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Evaluation of Biochemical effects of Famous Unani Herbal Product "Arq-e-Gulab" on Cardiac Enzymes

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Abstract
Prehistorically, the use of plants and plant-based products have often been considered for their medicinal properties in treating unhealthy states. The main reason of people preference of using plant based products is their low cost, accessibility, and minimal unwanted effects. There is widespread assumption that such products are safe to use. Furthermore, awareness and reporting culture of adverse events with plant-based 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 eye preparation to treat ophthalmic allergies and inflammatory condition. 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 cardiac enzymes to evaluate its effect on heart. 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 cardio-toxic effects.

Keywords:- Cardioprotective, Cardiotonic, Cardiotoxic, Rosa damascena Mill.

INTRODUCTION

The interest of human beings towards the plants and herbs primarily originated while fulfilling their necessity for food and shelter [1]. Prehistorically, the ancient people in order to achieve or maintain good physical and mental health, greatly relied on natural substances of mineral, plant and animal origins to ease sufferings and pain or to obtain therapeutic benefits in different ailments [2]. The most primitive kind of health care known are plants [3]. The use of natural products or herbal drugs is extremely old, probably as ancient as

human civilizations [4]. The evidence exists on use of plants as medicines about 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 herbal remedies has been increased vastly throughout the world. Sometimes when conventional therapies fails to treat chronic ailments effectively, people often go for unconventional remedies including the herbal preparations [7]. The main reason why public approaches complementary or traditional medicine system is to get relief from symptoms of chronic ailments, to treat chronic diseases or management of side effects of conventional therapies [8]. Also there is a general belief that natural products or herbal preparations are safe, with little or no adverse effects, are economical and locally available [9].

Among flowering plants, members of Rosaceae family are considered as one of the world's most renowned ornamental plants mainly due to their fragrances and visual beauty. Up till now more than 100 species of rose have been discovered and identified, among which Rosa damascena Mill. possesses wide importance 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 an excellent source of polyphenols, flavonoids, terpenes and other volatile components. AG is widely used as a flavoring agent in 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 heart by estimating cardiac enzymes levels in white albino rabbits.

MATERIALS AND METHODS

Preparation of Arq-e-Gulab

Water distillation was performed to prepare Arq-e-Gulab (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. 2.5 kg of air dried rose petals along with 5 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 1250 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 1500 to 2000 grams were separated from the breeding area of animal house Pharmacology Department of University of Karachi. Animals were kept in normal room temperature of 23 ± 3ºC and humidity 52 to 62% under 12 hour light (07:15 a.m. to 07:15 p.m.) and dark (07:15 p.m. to 07:15 a.m.) cycle with 24/7 free access to standard food and pure water. Rabbits 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 61st to observe the effect of AG on cardiac 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 cardiac enzymes including CPK and LDH. For estimation of these tests, standard kits were used which were purchased from the Human company [21][22].

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 $^{*y}p<0.05$, $^{**yy}p<0.01$ and $^{***yyy}p<0.001$ represents level of significance i.e. significant, very significant and highly significant difference in comparison to control and 250mg/kg dose group respectively.

RESULT

The effect of Arq-e-Gulab on serum LDH (Lactate dehydrogenase) is represented in table 1 and graph 1.
Data analysis and statistical calculations showed that in comparison to control, both single and double dose treated groups have insignificant effect on serum LDH (Lactate dehydrogenase) level.
No statistical significant difference was observed between the two treated groups.
The effect of Arq-e-Gulab on serum CK (Creatinine kinase) is represented in table 2 and graph 2.
Data analysis and statistical calculations showed that in comparison to control, both single and double dose treated groups significantly ($p<0.001$) decreased the serum CK (Creatinine kinase) level.
In comparison to single dose group, double dose group significantly ($p<0.05$) increased the serum CK (Creatinine kinase) level.

Table 1 Effect of Arq-e-Gulab on Serum LDH (Lactate dehydrogenase)

Groups	**LDH (Lactate dehydrogenase) (U/L)** X ± S.D
Control I	354.04±47.14
Test II (Single dose)	388.05±56.56
Test III (Double dose)	400.13±50.09

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

Graph 1

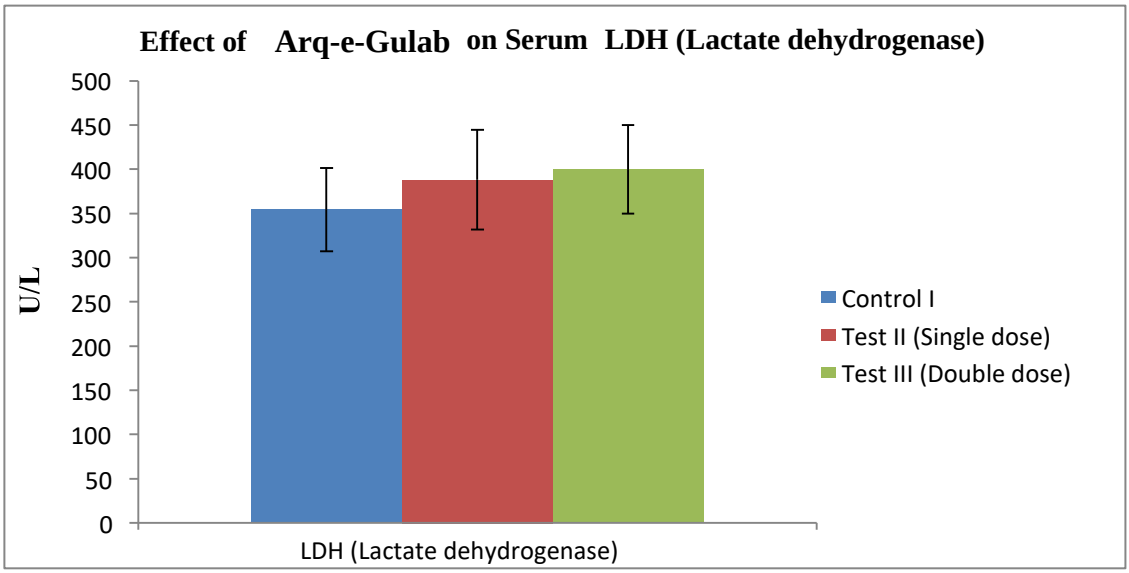
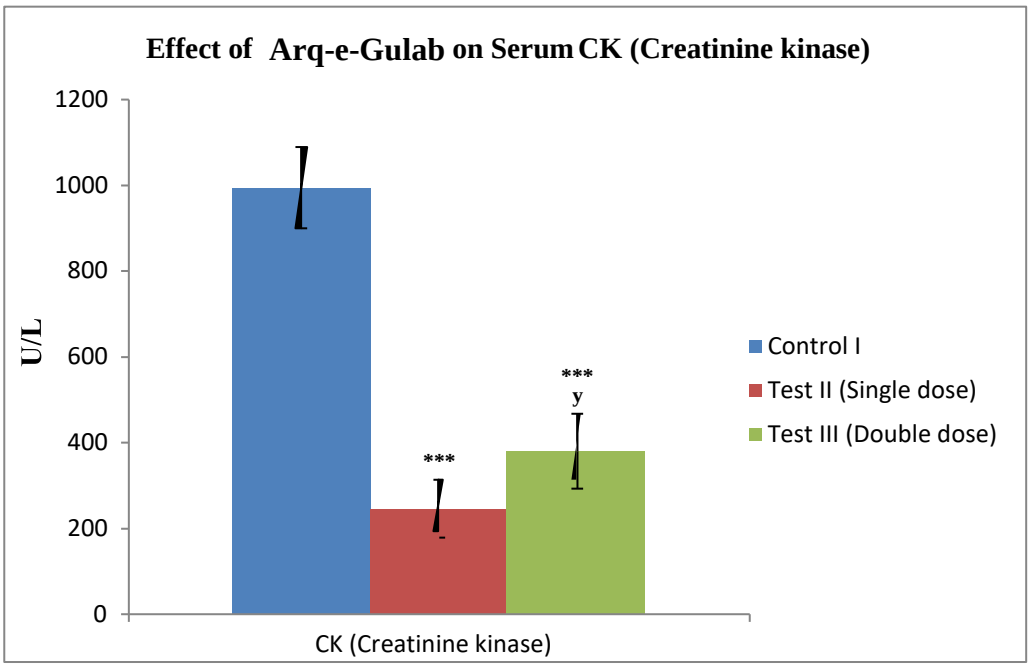


Table 2 Effect of Arq-e-Gulab on Serum CK (Creatinine kinase)

Groups	**CK (Creatinine kinase) (U/L)** X ± S.D
Control I	994.6±94.73
Test II (Single dose)	246.07±67.46***
Test III (Double dose)	380.1±87.30***y

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

Graph 2



DISCUSSION

World Health Organization in 2005 states that safety & efficacy determination of natural products is troublesome 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 [23][24][25][26].

To evaluate the effect of chronic dosing of AG on heart, serum LDH (Lactate dehydrogenase) and CK (Creatinine kinase) levels were checked. Serum CK and LDH are useful tools in monitoring patients of myocardial infarction [27]. Serum LDH and CK levels are often checked to evaluate any cardiac injury [28]. Our findings revealed that AG at both doses does not show any cardiac toxicity, does not affect serum LDH levels and drastically decreased serum CK levels. In a study conducted in 2017, ethanolic extract of *Rosa damascena* Mill. showed marked cardio-protective effects against isoproterenol induced myocardial lysosomal membrane destabilization [29]. Cardio-protective potential of AG might be due to its polyphenols rich nature and its constituents geraniol, linalool and kaempferol. Presence of polyphenols, geraniol, linalool and kaempferol in AG has already been established [30] [31]. Natural polyphenols show cardio-protective activities mainly by reducing cholesterol absorption, reduction in circulating plasma lipids, reducing the cytokines production and preventing the occurrence of inflammatory events [32]. In a study conducted in 2017, geraniol showed positive and protective effect on cardio-myocytes [33]. Cardio-protective effect of linalool against isoproterenol induced myocardial infarction has also been reported [34]. Another study reported cardio-protective potential of kaempferol against myocardial ischemia/reperfusion injury [35]. Hence these findings are suggestive of cardio-protective action of AG. However more detailed research is required in future to evaluate its efficacy in treatment and management of different cardio-vascular diseases.

CONCLUSION

According to the findings of this study, it is concluded that famous Unani Herbal product “Arq-e-Gulab” is safe to be used chronically as it does not possess any cardio-toxic effects. More detailed research is required in future to evaluate its efficacy in drug induced cardiotoxicity states and in different Cardiovascular diseases.

CONFLICT OF INTEREST

There is no conflict of interest.

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