Cardiotonic activity of methanolic extract of *Saussurea lappa* Linn roots

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Abstract: The cardiac activity of *Saussurea lappa* roots was evaluated in isolated perfused rabbit heart by the Langendorff's technique. Heart rate, contractility and coronary flow were determined in the presence of different concentrations of methanolic extract of *Saussurea lappa*, digoxin and diltiazem. The extract exhibited significant ($p<0.01$) positive inotropic effect at the first three doses (0.5/µg, 2.5/µg and 5.0/µg) while a significant negative chronotropic effect and coronary flow rates were observed at all the doses tested. These effects were comparable to the effects of digoxin and diltiazem. The increase in force of contraction with decrease in heart rate and coronary flow rates were also observed to be dose dependent as increase in the dose of test extract further enhanced the effects except contractility that started decreasing at higher doses. It is conceivable therefore, that *Saussurea lappa* roots contain certain pharmacologically active compounds that could be involved in the cardiotonic activity of the extract.

Keywords: *Saussurea lappa* Linn; methanolic extract; cardiotonic; digoxin; diltiazem.

INTRODUCTION

Cardiovascular diseases (CVDs) have become a serious medical concern and are prevalent throughout the world (Isselbacher *et al.*, 2001). The major risk factors for CVDs include family history, sex, raised lipid levels, hypertension, obesity, cigarette smoking, hyper-cholesterolemia and other lipid abnormalities. Most of these risk factors are widespread in developing countries because of the lack of resources and infra-structure available in health care system. Therefore, CVDs have become a very common problem in the affluent population of the developing countries due to their life styles. By the year 2020 it is expected that CVDs will be the most common cause of death (Trivedi and Nehra, 2004). In Pakistan, the condition is quite similar to other developing countries. According to National Health Survey of Pakistan, about one hundred thousand deaths occur per year in the country due to CVDs. This data alone does not depict the true picture, as there are many more people who are suffering from CVDs related disabilities. Many synthetic drugs have been commonly used for the treatment of these diseases but herbal medicines remain the popular choice in the third world countries. According to a survey by WHO, it has been estimated that about 60% of the world's population relys on traditional herbal medicine (Zhang, 2000).

Ethnobotanical surveys indicate that various medicinal plants have been used as traditional medicines for the treatment of CVDs such as *Digitalis purpurea, Digitalis lanata and Crataegus monogyna* (Hawthorn) for congestive heart failure (Jerie *et al*., 2007), *Rauwolfia serpentin* for systolic hypertension (Weng *et al*., 1984) and *Allium sativum* for atherosclerosis (Sutter *et al*., 2007). Traditional medicines though effective safe and potent in quite a number of diseases, require scientific evaluation in order to be used to their full extent.

Saussurea lappa, Linn. (Family: Compositae) commonly known as Qust-e-shireen is a long, erect herb found mostly in Northern mountainous regions of Pakistan and India (Gupta and Ghatak, 1967). Traditionally it has been used for the treatment of large number of diseases such as asthma, cough, throat infections, tuberculosis, leprosy, malaria, convulsions, fever, helminthic infestations and as antispasmodic (Nadkarni, 1997; Malik *et al*., 2011). This plant has been reported to contain certain phytochemical constituents like flavonoids and sesquiterpenes (Chang *et al*., 2003). This study was carried out to determine whether the plant contains some promising cardiotonic principle(s) which may ultimately provide novel and safe cardiotonic drug for future use in modern therapeutics.

MATERIALS AND METHODS

Chemicals and Drugs

Methanol, Digoxin and Diltiazem were purchased from Sigma Chemicals Co. All the chemicals and drugs used in the experiments were of standard grade.

Animals

Both male and female rabbits of local strain (*Oryctolagus cuniculus*) weighing 1-1.5 kg were used. All the animals were housed in controlled environment (23-25°C) at animal house of Department of Pharmacy, University of Sargodha, Sargodha. All animals were treated according to the standard procedures and the study protocol was approved by the local ethical committee.

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Table 1: The effect of different doses of methanolic extract of *Saussurea lappa* on various cardiac parameters in isolated perfused rabbit heart

Dose	Coronary flow (ml/min)		Force of contraction (g)		Heart rate (beats/min)	
	Mean	% Change from control	Mean	% Change from control	Mean	% Change from control
Control	17.17± 0.60A	0.00	2.34±0.008D	0.00	209.1±0.70 A	0.00
0.5µg	16.17± 0.60 AB	-5.83	2.46±0.010C	5.35	190±0.86B	-9.16
2.5µg	15.50± 0.43 B	-9.71	2.64±0.006A	13.06	170±0.58C	-18.73
5µg	13.67± 0.33 C	-20.13	2.52±0.007B	7.92	153.3±0.88D	-26.69
10µg	12.17± 0.40 D	-29.13	2.30±0.014E	-1.57	137.8±1.51E	-34.10
100µg	11.50.43D	-33	2.10±0.013F	-10.28	122.83F	-41.27

Results are expressed as means ± S.E.M (n=6). Mean sharing similar letters are statistically non-significant (P>0.05).

Analysis of variance

Cardiac Parameters	Source of variation	Degrees of freedom	Sum of squares	Mean squares	F-value
Heart Rate	Dose	5	31470.47	6294.095.13	1227.45**
	Error	30	153.83	5.13	
	Total	35	31624.31		
Force of contraction	Dose	5	1.096447	0.219289	374.14**
	Error	30	0.017583	0.00058	
	Total	35	1.114031		
Coronary flow	Dose	5	155.47	31.09	22.84**
	Error	30	40.83	1.36	
	Total	35	196.31		

**=Highly significant (P < 0.01)

Plant material

Dried roots of *Saussurea lappa* were purchased from a reputed commercial herbal supplier of Sargodha, Pakistan during the month of May, 2009. The plant was identified and authenticated by Dr. Amin Ullah Shah, Assistant Professor, Department of Botany, University of Sargodha, Sargodha and specimen was deposited in the Pharmacy department herbarium.

Plant extraction

Methanolic extract of *Saussurea lappa*, Linn (S. L) was prepared using cold maceration process. The grounded plant material (3/kg) was soaked in 7 liters of methanol for 72 hours at room temperature. After three days of occasional shaking, the whole material was filtered through Whatman qualitative grade 1 filter paper and the filtrate was evaporated under reduced pressure using rotary evaporator. The crude extract was then air-dried and a thick pasty mass was obtained with a percentage yield of 15.4%.

Study of Effect of Crude Extract on Various Cardiac Parameters

The experiment was performed according to the method prescribed by Langendorff (1895). Rabbits (n=6) received intraperitoneal injection of heparin (1000 units), 1 hour before dissection. The animal was then sacrificed by a blow on the neck with a metal rod. The thorax was cut open and the heart was immediately excised from the pericardial sac, with 1 cm aorta within 1-2 minutes so as to prevent ischemia. The heart was then transferred to a petri dish containing ice chilled perfusion solution, cleaned of any excessive tissue and mounted on the Langendorff apparatus. The aorta was tied to the glass cannula with a pressure transducer and at first perfusion fluid was passed at an increased flow rate. Then, the fluid flow rate was decreased and maintained at the same flow throughout the experiment. A clip was attached to the apex of the heart to measure the force of contraction (g). Force-displacement transducer was attached to the clip via a thread in order to measure force of contraction. Both the transducers were attached to the Power Lab and the recordings were measured using Chart 5.0 software. The recording channel was always calibrated before and after each experiment. The perfusion solution (Kreb's Hensleit solution) was used at constant pressure, aerated by carbogen (95% oxygen and 5% carbon dioxide). The preparation was then allowed to equilibrate for 30 minutes before starting the experiment. After stabilization, various doses (0.5µg, 2.5µg, 5.0µg, 10µg and 100µg) of the methanolic extract were applied to assess heart rate, forces of contraction and coronary flow. Similarly,

Table 2: The effect of different doses of digoxin on various cardiac parameters in isolated perfused rabbit heart

Doses	Coronary flow(ml/min)		Force of contraction (g)		Heart rate (beats/min)	
	Mean	% Change From control	Mean	% Change from control	Mean	% Change from control
Control	17.17± 0.60A	0.00	2.25± 0.013H	0.00	203.3±1.82A	0.00
1ng	16.17± .60AB	-5.83	2.34± 0.011G	3.17	199.67±1.74AB	-1.80
10ng	15.00± .52BC	-12.62	2.40± 0.008F	6.43	198.83±1.62B	-2.21
100ng	14.00± .52DC	-18.45	2.52± 0.012E	11.61	192.83±1.66C	-5.16
1µg	12.67± .49DE	-26.21	2.74± 0.015D	21.52	186.2±0.70D	-8.44
10µg	12.17± 0.31E	-29.13	2.99±0.011C	32.62	182.5±0.85D	-10.25
20µg	11.17± 0.31E	-34.95	3.12±0.011B	38.24	176.83±0.70E	-13.03
40µg	11.50± 0.43E	-33.01	3.16±0.013A	40.38	172.67±0.84F	-15.08

Results are expressed as means ± S.E.M (n=6). Mean sharing similar letters are statistically non-significant (P>0.05).

Analysis of variance

Cardiac Parameters	Source of variation	Degrees of freedom	Sum of squares	Mean squares	F-value
Heart Rate	Dose	7	5373.6	767.7	72.28**
	Error	40	424.8	10.6	
	Total	47	5798.5		
Force of contraction	Dose	7	5.563	0.794	925.47**
	Error	40	0.034	0.0008	
	Total	47	5.597		
Coronary flow	Dose	7	207.31	29.62	21.09**
	Error	40	56.17	1.40	
	Total	47	263.48		

** = Highly significant

different doses of digoxin and diltiazem were also assessed in order to determine the mechanism of action of crude extract. Each heart served as its own control. Changes in heart rate, force of contraction and coronary flow were expressed as means ± S.E.M. (Farid *et al*., 1992).

STATISTICAL ANALYSIS

The results were expressed as means ± standard error of mean (SEM) and statistical analysis was done by One-way analysis of variance and Duncan's multiple range test. Probabilities of < 0.05 were considered significant.

RESULTS

Effects of methanolic extract of Saussurea lappa on force of contraction, heart rate and coronary flow in isolated rabbit heart

Following the administration of various doses (0.5µg, 2.5µg, 5.0µg, 10µg and 100µg) of the methanolic extract of S. L, a dose dependent (P<0.01) positive inotropic effect was seen at doses of 0.5µg, 2.5µg and 5µg with maximum increase at 2.5µg (13%). However the extract exhibited a significant (P<0.01) negative inotropic effect at last two doses i.e. 10µg and 100µg. The extract produced a dose dependent (P<0.01) negative chrono-tropic effect with a maximum decrease at 100µg (-41%). Similarly all the tested doses of the extract showed a gradual decrease in coronary flow (CF). The first dose 0.5µg has no significant effect on CF as compared with baseline value (P>0.05) and like this last two doses i.e. 10µg and 100µg of extract showed similar non-significant effects. A highly significant (P<0.01) decrease in CF was observed at a dose of 5.0µg with the decrease of 20.39 % (Table 1).

Effects of digoxin on force of contraction, heart rate and coronary flow in isolated rabbit heart

Various doses of digoxin (1ng, 10ng, 100ng, 10µg, 20µg and 40µg) produced a significant (P<0.01) dose dependent positive inotropic effect. The maximum increase was seen at 40µg (40.38%). Moreover, a significant dose dependent decrease in heart rate was seen with maximum decrease at 40µg (15.08%). A significant (P<0.05) decrease in CF was observed at a dose of 1µg while a highly significant (P<0.01) decrease was exhibited at all other doses (Table 2).

Effects of diltiazem on force of contraction, heart rate of isolated rabbit heart

Diltiazen showed a dose dependent decrease in force of

Table 3: The effect of different doses of diltiazem on various cardiac parameters in isolated perfused rabbit heart

Doses	Force of contraction(g)		Heart rate(beats/min)	
	Mean	% Change from control	Mean	% Change from control
Control	2.33±0.007A	0.00	208.5±0.76A	0.00
1ng	2.17±0.026B	-7.08	195.00±1.06B	-6.47
10ng	1.61±0.027C	-30.88	182.00±0.97C	-12.71
100ng	1.00±0.007D	-56.97	166.00±1.26D	-20.38
1mg	0.58±0.02E	-75.27	154.67±1.41E	-25.82

Results are expressed as means ± S.E.M (n=6). Mean sharing similar letters are statistically non-significant (P > 0.05).

Analysis of variance

Cardiac Parameters	Source of variation	Degrees of freedom	Sum of squares	Mean squares	F-value
Heart Rate	Dose	4	11228.53	2807.13	375.62**
	Error	25	186.83	7.47	
	Total	29	11415.37		
Force of contraction	Dose	4	13.443	3.360	1313.53**
	Error	25	0.063	0.002	
	Total	29	13.507		

**= Highly significant (P < 0.01).

contraction and heart rate in isolated perfused heart. The maximum decrease in force of contraction and heart rate was observed at 100µg with percentage decrease of 75% and 25% respectively (Table 3).

DISCUSSION

Traditionally used medicinal plants have always remained a major tool for drug development. The medicinal values of traditional medicinal plants cannot be ignored and studies have been carried out in order to investigate various active principles of the extracts with intensive follow up studies to establish their exact mechanism of action. One of the most important area in which compounds from plant sources have contributed successfully, is the cardiovascular research. *Saussurea lappa*, Linn. a plant indigenous to Pakistan has been extensively used for the treatment of various ailments. This study was conducted to evaluate the cardiotonic activity of methanolic extract of S. L in isolated perfused rabbit heart as it has been reported to contain flavonoids i.e., flavone glycosides and terpenes i.e., sesquiterpene lactones as a constituent (Chang *et al*., 2003).

The data obtained have clearly indicated that methanolic extract of *Saussurea lappa* roots exerted a dose dependent negative chronotropic effect. The extract showed a highly significant (P<0.01) positive inotropic effect at first three doses and a negative inotropic effect on following two doses. There was also a dose dependent decrease in coronary flow of the isolated perfused heart.

The observed dose dependent positive inotropic effect of the plant might be due to the presence of flavonoids in the root extract as previously reported (Chang *et al*., 2003). However, the positive inotropic effect at low doses and negative chronotropic effect at high doses may be due to the presence of sesquiterpene lactones in the extract (Picman *et al*., 1981).

In order to assess the possible mechanism of action the methanolic extract of S. L, the effects were compared with digoxin and diltiazem. The positive inotropic effects of extract at low doses appear to be comparable with digoxin (Farid *et al*., 1992). At a dose of 10µg and 100µg, the test extract produced a gradual decline in force of contraction. This decrease in force of contraction at higher doses may be due to the presence of calcium antagonist constituents in root extract (Gillani *et al*., 1999). Moreover, the methanolic extract produced highly significant (P<0.01) dose dependent negative chronotropic effect similar to that of diltiazem. The middle dose of methanolic extract, i.e. 5.0µg showed 26.69% decrease in heart rate which was almost equal to the effect of last dose of diltiazem (100mg). This pronounced negative chronotropic effect of methanolic extract of S. L might be due to the involvement of cholinergic and calcium antagonist compounds present in root extract (Gilani *et al*., 1999). Similarly, a decrease in coronary flow by the extract might also be due to certain calcium antagonist components of plant extract (Dagenais *et al*., 1997).

It is concluded therefore, that the cardiotonic effects of methanolic extract of *Saussurea lappa*, Linn. roots might be due to the presence of flavonoids, sesquiterpene lactones, calcium channel blocker and cholinergic constituents. However further studies are required to isolate these biologically active compounds in order to determine its exact mechanism of action.

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