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Cardioprotective and Antilipidemic Effect of Gemmotherapeutically Treated *Glycyrrhiza glabra* against Isoproterenol Induced Myocardial Injury

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Authors' contributions

This work was carried out in collaboration among the authors. Author MH prepared synopsis, performed statistical analysis, research work, wrote the protocol and draft of the manuscript. Author KR managed the laboratory facilities and funds to conduct the research and approved the final manuscript. Author NJ surveyed the literature. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: To evaluate preventive (pre- treated) and curative (post treated) potential of gemmomodified and native extract of *Glycyrrhiza glabra* for alleviating harmful changes in lipid profile (HDL, LDL, TG, TC) and cardiac enzymes (CK-MB, LDH, SGOT, SGPT) against isoproterenol (ISO) induced myocardial injury in rabbits.
Study Design: *In vivo* study.
Place and Duration of Study: Department of Chemistry and Biochemistry, University of Agriculture, Faisalabad, Pakistan, between February 2011 and April 2011.
Methodology: Thirty six rabbits weighing 1.25 ± 0.2 Kg were allocated into six groups (Control, Ischemia, Gemmo curative, Native curative, Gemmo preventive and Native preventive) having six animals each. Rabbits were fed normal diet for 20 days. Gemmo preventive and Native preventive groups were also given gemmo modified and native extract (100 mg kg^{-1}). On 20^{th} day and 21^{st} day rabbits were given ISO (50 mg kg^{-1}). Five days after the ischemia the Gemmo curative and Native curative groups were given gemmo and native extracts (100 mg kg^{-1}). Serum activities of lipid profile and cardiac enzymes were determined.

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Results: ISO administration significantly lowered *(P=.05)* HDL level and increased (*P=.05*) LDL, TG and TC as compared with control rabbits. ISO injury significantly increased (*P=.05*) the levels of cardiac enzymes CK-MB. LDH, SGOT and SGPT as compared with control rabbits. Curative treatment with gemmo and native extracts of *Glycyrrhiza glabra* significantly increased (*P=.05*) level of HDL and lowered *(P=.05)* the level of LDL, TG, TC and cardiac enzymes as compared with ischemic rabbits. Pre treatment with gemmo and native extracts prevented the reduction (P=.05) in HDL level and resisted the rise (*P=.05*) in other lipid parameters and cardiac enzymes as after ISO induced myocardial injury. Pretreatment with extracts was significantly better (*P=.05*) than curative treatment. Gemmo extract was significantly better (*P=.05*) than native extract in preventive and curative treatment in normalizing serum levels of lipid parameters and cardiac enzymes in ISO injured rabbits.
Conclusion: The results provide evidence for the first time that gemmo extract of *Glycyrrhiza glabra* prevents myocardial injury induced by ISO in rabbits.

Keywords: Glycyrrhiza glabra; isoproterenol; lipid profile; cardiac enzymes.

1. INTRODUCTION

Cardiovascular disease poses serious problems to human health. In fact, it is the main cause of death throughout the world [1]. Not only the underdeveloped but also the developed countries have not been able to control this disease. The Indian subcontinent (including India, Pakistan, Bangladesh, Sri Lanka, and Nepal) has the highest rate of cardiovascular disease (CVD) globally. Many reports have highlighted the high CVD rates among south Asian immigrants living in western countries, but the enormous CVD burden within the Indian subcontinent itself has been under-emphasized. At present it is the highest killer in US [2].

Lately, the medicinal plants have attracted a great deal of attention because of several possible cardioprotective mechanisms besides antioxidant activity [3]. Medicinal plants contain many bioactive chemical compounds such as saponins, flavonoids, glycosides and tannins. These phytoconstituents act as blood thinner, help to decrease the blood cholesterol level, and prevent the deposition of cholesterol in blood vessels, which in turn prevent the formation of thrombus in blood vessels and protect from acute myocardial infarction.

Gemmotherapy is a therapeutic method that uses plant bud extracts and other young vegetative tissues, freshly harvested leaves from growing plant to stimulate elimination of toxic compounds from the body. Gemmotherapy remedies act to gently stimulate and promote elimination. Gemmotherapy works because of the presence of gibberellins, plant growth hormones, which act on the organs to be stimulated. Gemmotherapy became accepted form of herbal medicine in France (entering the Pharmacopie e Francaise in 1965). There has been scientific research since 1950s, describing, explaining and demonstrating the effects of the gemmotherapy remedies. The remedies by gemmotherapy are distinctive in their intense combination of minerals, vitamins and other powerful phytochemical properties of whole plant [4,2].

Glycyrrhiza glabra (mulathi) also known as licorice and sweet wood, is native to the Mediterranean and certain areas of Asia. *Glycyrrhiza glabra* extract has been used for more than 60 years in Japan to treat the chronic hepatitis, also has the therapeutic benefit against

some viruses, including Human Immuno deficiency Virus (HIV), cytomegalo Virus (CMV), and Herpes simplex virus (HSV). It is also effective against different types of ulcers.

A number of components has been isolated from *Glycyrrhiza glabra*, these are triterpene, saponins, flavonoids, including (liquiritin, iso liquiritin, a chalcone) flavones, flavonols, isoflavones, bihydroflavones, bihydrochalcones [5]. Pharmacological investigation indicates that *Glycyrrhiza glabra* has antioxidant, antibacterial, anti inflammatory and estrogen likes activities [6].

Many factors are involved in the causation and progression of heart diseases. Among them dyslipidemia, hypertension, diabetes and smoking are associated with increased vascular production of reactive oxygen species (ROS). Myocardial damage induced by ischemia reperfusion is due to the generation of ROS [7]. The plants are the main source of such molecules. Although modern drugs are effective in preventing cardiovascular diseases, due to their side effects their use is limited. With this basic information it might be interesting and possibly fruitful to study the local medicinal plants (native and modified through gemmotherapy) to discover their cardioprotective potential in animals (rabbits).

The present study was designed to evaluate the efficacy of preventive and curative cardioprotective potential of gemmotherapeutically modified *Glycyrrhiza glabra* in the experimental model of isoproterenol (ISO) induced myonecrosis as compared to native extract of *Glycyrrhiza glabra*.

2. MATERIALS AND METHODS

2.1 Plant Material and Preparation of Plant Extracts

The plant *Glycyrrhiza glabra* (mulatthi) Solanaceae family was selected for the study. The fresh growing shoots, leaves and mature leaves were collected from the new Botanical garden of University of Agriculture, Faisalabad, Pakistan. The plant was identified by the Taxonomist, Department of Botany, University of Agriculture, and Faisalabad, Pakistan.

2.2 Plant Extract

2.2.1 Native extract

The plant extract was prepared by following [8] procedure with some modifications. Mature parts of *Glycyrrhiza glabra* were collected, washed with distilled water. The leaves were completely dried in shady place and ground in herbal grinder. The measured amount of powdered material was soaked in alcohol (70% ethanol). The material was filtered through filter paper after one month. The filtrate was concentrated in a rotary evaporator. The remaining alcohol was evaporated. The amount of extract was measured on per gram of crude powder basis [2].

2.3 Gemmotherapeutically Treated Extract

The young growing shoots and leaves to be used were cleaned and weighed. The dry weight of plant material was determined by drying it at 105ºC in an oven till constant weight. The measured amount of the plant material was blended in an equal mixture of alcohol and glycerine having ratio of 2:1, respectively. The quantities were calculated so

that the weight of this mixture was 20 times that of an equivalent amount of dried sample. This mixture was allowed to stand for one month at room temperature and was shaken from time to time to help the maceration process. It was then filtered and concentrated under constant pressure. The filtration process was done again after a period of 48 hr. The filtrate was concentrated in rotary evaporator till the maximum alcohol was evaporated. The amount of extract in glycerine was calculated on per gram of dry weight of crude powder as described by [9].

2.4 Phytochemicals Screening

The identification of the major phytoconsistuents (alkolids, flavonoids, glycosides, sapnonins, steroids, tannic acid) was carried out by following the procedures of [10].

2.5 Experimental Animals

Thirty six rabbits with average weight 1.25 ± .20 kg were used in the study. Throughout the investigation, animals were housed individually in standard metallic wire guage cages at 25 ± 5ºC with 12 hr light-dark cycle and maintained at humidity of 50 ± 5 %, with free access to food and water. Animals were weekly weighed.

2.6 Evaluation of Cardioprotective and Antilipidemic Activities of *G. glabra*

2.6.1 Experimental protocol

The animals were randomly allocated into six groups comprising six animals each.

Group 1 (G-1): Control group

Rabbits were administered normal diet daily for 20 days.

Group 2 (G-2): Ischemia or damage group

The animals were orally fed normal diet for 20 days and in addition administered ISO (isoproterenol) (50mg kg^{-1}) on 20^{th} and 21^{st} day at an interval of 24 hr.

Group 3 (G-3): Gemmo curative group of *Glycyrrhiza glabra*

Rabbits were given normal diet for 20 days and administered ISO (isoproterenol) (50mg kg^{-1}) on 20^{th} and 21^{st} day at an interval of 24 hr. Following ISO injury, post-treatment of gemmo-extract of *glycyrrhiza glabra* (100mg kg^{-1}) was given to the rabbits for five days to limit myocardial injury.

Group 4 (G-4): Native curative group of *Glycyrrhiza glabra*

Rabbits were treated as in group 3 upto 21 days. Then post treatment of native extract of *glycyrrhiza glabra* (100 mg kg^{-1}) was given for five days to limit myocardial injury.

Group 5 (G-5): Gemmo preventive group of *Glycyrrhiza glabra*

The animals were kept on normal diet and pre-treated with gemmo-extract of *Glycyrrhiza glabra* (100mg kg^{-1}) for 20 days. On 20^{th} and 21^{st} day, the animals were administered ISO (50mg kg^{-1}) to induce myocardial injury, at an interval of 24 hr.

Group 6 (G-6): Native preventive group of *Glycyrrhiza glabra*

The animals were kept on normal diet and pre treated with native extract of *Glycyrrhiza glabra* (100mg kg^{-1}) for 20 days. On 20^{th} and 21^{st} day, the animals received ISO (50mg kg^{-1}) to induce myocardial injury, at an interval of 24 hr.

2.7 Determination of Heart Rate (Beat per 30 sec) of Rabbits

Heart beat was checked regularly at an interval of 24 hr before and after administration of ISO, and plant extracts [2].

2.8 Biochemical Analysis

Serum samples were analyzed for estimation of the lipid profile and serum enzymes levels [(high density lipoprotein (HDL), low density lipoprotein (LDL), triglycerides (TG), serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), creatine kinase (CK-MB), lactate dehydrogenase (LDH)] using the Micro 200 (Merck, Germany). Diagnostic kits were used in automated analyzer for the quantitative estimation of biochemical parameters.

2.9 Statistical Analysis

The results are expressed as mean ± SD from six animals in each group. Results were statistically analyzed using one- way ANOVA followed by Duncan's Multiple Range Test (DMR) for individual comparision [11].

3. RESULTS AND DISCUSSION

3.1 Phytochemical analysis of *Glycyrrhiz galabra*

The results obtained after phytochemical analysis of *G. galabra* are given in Table 2.

Table 2. Phytochemical analysis of *Glycyrrhiza glabra*

Serial No.	Phytoconstituents	Results	Native extract %	Gemmo extract %
1	Flavonoids	+	21	23
2	Glycosides	+	10	12
3	Saponins	+	8	10
4	Tannic Acid	+	11	12
5	Alkaloids	+	6	7
6	Steroids	-	0	0

3.2 Curative effect of *Glycyrrhiza glabra* on Lipid Profile

The curative effect of gemmotheraputically modified and native extracts of *Glycyrhiza glabra* on levels of lipid profile (HDL, LDL, TG, TC) in serum of rabbits (control and isoproterenol induced infarction) is given in Fig 1. In control rabbits, the level of HDL in serum ranged from 62.00 to 70.00 mg dL^{-1}. Administration of isoproterenol (ISO) (ischemia) resulted in significant) reduction (*P*=.05) in HDL and they further decreased with time. Curing the ischemic rabbits with gemmo modified extract of *Glycyrhiza glabra* significantly increased (*P*=.05) HDL level and they improved remarkably with time. After five days level of HDL in serum of gemmo curative group rabbits increased from 50.00 mg dL^{-1} to 78.00 mg dL^{-1} on post treatment with gemmo modified extract of *Glycyrrhiza glabra*. In native curative animals treated with native extract of *Glycyrhiza glabra* HDL level in serum increased as compared to ISO ischemic group. The group means showed a significantly (*P* =.05) higher HDL level (68.6 mg dL^{-1}) on post treatment with gemmo modified *Glycyrhiza glabra* plant extract as compared with native plant extract (66.4 mg dL^{-1}). The group means also showed that gemmo modified extract was significantly (*P*=.05) better than native extract in curative treatment.

In control group LDL ranged between 93.00 to 95.00 mg dL^{-1} (Fig 1). In ischemic group LDL level remarkably increased ranging from 138.0 to 175.00 mg dL^{-1} and it decreased with time significantly (*P*=.05). In rabbits post treated with gemmo modified *Glycyrhiza glabra* extract (gemmo curative group) the LDL level decreased significantly (*P*=.05) ranging from 158.00 to 130.00 mg dL^{-1}. The LDL level in rabbits post treated with native extract (native curative group) was higher than in gemmo - curative group ranging from 132.00 to 170.00 mg dL^{-1}. The group means showed that gemmo modified *Glycyrrhiza glabra* extract caused significantly (*P*=.05) higher decrease in LDL level as compared to native extract.

These results are in agreement with other studies conducted on *Glycyrrhiza glabra* [12, 13].

Triglycerides (TG) level in serum of normal rabbits was 175.00 - 178.00 mg dL^{-1} (Fig 1). In ischemic group the TG levels ranged from 200.00 mg dL^{-1} to 280.00 mg dL^{-1} which was 37% to 57% higher as compared to control rabbits. Curative treatment with gemmo modified extract of *Glycyrrhiza glabra* decreased the TG significantly (*P*=.05). In native – curative group TG level was also lower than that in ischemic group. The mean values decreased by 3.8 to 10% in gemmo curative and 0 to 5% in native - curative group, respectively. Group means showed that TG level was lowest in gemmo curative group, that is post treatment with gemmo modified extract of *Glycyrrhiza glabra* caused reduction in TG more than native extract (*P* =.05).

In normal rabbits mean values of serum total cholesterol (TC) ranged between 225-230 mg dL^{-1}. Ischemia induced with isoproterenol caused considerable increase in TC and mean values ranged from 310 to 400 mg dL^{-1}, corresponding to 37.7 to 73.9% increase as compared to normal rabbits. The post treatment with gemmo modified and native extracts of *Glycyrrhiza glabra* given to Ischemic rabbits caused a significant (*P* =.05) reduction in TC. The group means showed native extract caused significantly (*P* =.05) higher reduction than gemmo extract and TC was 337.2 mg dL^{-1}.

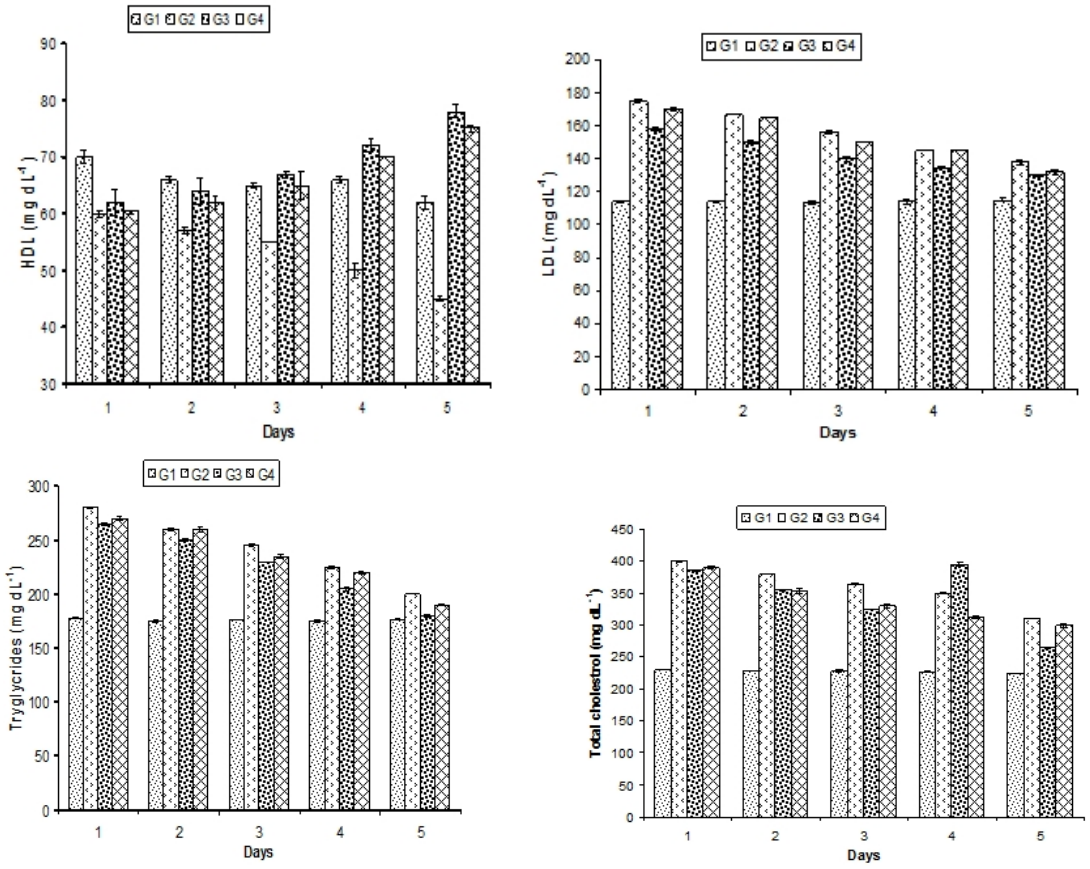

Fig. 1. Curative effect of gemmo - modified and native extracts of *Glycyrrhiza glabra* on lipid profile (HDL, LDL, TG. TC) in ISO infarcted rabbits

G1= Control, G2= Ischemia, G3= Gemmo curative, G4= Native curative. Results expressed as mean ± SD for six rabbits in each group. Values differ significantly among each other according to Duncan's Multiple range Test at P=.05

3.3 Pre Treatment (Preventive) Effect of *Glycyrrhiza glabra* on Lipid Profile

The preventive effect of gemmo modified and native extracts of *Glycyrrhiza glabra* on HDL, LDL, TG, and TC in rabbits is presented in Fig 2**.** The HDL decreased in ischemic animals significantly (*P* = .05) and 14.3% reduction was recorded as compared with normal animals. In Gemmo-preventive group the pre treatment with gemmo modified *G. glabra* extract significantly increased (*P* = .05) the HDL by 58.3% as compared to Ischemic group, and 50% increase in HDL was recorded in native preventive group.

The LDL level in serum increased significantly (*P* = .05) by 88.17% in ischemic group as compared with normal animals. Pre-treatment given with modified and native extracts of *Glycyrrhiza glabra* resulted in 22.3% and 10.2% reduction in LDL, respectively as compared to ischemic group of rabbits. TG also increased in ISO infarcted rabbits as compared with control group animals (*P* = .05), corresponding to 55%. The preventive treatment with gemmo modified *G. glabra* extract resulted in 28.3% reduction (*P* = .05) in TG in gemmo-

preventive animals and native preventive treatment caused 19.3% reduction (*P* = .05) in TG in native preventive animals.

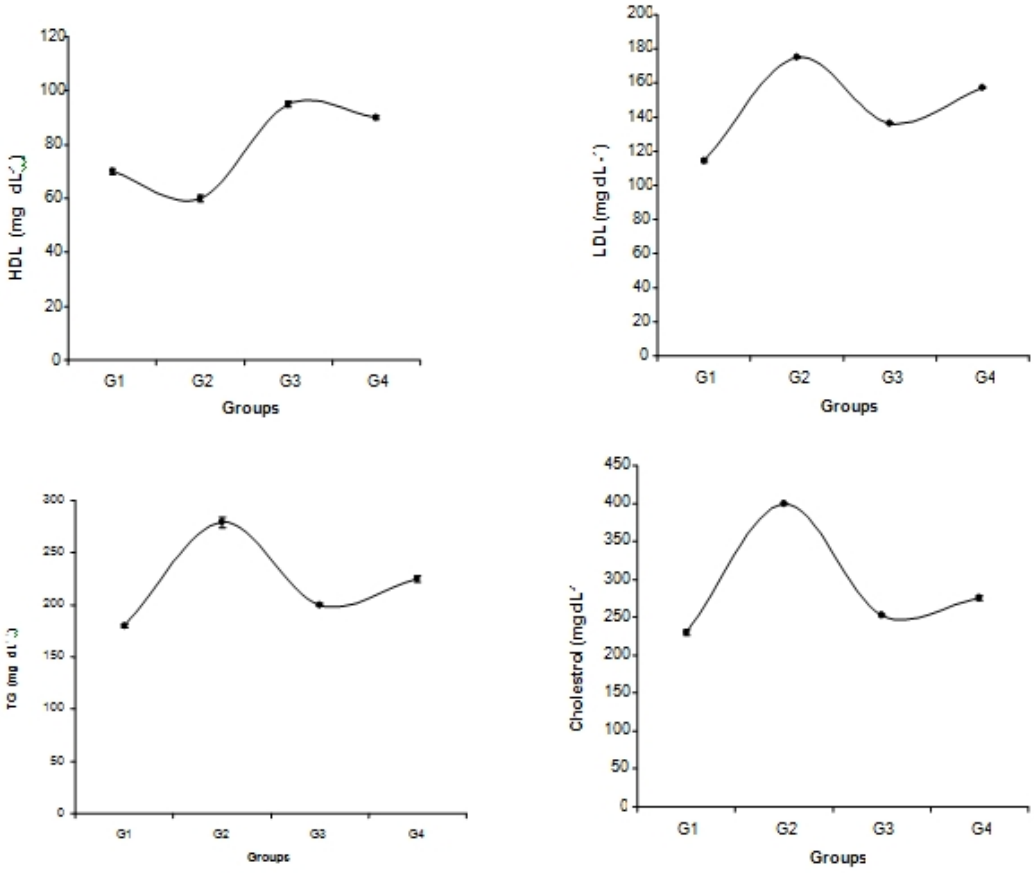

Fig. 2. Preventive effect of gemmo modified and native extracts of *Glycyrrhiza glabra* on serum lipid profile levels (LDL, HDL, TG, TC) (mg dL^{-1}) in ISO infarcted rabbits

G1= Control, G2= Ischemia, G3= Gemmo preventive, G4= Native preventive. Results expressed as mean ± SD for six rabbits in each group. Values differ significantly among each other according to Duncan's Multiple Range Test at P =.05

The TC level in normal animals was 230 mg dL^{-1}, it significantly (*P* = .05) increased to 400 mg dL^{-1} in ischemic group, corresponding to 73.9% rise. The preventive treatment with gemmo-modified and native *G. glabra* extracts caused reduction in TC by 37.0% and 31.3%, respectively (*P* = .05).

These results are consistent with other studies conducted on different plant extracts [7,12,13,14] Infarcting rabbits with ISO elevated the LDL cholesterol, TG and TC and decreased the HDL cholesterol level in the serum of ischemic rabbits. The increase in levels of TC, LDL, TG cholesterol and a decrease in HDL cholesterol are associated with raised risk of myocardial infarction [15]. High level of circulating cholesterol and its accumulation in heart tissues is accompanied with cardiovascular damage. The extract of (gemmo modified and native) *Glycyrrhiza glabra* elevated HDL level and decreased LDL level. There is an

evidence from epidemiologic, clinical and laboratory data indicating that elevated TG levels are independent risk factors for cardiovascular disease. Hypertriglycridemic patients, at a risk for cardiovascular disease, often develop a lipoprotein profile characterized by elevated TG, LDL and low HDL cholesterol which causes myocardial membrane damage [16]. Hypertriglyceridemia observed in ISO infarcted rabbits is clinically reported as ischemic heart disease. Pretreatment with *Glycyrrhiza glabra* extract prevented the elevation of TG cholesterol, LDL cholesterol and TC cholesterol signifying that myocardial membrane was intact and not damaged [17]. The cardioprotective properties of *Glycyrrhiza glabra* observed in this study add to the accumulating evidence for therapeutic potentional of this plant.

There are many useful compounds in licorice root such as, glycyrrhizin and aglycone, glycyrrhetinic acid which are clinically used for hyperlipidemia [5]. Licroice flavonoid constituents mainly include flavones, flavonals, isoflavones, calcones, bihydroflavones and bihydrochalcones. Pharmacological investigations indicate that they have antioxidant, antibacterial and anti-inflammatory activities [6]. Glycyrrhizin and glabridin inhibit the generation of reactive oxygen species (ROS) by neutrophils [18].

In the present study gemmo modified extract was more effective in lowering LDL, TG, and TC and increasing HDL than the native extract. There is no study evaluating the cardioprotective potential of the gemmo modified extract of buds and young growing shoots of *Glycyrrhiza glabra*. This is because the gemmo contain many active principles that start to disappear after a plant reaches a certain point in its development. Two phytohormones auxins, which stimulate cell growth and gibberellins which stimulate RNA and protein synthesis are present in buds and growing shoots. These compounds are not present in mature plant. Additionally they are rich in vitamins, anthocyanins (various water soluble pigments) and essential minerals and trace elements.

3.4 Curative Effect of *Glycyrrhiza glabra* on Cardiac Enzymes

The curative effect of gemmo modified and native extract of *Glycyrrhiza glabra* on cardiac enzymes (CK-MB, LDH, SGOT and SGPT) in rabbits is presented in Fig 3. The CK-MB means in control group ranged from 120.3 U mL^{-1} to 121.00 U mL^{-1} which differed significantly (*P*=.05) over days. In Ischemic group, CK-MB level increased considerably ranging from 270 U mL^{-1} to 320 U mL^{-1} that is 2.3 to 2.6 fold that of the normal animals. The post treatment with gemmo modified extract of *Glycyrrhiza glabra* resulted in decrease (*P* = .05) in CK-MB varying from 170 mg dL^{-1} to 300 mg dL^{-1}, corresponding to 6.3 to 38.18% reduction. The native extract used as curative could decrease CK-MB by 3.2 to 27.2% over days. The group means showed that gemmo modified extract was superior to native extract in lowering CK-MB level in Ischemic animals (*P*=.05).

The mean values of LDH in normal rabbits ranged between 245.0 U mL^{-1} and 270.0 U mL^{-1}, which differed significantly (*P*=.05) over the days. Administration of ISO to rabbits resulted in remarkable increase in LDH level varying between 290.0 U mL^{-1} and 380.0 U mL^{-1}. The means decreased with gemmo modified extract of *Glycyrrhiza glabra* and differed significantly (*P*=.05) within the group. The post treatment with native extract also caused reduction in LDH level but lower than gemmo modified extract. The gemmo modified extract was significantly (*P*=.05) better than native extract in reducing the LDH level in ISO induced Ischemic animals.

The mean values of SGOT in normal animals varied between 16. U mL^{-1} and 19.0 U mL^{-1} which did not differ significantly (*P*=.05) in control group over the days. Administration of ISO

resulted in considerable increase in SGOT levels upto 38.2% to 76.4% as compared with control animals. The curative treatment with gemmo modified extract of *Glycyrrhiza glabra* decreased the SGOT levels by 6.6% to 14.9%. The post treatment with native extract of *Glycyrrhiza glabra* was as effective as gemmo modified extract in reducing the SGOT level in ischemic animals.

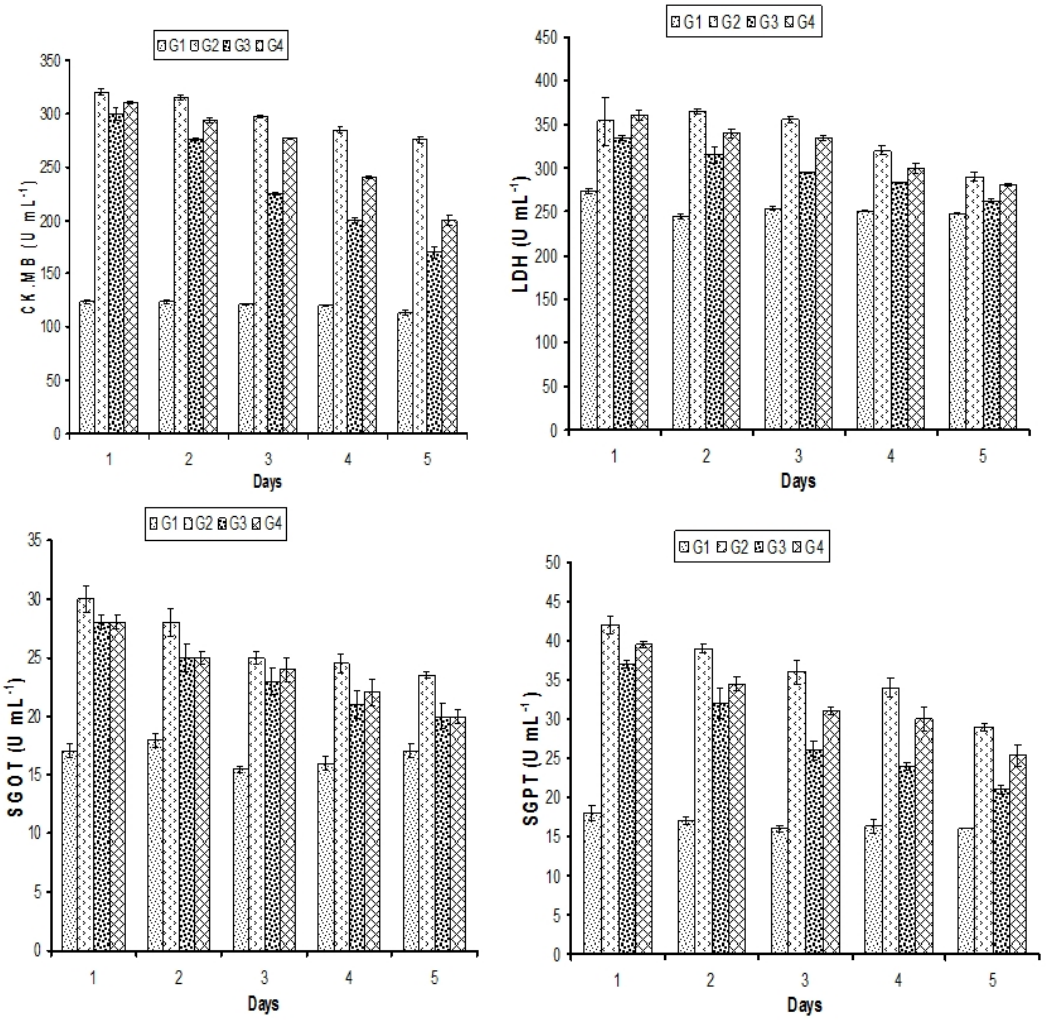

Fig. 3. Curative effect of gemmo - modified and native extracts of *Glycyrrhiza glabra*, on cardiac enzymes (CK-MB, LDH, SGOT, SGPT) in ISO infarcted rabbits

G1= Control, G2= Ischemia, G3= Gemmo curative, G4= Native curative. Results expressed as mean ± SD for six rabbits in each group. Values differ significantly among each other according to Duncan's Multiple Range Test at P=.05

The mean values of SGPT in normal rabbits ranged from 17.00 to 18.00 U mL^{-1} and did not differ significantly (*P* =.05) over days. Ischemia induced with ISO increased the SGPT 1.8 to 2.3 times compared with control animals. The SGPT level decreased by 11.9% to 29.4% with post treatment of the animals with gemmo modified extract of *Glycyrrhiza glabra*. Whereas, the curative treatment with native extract reduced the SGPT levels by 5.9 to

13.8% as compared with ischemic animals. The group means showed that the gemmo modified extract was significantly (*P* =.05) superior to native extract in decreasing the SGPT levels in infarcted animals.

3.5 Pre treatment (Preventive) Effect of *Glycyrrhiza glabra* on Cardiac Enzymes

The CK-MB level in control rabbits was 121.33 U mL^{-1}. The gemmo modified extract of *Glycyrrhiza glabra* as pre treatment caused 48.4 % reduction in CK-MB levels as compared with ISO infarcted animals (Fig 4). Whereas, native extract given as preventive treatment reduced CK-MB level by 40.6% (*P* = .05) . The LDH level in control animals was 270.0 UmL^{-1}, it increased to 380.0 U mL^{-1} in ISO induced ischemic group of animals which was 1.4 time higher than that in the normal animals. The preventive treatment with gemmo modified extract of *Glycyrrhiza glabra* decreased the LDH by 13.2%. The SGOT level in control rabbits was 16.0 UmL^{-1} which increased 1.76 fold that in ISO infarcted animals. The preventive treatment given with gemmo modified and native extract of *Glycyrrhiza glabra* decreased the level of SGOT by 26.6% and 15.56%, respectively. The SGPT level in control animals was 18.00 mg dL^{-1} and it was 2.3 time higher in ISO ischemic animals. The preventive treatment with extract of *Glycyrrhiza glabra* (gemmo modified and native) lowered the SGPT level by 28.5%and 9.5%, respectively. In preventive treatment the gemmo extract was significantly better than native extract (*P*=.05) in resisting the elevation of cardiac enzymes after ISO induced infarction in rabbits.

Raised serum LDH and creatine kinase (CK-MB) is the result of free radicals generated by ISO, initiate lipid peroxidation of the membrane bound polysaturated fatty acids, damaging membrane structural and functional integrity. The metabolic impairment of myocardium results in increase in the concentrations of the marker enzymes like LDH and CK-MB [14]. This occurred in the present study too.

The *Glycyrrhiza glabra* administration restored the myocardial LDH and CK-MB iso-enzyme activities, which indicated protection of the myocardium depletion in myocardial LDH, and CK-MB isoenzyme levels during ISO induced myocardial necrosis, indicated altered membrane permeability and leakage of these soluble enzymes [19]. This significant depletion in myocardial LDH and CK-MB iso-enzyme activities observed in the present study is in agreement with similar findings in other studies [20,21].

Therefore, it is very likely that *Glycyrrhiza glabra* provided cardioprotection due to its antioxidant and anti-peroxidative properties. Similarly the lyophilized hydroalcoholic extract of a *Bacopa monniera* provided significant cardioprotection against ISO induced exogenous stress in rats [22,23,24,25]. The exact mechanism for such cardioprotection by *Bacopa monniera* extract is not clear, but may involve its antioxidant and peroxidative actions. Herbal medicines possessing antioxidant and free radicals scavenging activities may, therefore, have protective role in cardiovascular disease and provide viable alternatives. Comparative safety, lack of significant side effects and lower cost compared to conventional medicine add to the advantages of the plants [26,27].

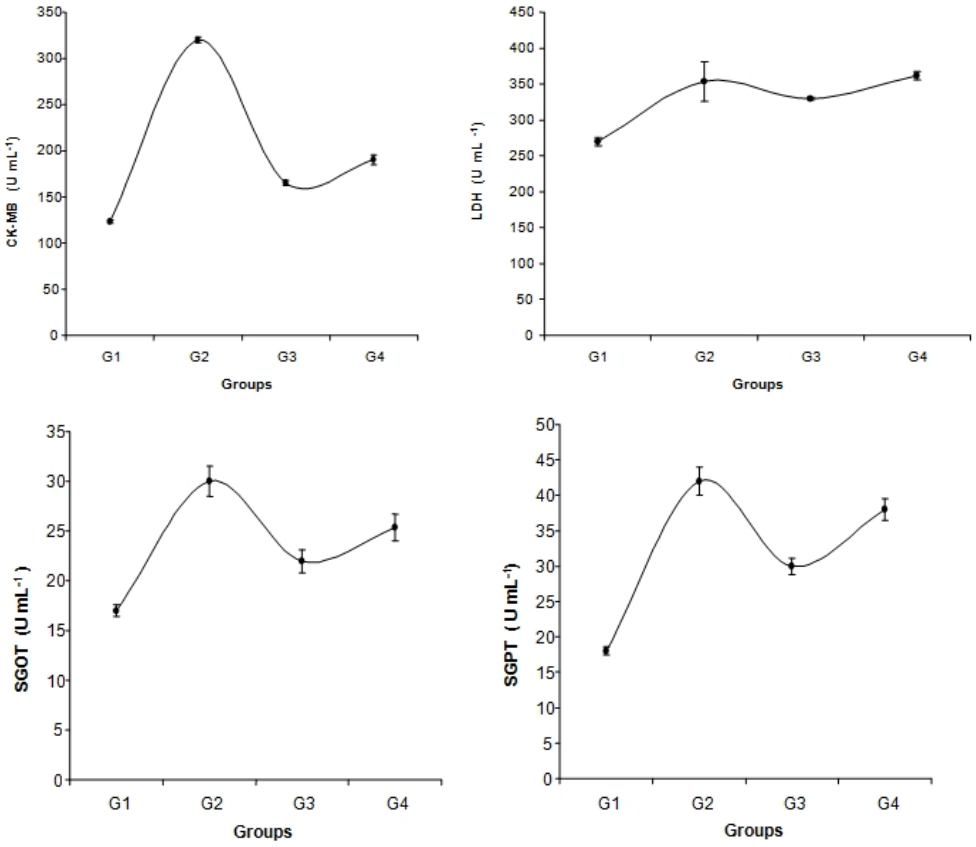

Fig. 4. Preventive effect of gemmo - modified and native extracts of *Glycyrrhiza glabra*, on cardiac enzymes (CK-MB, LDH, SGOT, SGPT) in ISO infarcted rabbits

G1= Control, G2= Ischemia, G3= Gemmo preventive, G4= Native preventive. Results expressed as mean ± SD for six rabbits in each group. Values differ significantly among each other according Duncan's Multiple Range Test at P = .05.

There was a significant (*P*=.05) increase in serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) in infarcted animals relative to control. These changes were however remarkably resisted in the *Glycyrrhiza glabra* treated infarcted animals.

The elevated levels of SGOT and SGPT in serum after intoxication were used as indicators of damage and protective effect of the plant extract was demonstrated in lowering the raised serum levels of SGOT and SGPT [28,29,30].

Increases in SGOT and SGPT are usually secondary to tissue damage. This is because such damage results in the leakage of these enzymes from their intracellular stores into plasma. SGPT is the most prevalent in the liver, whereas SGOT may also be found in heart and skeletal muscles and liver to nearly the same extent. Increases in SGOT are often seen in hemolytic anemia, myocardial infarction and cholestatic disease of the liver [31,32,33,34,35]. The fractional increase in SGOT and SGPT or the ratio of the SGOT: SGPT may be a useful tool in assessing the extent of damage.

The changes in ISO infarcted rabbits observed in the present study may have been complicated by multisystem involvement [36,37]. The exact mechanism by which *Glycyrrhiza glabra* exerts its effect is not clear. This activity can be readily ascribed to anyone of the many biologically active compounds present in this plant.

Phytochemical screening examination of the herb revealed that it is rich in flavonoids and terpenes and the pharmacological actions of *Glycyrrhiza glabra* are believed to be due to the presence of these phytochemicals [38,39]. Glycyrrhizic acid inhibits cyclooxygenase activity and prostaglandin formation besides indirectly inhibiting platelet aggregation [40].

It is known that SGOT can be found in the liver, cardiac muscles, kidney, brain, pancreas, lungs, skeletal muscles, leukocytes and erythrocytes [41]. Whereas SGPT is present in high concentration in the liver. In tissues SGPT occurs in two locations, cytosol and mitochondria [42]. SGPT appears to be a more sensitive and specific parameter of acute hepatocellular damage than SGOT [43]. Therefore, the possible cardioprotective mechanism of *Glycyrrhiza glabra* extracts on the ISO induced infarction may be through the following action, inhibition of the cytochrome P-450 activity, stopping the process of the lipid peroxidation, stabilization of the heart membrane and increasing the protein synthesis [44,45].

3.6 Effect of *Glycyrrhiza glabra* on Heart Rate (Tachycardia) of Rabbits Infarcted with ISO

The effect of gemmo modified and native extracts of *Glycyrrhiza glabra* on heart rate (tachycardia) was also investigated in the present study. The mean heart rate in control animals was 83 - 84 beats per 30 sec recorded at different time intervals (Table 2 which increased 1.48 to 1.58 fold in ISO infarcted animals (ranging from 125-134 beats per 30 sec). Administration of gemmo - modified extract of *Glycyrrhiza glabra* significantly (P =.05) lowered the heart rate by 9.7 % to 16.8 % (heart rate varied from 120 to 104 beats per 30 sec). Similarly native extracts treatment reduced the heart rate by 6.0 to 12.0 % (heart rate ranging from 125 – 110 beats per 30 sec). The means and group means showed that gemmo modified extract of *Glycyrrhiza glabra* was significantly (P =.05) better than native extract. The preventive treatments given with gemmo modified extract and native extract of *Glycyrrhiza glabra* did not differ significantly (P =.05) in reducing the heart rate (Table 3).

Table 2. Curative effect of gemmo - modified and native extracts of *Glycyrrhiza glabra* on heart rate (beats per 30 sec) of ISO infarcted rabbits.

Time hr.	G 1	G 2	G 3	G 4
12	$84.00^{j} \pm 4.58$	$133.00^{a} \pm 3.00$	$120.00^{defg} \pm 2.00$	$125.00^{cd} \pm 4.58$
24	$83.00^{J} \pm 3.60$	$134.00^{a} \pm 4.00$	$119.00^{defg} \pm 1.73$	$123.00^{cde} \pm 3.60$
36	$83.00^{j} \pm 2.64$	$132.00^{ab} \pm 2.64$	$117.00^{efg} \pm 1.73$	$121.00^{cdef} \pm 1.00$
48	$83.00^{J} \pm 3.00$	$131.00^{ab} \pm 3.00$	$116.00^{fg} \pm 1.72$	$119.00^{defg} \pm 4.58$
72	$83.00^{j} \pm 2.64$	$127.00^{bc} \pm 3.00$	$114.00^{gh} \pm 3.60$	$117.00^{efg} \pm 2.64$
96	$84.00^{j} \pm 4.00$	$125.00^{cd} \pm 5.00$	$104.00^{i} \pm 3.60$	$110.00^{h} \pm 3.60$
	83.33^{D}	130.3^{A}	115.00^{C}	119.2^{B}

Results expressed as mean ± SD for six rabbits in each group.

G1= Control, G2=Ischemia, G3= Gemmo curative, G4= Native curative

Means followed by the different letters differ significantly at P=.05 according to Duncan's Multiple range Test

Table 3. Preventive effect of Gemmo modified and native extracts of *Glycyrrhiza glabra* on heart rate (beats per 30 sec) of ISO infracted rabbits

Time hr.	G 1	G 2	G 3	G 4
12	$84.00^{j} \pm 4.58$	$133.00^{a} \pm 3.00$	$110.00^{de} \pm 3.46$	$112.00^{d} \pm 3.46$
24	$83.00^{j} \pm 3.60$	$134.00^{a} \pm 4.00$	$108.00^{def} \pm 4.00$	$109.00^{de} \pm 2.64$
36	$83.00^{j} \pm 2.64$	$132.00^{ab} \pm 2.64$	$105.00^{ef} \pm 2.64$	$106.00^{efg} \pm 4.00$
48	$83.00^{j} \pm 2.64$	$131.00^{ab} \pm 3.00$	$101.00^{ghi} \pm 4.00$	$103.00^{fgh} \pm 3.00$
72	$83.00^{j} \pm 2.64$	$127.00^{bc} \pm 3.00$	$97.00^{ij} \pm 1.73$	$99.00^{hi} \pm 3.00$
96	$84.00^{j} \pm 4.00$	$125.00^{c} \pm 5.00$	$91.00^{k} \pm 3.60$	$93.00^{jk} \pm 3.00$
	Mean 83.33^{D}	130.3^{A}	102.00^{B}	103.7^{B}

Results expressed as mean ± SD for six rabbits in each group.
G1= Control, G2= Ischemia, G3= Gemmo preventive, G4= Native preventive, Means followed by the different letters differ significantly at P = .05 according to Duncan's Multiple range Test

The exact mechanism by which *Glycyrrhiza glabra* extracts (gemmo modified and native) affect the heart rate in ISO ischemic rabbits is still not clear but may be due to the phytoconstituents present in this plant.

Isoproterenol (ISO) a synthetic catechol amine is a beta- adrenergic receptor agonist. Its high dose has the ability to destroy myocardium and causes cardiotoxicity due to cytosolic Ca^{+2} overloads. The metabolism of ISO produces an oxidative stress due to excessive production of free radicals that initiates the peroxidation of polyunsaturated fatty acids present in membrane which may result in the loss of function of myocardial membrane. As a result of this myocardium destruction, cytosolic enzymes (CK-MB, LDH, SGOT, SGPT) are secreted into the blood and serve as diagnostic markers of cardiotoxicity. Pathophysiological changes including cell necrosis, contractile failure and ventricular arrhythmias were observed [24]. Possible side effects of ISO include tachycardia which may lead to myocardial infarction [46].

In the present study the *Glycyrrhiza glabra* showed high flavonoids contents which strongly suggest the antioxidant and cardioprotective nature.

Cardiovascular disorders such as atherosclerosis and arterial thrombus formation leads to endothelial dysfunction. The intake of flavonoids may stop endothelial dysfunction by increasing the vasorelaxant process leading to reduction of arterial pressure [47]. Flavonoids may directly scavenge some radical species and also help in uptake of oxidatively modified LDL through scavenger receptors. Flavonoids also suppressed the LDL oxidation and exerted significant vesorelaxation [2].

The exact mechanism by which *Glycyrrhiza glabra* extracts (gemmo modified and native) affect the heart rate in ISO induced infarction in rabbits is still not clear but may be due to the phytoconstituents present in *Glycyrrhiza glabra*.

4. CONCLUSION

These studies on the cardioprotective and antilipidemic effects of *Glycyrrhiza glabra* are preliminary. However, the evidence for the beneficial effects is encouraging. The preventive or curative application of *Glycyrrhiza glabra* extracts (gemmo and native) significantly prevents the damage induced by isoproterenol on biochemical changes in rabbit's model of myocardial infarction. Observations on the effectiveness of the extracts suggest that by the

usage of this medicinal plant in our daily life we could minimize the risk of CVD but more studies are warranted in clinical set up.

CONSENT

Not applicable.

ETHICAL APPROVAL

Synopsis and thesis approved by synopsis and thesis approval committee of university of Agriculture Faisalabad, Pakistan.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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