild laxative activity [14].

The perception of safety in natural remedies is often based on subjective evidence or tradition knowledge. The underreporting of adverse effects of natural products is a global issue. This study investigates the impact of chronic daily dosing of AG on liver by estimating liver enzymes levels in white albino rabbits.

MATERIALS AND METHODS

Preparation of Arq-e-Gulab

Water distillation was performed to obtain AG. Fresh flowers of Rosa damascena Mill. were plucked from the botanical garden of University of Karachi which were identified and authenticated by Department of Pharmacognosy, Faculty of Pharmacy & Pharmaceutical Sciences, University of Karachi [Voucher no: RDF-01-16/17]. Petals were separated from flower and allowed to dry at room temperature. The distillation apparatus comprises of a stainless steel tank, a cohobation column, a condenser and a receiver. Dried petals with distilled water was added in the distillation apparatus in the ratio of 1:2. 3 kg of air dried rose petals along with 6 liters of water was added in the distillation apparatus. Air vents were closed after complete removal of air and the apparatus was then operated as a closed system to distill the rose petals under maintained high temperature and pressure. The vapors were generated in cohobated column which were then condensed with circulating chilled water in a condenser and finally received in the receiver. The process of distillation was completed after collection of 1300 ml of distillate. The water distillate of Rosa damascena Mill. flower received was of concentration 0.5gm/ml [15][16][17].

Selection of experimental animals

Male albino rabbits weighing between 1800 to 2200 grams were selected for this study which were separated from the breeding area of animal house of Department of Pharmacology, University of Karachi. Throughout the entire study period, the animals were kept in normal room temperature of 21 ± 4ºC and humidity 53 to 63% under 12 hour light (06:15 a.m. to 06:15 p.m.) and dark (06:15 p.m. to 06:15 a.m.) cycle with 24/7 free access to standard food and pure water. Animals were handled as per the specifications of National Research

Council (NRC) [18]. This study was conducted after the approval of BASR (Board of Advanced Studies and Research), University of Karachi [BASR/No./03460/ Pharm.Resol.No. 10(P) 04].

Animal grouping and dosing protocol

3 groups of 10 rabbits were set for biochemical screening. Group I was control and was given 1ml distilled water, Group II and III were test groups and were given AG in the doses of 250mg/kg and 500mg/kg respectively [19][20]. The dosing was done by oral route for 60 consecutive days and blood was withdrawn for biochemical testing on day 61 to observe the effect of AG on liver enzymes.

Biochemical Investigation

For biochemical testing, blue capped siliconized glass tubes were used in which the blood samples were taken. After blood withdrawal, these tubes were centrifuged for 700 to 950 seconds at 3000 RPM to get the pure plasma which was then analyzed using Humalyzer- 3000 (Human-Germany) for the estimation of hepatic enzymes such as direct and total bilirubin, GGT, ALP, SGPT and SGOT. For estimation of these tests, standard kits were used which were purchased from the Human company [21][22][23][24][25].

STATISTICAL ANALYSIS

Statistical analysis was carried out using SPSS software version 22. Values were presented as Mean ± Standard Deviation (S.D). One-way ANOVA followed by multiple comparison post hoc Tukey's test was performed for statistical calculations. All p-values less than 0.05 were considered significant where $^{*\gamma}p<0.05$, $^{**\gamma\gamma}p<0.01$ and $^{***\gamma\gamma\gamma}p<0.001$ represents level of significance i.e. significant, very significant and highly significant difference in comparison to control and 250mg/kg dose group respectively.

RESULT

Table 1 represents effect of Arq-e-Gulab on Liver enzymes including TB, DB, ALP, ALT and GGT respectively. One-way ANOVA followed by Post-hoc tukey's test showed that in comparison to control group both treated group did not affect the serum levels of TB, DB, ALT and GGT not there was any significant difference between the treated groups. However in comparison to control both treated groups positively decreased the levels of serum ALP.

Table 1 Effect of AG chronic dosing on Liver enzymes

Groups	TB Total Bilirubin (mg/dL)	DB (Direct Bilirubin) (mg/dL)	ALP (Alkaline Phosphatase) (U/L) X ± S.D	ALT (Alanine-amino-transferase) (U/L) X ± S.D	GGT (Gamma glutamyl transferase) (U/L) X ± S.D
Control I	0.29±0.08	0.05±0.03	43.02±5.9	51.09±9.43	5.1±1.19
Test II (Single dose)	0.25±0.03	0.04±0.02	25.01±8.43***	57.5±18.44	6.5±1.58
Test III (Double dose)	0.26±0.04	0.05±0.02	31.04±11.58*	47.01±9.48	4±1.33$^{\gamma}$

n=10, Values are presented as Mean (X) ± Standard Deviation (S.D).

DISCUSSION

World Health Organization in 2005 states that safety & efficacy evaluation of herbal remedies is worrisome and needs critical scientific methodologies and research. Safety is considered to be an essential aspect of any drug which is expected to cause no unwanted and harmful effects under the labelled use. The literature available on toxicity, adverse effects and safety of natural therapies is very confined and required more detailed screening which will aid in identifying the safety profile of medicinally active compounds in a plant [26][27][28][29].

To evaluate the effect of chronic dosing of AG on liver, liver function test (LFT) was performed. Liver function test is an important diagnostic tool to detect liver diseases and hepatic dysfunction. In liver function test, serum bilirubin (total and direct), ALP (Alkaline Phosphatase), ALT (Alanine-amino-transferase) and GGT (gamma glutamyl transferase) levels are checked. Hyperbilirubinemia or elevated serum bilirubin levels are

observed in alcoholic hepatitis, jaundice, acute liver failure and primary biliary cirrhosis. An elevated ALP (Alkaline Phosphatase) levels indicate biliary obstruction or cirrhosis, drug induced cholestasis and metastatic liver disease. An elevated serum ALT (Alanine-aminotransferase) levels indicate non-alcoholic steato-hepatosis, chronic hepatitis and other liver diseases. An elevated GGT (gamma glutamyl transferase) levels indicate alcoholism, pancreatic and hepato-biliary disease [30][31].

Our findings revealed that AG at both doses does not show any hepato-toxicity and does not affect the serum bilirubin, ALT (Alanine-aminotransferase) and GGT (gamma glutamyl transferase) levels, however serum ALP (Alkaline Phosphatase) levels very significantly decreased. Hepato-protective potential of ethanolic extract of *Rosa damascena* has been reported in 2008 [32]. Hepatoprotective potential of AG might also be due to its constituents quercetin, kaempferol, geraniol and ellagic acid. Presence of kaempferol, quercetin, ellagic acid and geraniol in AG has been reported [33][34]. A study in 2010 reported hepato-protective potential of quercetin against alcohol induced liver damage [35]. Another study reported hepato-protective potential of kaempferol against alcohol induced liver injury [36]. In a study conducted in 2016, geraniol showed hepato-protective effects and ameliorates diet induced non-alcoholic steatohepatitis [37]. Hepato-protective activity of ellagic acid against carbon tetra-chloride induced hepatotoxicity has also been reported [38]. Hence these findings suggest hepato-protective action of AG. However more detailed research is required in future to determine its exact mechanism of liver protection.

CONCLUSION

In the light of above discussed evidences and findings of our study, it is concluded that “Arq-e-Gulab” is safe to be used chronically as it has no hepato-toxic effect. In future more detailed research is required to evaluate its efficacy in disease associated and drug induced hepatotoxicity states.

CONFLICT OF INTEREST

There is no conflict of interest.

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