Cardio Protective Role of Novel Gemmo Therapeutically Treated *Glycyrrhizaglabra* Against Isoproterenol Induced Myocardial Injury

ABSTRACT

Aim: To explore cardio protective potential of *Glycyrrhiza glabra*as against chemically induced myocardial injury in experimental animals by using their blood (serum) for subsequent determination of enzyme levels. **Methodology:** *Glycyrrhizaglabra* with known cardio protective potential was used against chemically induced myocardial injury in experimental animals. These animals were divided into seven groups (Control, damage, Gemmo-curative, Gemmo-preventive, Native-curative, and Native-preventive, base-line) having ten animals each. Seventy rabbits of both sexes, 1-1.5kg weight was included while pregnant rabbits weight >1.5kg were excluded. *Glycyrrhiza glabra*leaves after gemmo-therapeutic treatment were subjected for phytochemical screening. Then, 100 mg/kg gemmo and native extract was given to native-preventive and gemmo-preventive groups respectively for 20 days. On 21st-25thday isoproterenol (50mg/kg) were given to rabbits for induce ischemia. On 26th day native and gemmo modified extract (100mg/kg) were given to native-curative and gemmo-curative groups respectively. The blood was drawn from the neck region; serum was separated for biochemical analysis. Pretreatment with native and gemmo-extract resisted to rise (P≤0.05) cardiac enzymes (CK-MB, SGOT, SGPT, LDH).
Results: It was studied that in isoproterenol injured rabbits, gemmo-extract was significantly better (P≤0.05) than native-extract in curative as well as in preventive treatment to normalizing serum cardiac enzymes.

Keywords: Cardiovascular diseases, cardiac enzymes, CK-MB, *Glycyrrhiza glabra,* Isoproterenol, LDH, SGOT, SGPT

1. INTRODUCTION

Cardiovascular diseases (CVDs) are related to heart or a blood vessel, their prevalence is higher in western populations. In America, CVDs is responsible for high mortality rate in comparison to cancer. The CVDs alone accounts for 30% of all deaths along with other CVDs related diseases causing significant further disability and death. According to the WHO, the Ischemic heart diseases is leading cause of death worldwide and it is expected that in 2030 it would be the major cause of morbidity[1]. The occurrence of ischemic heart disease increases the mortality from a myocardial heart disease[2].

Several cardiac biomarkers are found to be associated with the heart function, damage or failure such as CK-MB, SGOT, SGPT and LDH. They are employed for detection of presence and severity of CVDs. So that appropriate treatment can be started earlier[1,3]. Their levels vary with time therefore, help in detection and estimation of disease progression and monitored to relapse. Very few cardiac biomarkers are being employed by physicians to carry out routine tests[2, 4, 5].

According to WHO the use of medicinal plants for therapeutic purpose has reached to approximately 70-80%.[2, 4]. These plants are blessed with therapeutically

active constituents[4, 6, 7]. *Glycyrrhiza glabra* is one of the medicinal plants having significant potential against cardiovascular diseases[2, 5, 8]. It constitutes glycyrrhizin (saponin-like glycoside), aglycone, glycyrrhetinic acid (useful for treatment of hyperlipidemia)[6, 9, 10] and a flavonoid Isoliquiritigenin (ISL) known for anti-oxidant, anti-tumor, anti-platelet, vasorelaxant, antiviral, anti-allergic and estrogenic properties[7, 11, 12]**.** Gemmo-therapy method was developed in France and officially entered in French pharmacopeia[9]. It is one of the modern methods used to get better amount of phyto-constituents (vitamins, hormones, enzymes and other nutrient) [1,5,7] as remedial source.

The aim of the study to explore cardio protective potential of *Glycyrrhiza glabra*as against chemically induced myocardial injury in experimental animals by using their blood (serum) for subsequent determination of enzyme levels.

2. MATERIAL AND METHODS

All chemicals and kits were purchased from a certified medical store, Merck and Sigma company.The research plan was completed in following steps after the approval of ethical review committee PUMHS Nawabshah.

Plant Sampling and Treatment

Fresh growing leaves of *Glycyrrhiza glabra* were identified with the help of plant taxonomist. Leaves were carefully washed with double distilled water followed by drying and weighing. The samples were then grinded to perform the phytochemical analysis after drying in shaded cool environment by adopting the method of Lopez and Murrau[1].

Gemmotherapeutic Treatment

Leaves (as native) were blended in a mixture of glycerin and alcohol (1:2 V/V) and mixture was kept in a shaded and cool environment for one month with time to time shaking for complete maceration. The sample was then filtered under constant pressure, re-filtered after 48 hours and the resulting liquid was labeled as stock. To remove alcohol from the stock solution, it was evaporated in rotary and the resulting solution was kept in an incubator at 65°C to remove remaining alcohol if present. This gemmotherapeutic solution or extract could be used within five years from the date of extraction from plant material[9]

Animal selection

A total of 21 rabbits (1.25±0.10 kg) were selected as experimental animals and were acclimatized for fortnight under laboratory condition before use. The animals were provided with water and standard diet and placed under standard environmental conditions of humidity (50%) and temperature (30 °C) with an alternating 12-h dark:12-h light cycles and were weighed weekly. The experimental studies were performed in conformity by following the guidance for care and standard experimental animals study ethical protocols.

Dose Preparation and Drug Administration

Plant doses were prepared and given according to the standard method that was according to body weights of rabbits (100mg of plant dose/kg body weight). Doses were administered orally by dissolving plant material into distilled water (Tab. 1).

Table 1: Treatment protocol of experimental rabbits in all groups

Groups	Days		
	1-20	21st-25th	Five days after damage or ischemia
1	Normal diet	Normal diet	---
2	Normal diet	Isoproterenol(50 mg/kg)	---

3	Gemmo-extract(100 mg/kg)	Isoproterenol(50 mg/kg)	---
4	Normal diet	Isoproterenol(50 mg/kg)	Gemmo-extract(100 mg/kg)
5	Native extract(100 mg/kg)	Isoproterenol(50 mg/kg)	---
6	Normal diet	Isoproterenol(50 mg/kg)	Native-extract (100 mg/kg)
7	Gemmo-extract(100 mg/kg)	---	---

Experimental Protocol

Animals were divided into 7 groups having ten animals in each group.

Collection of Blood Samples

The blood samples were collected in centrifuged glass tubes from the neck vein (jugular vein) of the rabbits, which were kept at fasting overnight. The blood samples were then centrifuged and serum was separated, collected and stored at low temperature in deep freezer for further biochemical analysis.

Biochemical Analysis

Enzyme level was determined by using atomic absorption spectrophotometer. Different kits of enzymes (CK-MB test, SGOT-AST Test, SGPT-ALT Test, LDH (DGKC) Test) were used.

Statistical Analysis.

All statistical analysis was performed by using SPSS version 21 (SPSS Inc., Chicago, IL, USA). ANOVA was used to compare applications[13] and all the values were represented as mean±S.D. (n = 3). A probability of $p < 0.05$ was considered as significant.

3. RESULTS AND DISCUSSION

Phytochemical screening

Phytochemical screening for alkaloids, flavonoids, glycosides, steroids, triterpenoids, tannic acid, and saponins has been summarized on qualitative and quantitative basis in tables 2. Where gemo-treated extract maintained its superiority for almost all metabolites.

Table 2: Phytoconstituents and nutritive characters (proximate analysis) of gemmo and native phytoextract of *Glycyrrhizaglabra*

		NE	GE
Phytoconstituents	**Alkaloids**	**7**	**7.5**
	Flavonoids	**21**	**24**
	Glycosides	**16**	**20**
	Saponins	**8**	**15**
	Steroids & Triterpenoids	**-**	**-**
	Tannic Acid	**10**	**15.5**
Nutritive parameters	**Dry matter**	**96**	**87**
	Fat	**4**	**5**
	Crude fiber	**3**	**3.5**
	Ash	**5**	**4.7**
	Crude protein	**26.38**	**26.68**

NE: Native, GE: Gemmo treated

Proximate analysis

Nutritional activity of *G. glabra*in terms of proximate analysis was executed and compared for gemmo and native extracts.Gemmo-extract exhibited better results for fat as compared to native-extract but in case of crude fibers, ash and crude protein content, gemo & native extract showed almost same results. However, native-extract significantly showed more dry matter than gemmo (Tab. 2).

Myocardial injury in animal samples was determined by heart beat rate per 30 second after various time intervals. Isoproterenol application (G2) manifested marked rise in heart beat rate as compared to the control (G1). Further it was observed that heart beat rate of G4 & G6 was significantly high after the administration of isoproterenol but beat moved toward normal when those rabbits were treated with native/gemmo-extract (Tab 3).

Table 3: Tachycardia rate of rabbits after starting treatment

	Heart beat rate/30 sec after time interval					
Groups	12 hrs	24 hrs	36 hrs	48 hrs	72 hrs	96 hrs
G1	84.33±1	83.67±1.4	84±1.3	81.33±1	82±2.14	83±2.4
G2	132±3.45	131.66±2.65	132.33±3.94	127.66±4.1	129.67±4.12	131±3.12
G3	110±1.2	108±1.42	98±1	89±0.84	83±0.89	80±1.24
G4	120.33±1.4	117.33±1.43	109.67±1.4	104.67±1.5	90.33±1.1	83±1.02
G5	111±1.1	106.67±1.3	102±1.4	96.67±1.7	86±1.0	82±1.03
G6	120.33±2.5	117.33±3.4	109.67±3.4	104.6±3.12	90.33±3.1	83±2.3
G7	87.33±2.4	81.66±1.6	85.33±1.4	85.67±1	83.33±1.02	81.3±1.35

Gross pathology:

Immediately after sacrificing the animals' gross pathology was performed with the help of veterinary doctor, results shown in table 4.

Table 4 Gross pathology of rabbits in all groups

Organelles		Pathological studies	Wt. In grams
Heart	G1	Normal	2.13
	G2	Hard (damage)	2.63
	G3	Normal	2.8
	G4	Normal	2.22
	G5	Normal	3.3
	G6	Normal	3.2
	G7	Normal	2.9
Lungs	G1	Pale red	2.75
	G2	Pale yellow	5.03
	G3	Pale red	6.7
	G4	Pale yellow	6.48
	G5	Pale red	7.2
	G6	Deep red	7.3
	G7	Pale red	6.6
Liver	G1	Normal	7.85
	G2	Decolorized	29
	G3	Normal	26
	G4	Normal	28.49
	G5	Normal	31

	G6	Normal	29
	G7	Normal	27.78
Kidney	G1	Normal	26.17
	G2	Normal	7.9
	G3	Normal	5.9
	G4	Normal	6.19
	G5	Normal	8.4
	G6	Normal	6.99
	G7	Normal	6.89

Estimation of cardioprotective activity

Cardioprotective activity was determined by the estimation of markers (cardiac enzymes) in the serum of the control, native &gemmo treated animals.

Serum CK-MB level was significantly increased after oral administration of Isoproterenol in gemmo (276.00±37.04U/L) and native (288.33± 20.232U/L) curative groups when compared with control (105.33±7.095 U/L) (Fig.1a). Aspartate transaminase (AST/SGOT) is another important biochemical marker for the estimation of damaged heart (myocardial infarction). It was observed considerable amount of serum AST was increased when Isoproterinol was orally administered in native-curative (32.667±2.082 U/L) &gemmo-curative (28.33±3.055U/L) rabbits as compared to control (16.00±2.00U/L) (Fig 1b).Similar results were also observed by other researchers [14,15,16]. Zaman (2014) investigated thatRaphanassativus fruit powder and its ethanoic extracts showed protective effect against cardiac disorders[17].Chauhan and Naik (2015) evaluated that isoproterenol induced ischemic rats were recovered by A.V. Circulo extract[18]. It was observed that extract significantly reduce the level of serum markers (CK, LDH & SGOT).ALT is a crucial enzyme to diagnose damage liver cells but myocardial infarction is also determined by this biochemical marker. It was revealed that Isoproterinol significantly enhanced the ALT level in native-curative (27.667±4.509 U/L) & gemmo-curative (27.000±2.00 U/L) rabbits as compared to control (12.667±2.082 U/L) group (Fig 1c). Anthony *et al*, (2015) revealed that grape seed proanthocyanidin extract significantly reduced biochemical markers and showed its cardio protective effect (ALT, AST, CK and LDH).Such type of investigation was also performed by Panda and Naik (2018)[16, 19]. LDH level was significantly increased after administration of Isoproterinol in native curative and gemmo curative groups 402.00± 17.349 U/L 388.667±14.978 respectively as compared to control group (267.00±11.00 U/L) (Fig 1d). It has been studied by various researchers that HCl extract of *Withaniasomni fera*used to protect isoproterenol induced ischemic heart in rats[19, 20]. Cheng *et al.* (2015) also observed that Curcumin significantly recover the myocardial reperfusion injury by reducing the activity of biochemical markers (LDH, CK-MB,CK).

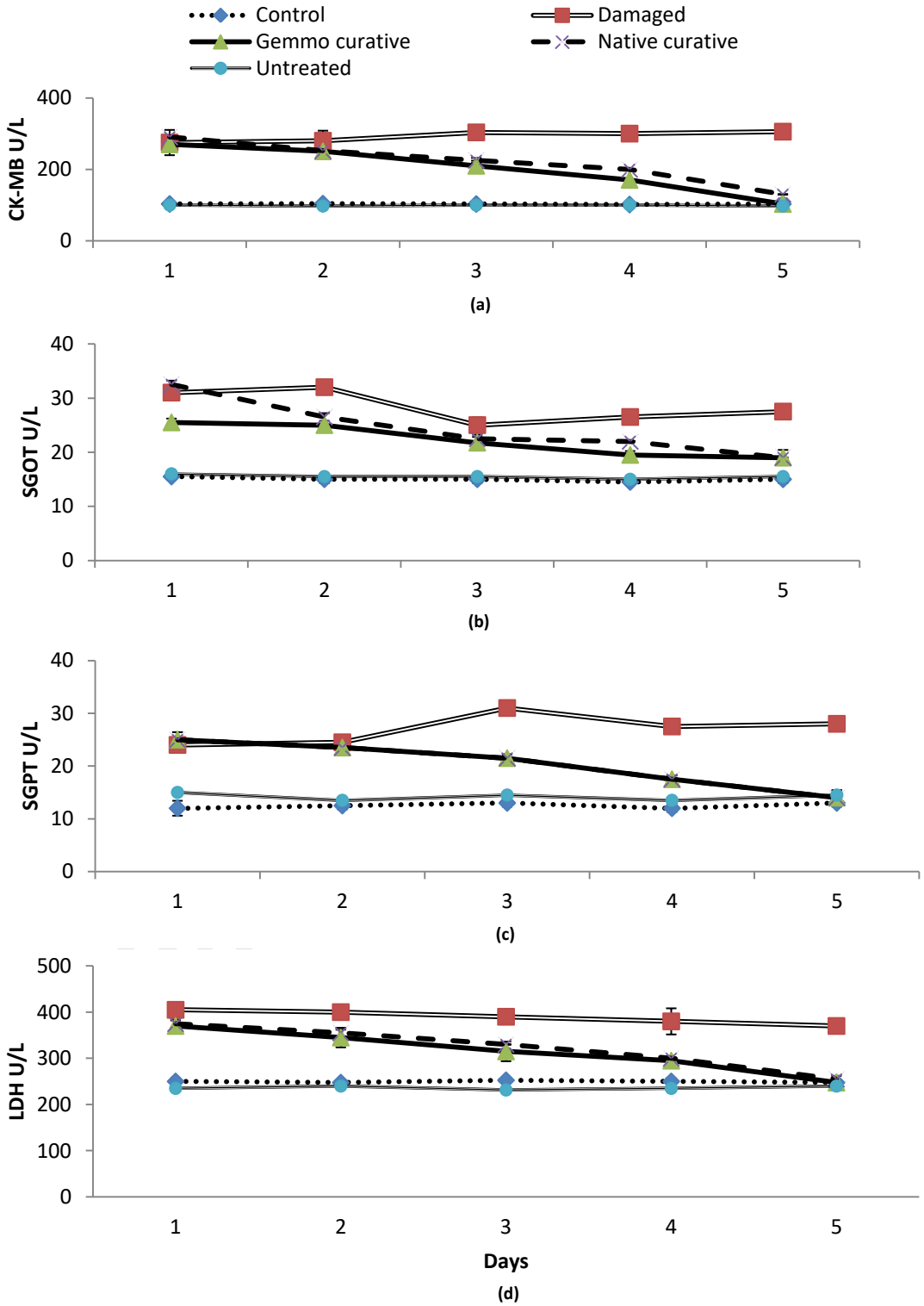
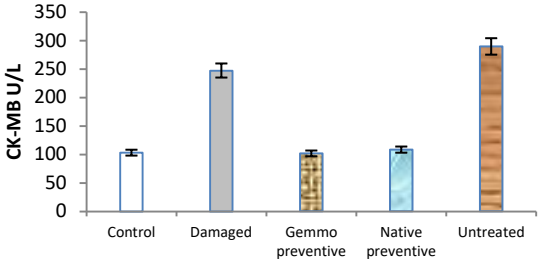
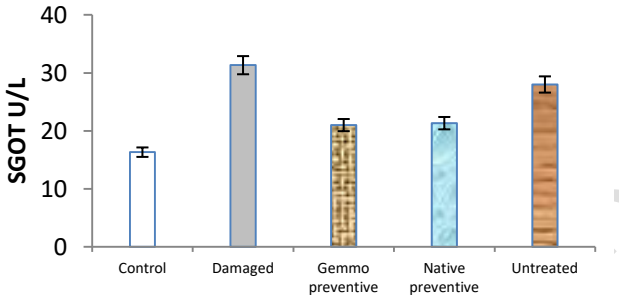


Fig. 1: Time course changes in biochemical markers of curative groups and control in ISO infarcted

Oral administration of isoproterinol significantly increased the serum level of biochemical markers i.e CK-MB, SGOT, SGPT and LDH in damage group 247.33±39.703 U/L, 31.33±5.508 U/L, 24.33±3.055 U/L, 402.667 ±17.786 U/L respectively as compared to control. But the serum level of these biochemical markers resorted towards normal level due to pretreatment of rabbits with preventive doses of native preventive and gemmo preventive as compared to untreated group (Fig 2a-d).



(a)



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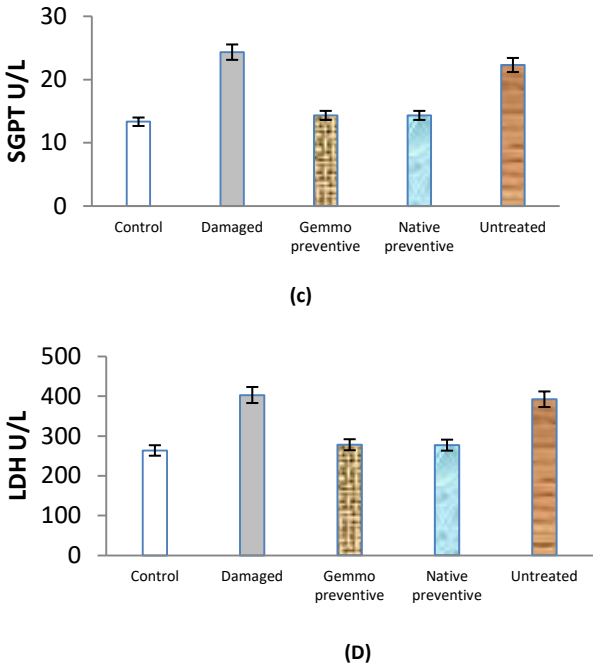

Fig. 2: Comparative values of biochemical markers in preventive groups

Shen*et al*, (2014) found similar results by evaluating cardioprotective efficacy of Ginkgo bilobaextract. After 5 days' oral administration of ginkgo biloba extract to ischemic animals showed normal levels of CK-MB and SGOT. **Jiangning *et al*. (2014) also** examined the similar kind of cardio protective effects when surgically inducing injured rabbits were treated with caffeic acid phenethyl ester[22].Tajuddin*et al* (2016) also conducted a study to evaluate cardioprotective effect of Unani formulation on preventive measures[11]. They pretreated the animals with unani formulation dose of 1g /kg body weight for sixty days before oral administration of isoproterenol. They revealed that SGOT level was statistically same in controlled and treated animal groups which showed a strong preventive effect. Such type of investigations was also performed by other researchers[19, 21, 23]**.** Other scientists observed Sasanquasaponin (SQS) which act as cardio protective compound [24]. They revealed that pretreated rats with SQS when induced by isoproterenol maintained LDH level near to normal.

4. CONCLUSION

It was concluded that in ISO injured rabbits, gemmo modified extract was significantly better (P≤.05) than native extract in curative as well as in preventive treatment to normalizing serum cardiac enzymes.

CONSENT

All authors declare that 'written informed consent was obtained from the patient.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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