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COMPARATIVE CARDIOTONIC ACTIVITY OF *MORINGA* LEAVES BY USING FROG'S ISOLATED HEART

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Abstract:

The present study was undertaken to evaluate the cardio tonic activity of the alcoholic extract of leaves Moringa oleifera. The leaves are believed to contain some antioxidants and hence posedto be used in the prevention of cardiovascular diseases. The cardiotonic effect of alcoholic extract of leaves of Moringa oleifera was studied by using isolated frog heart perfusion technique (IFHP). Ringer solution without calcium was used as a vehicle for administration of alcoholic extract as test and digoxin as standard. A significant increase in the height of force of contraction (positive ionotropic effect) and decrease in heart rate (negative chronotropic effect) was observed at smaller doses (0.1mg). The effect increased as dose was increased. The test extract had not produced cardiac arrest even at a dose of 2 mg, a higher concentration as compared to standard, digoxin that showed cardiac arrest at dose of 0.2 mg. Hence, as compared to standard, test drug showed wide therapeutic index.

Keywords: *Digoxin, isolated frog heart perfusion technique, therapeutic index, Moringa oleifera.*

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1.INTRODUCTION:

A natural product is a chemical compound or substance produced by a living organism found in nature that usually has a pharmacological or biological activity for use in pharmaceutical drug discovery and drug design. A natural product can be considered as such even if it can be prepared by total synthesis. These small molecules provide the source of inspiration for the majority of FDA-approved agents and continue to be one of the major sources of inspiration for drug discovery. In particular, these compounds are important in the treatment of life-threatening diseases. Natural products may be extracted from tissues of terrestrial plants, micro organisms or micro organism fermentation broths. A crude (untreated) extract from any one of these sources typically contains novel, structurally diverse chemical compounds, which the natural environment is a rich source of. Chemical diversity in nature is based on biological and geographical diversity, so researchers travel around the world obtaining samples to analyze and evaluate in drug discovery screens or bioassays. This effort to search for natural products is known as bioprospecting. Plants have always been a rich source of lead compounds. Many of these lead compounds are useful drugs in themselves and others have been the basis for synthetic drugs. Clinically useful drugs which have been recently isolated from plants include the anticancer agent paclitaxel (Taxol) from the yew tree, and the anti malarial agent artemisinin from Artemisia annua.[1]Rutaceae, commonly known as the rue or citrus family, is a family of flowering plants, usually placed in the order Sapindales.Species of the family generally have flowers that divide into four or five parts, usually with strong scents. They range in form and size from herbs to shrubs and small trees. About 346 various plants belonging to family Rutaceae have been identified & widely used as medicinal agents.[1-3]

2.MATERIALS AND METHODS:

Materials

Standard Drug: Digoxin [SAMARTH LIFE SCIENCES PVT.LTD (0.25mg)]

Test Drug: Aqueous extract of leaf of *Moringa oleifera.*

Physiological solutions: Ringer solution, Hypodynamic ringer solution.

Animal*: RanaTigrina* (frog)

Instruments: Sherrington Rotating Drum, Starling heart lever.

Preparation of extract:

The leaves of *Moringa oleifera* was collected from local area. The leaves was washed thoroughly to remove adhered material and dried, fine powder was made by using hand grinder. 1gm of the powder was dissolved in 100ml of alcohol with the help of magnetic stirrer for half an hour, maceration for 12 hours. The material was filtered thtough Whatmann filter paper and filtrate was collected. The prepared infusion was diluted with the help of distilled water in varying proportions and labeled as follows:

MK1- Undiluted filtrate
MK2- 1:1 (filtrate: distilled water)
MK3- 1:2 (filtrate: distilled water)
MK4- 1:4 (filtrate: distilled water)

All the preparations were evaluated for their cardio tonic activity by using isolated frog heart assembly. The rate and force of heart contraction was determined. [4-8]

Evaluation of Cardiotonic activity:

The frog of species Ranatigrina was pithed and pinned it to the frog board. A midline incision was given on the abdomen, the pectoral girdle was removed and the heart was exposed. The pericardium was carefully removed and put a few drops of Hypodynamic frog ringer over the heart. The inferior venacava was traced, put a thread around it and given a small cut in order to insert the venous cannula. The cannula was inserted in the vein and the thread was tied to assure the cannula in place which is in turn connected to a saline bottle containing Hypodynamic frog ringer solution. A small cut in one of the aorta was given for the ringer to come out. Heart was isolated and attached to the stand with moderate flow of ringer. A thin pin hook was passed through the tip of the ventricle and with the help of a fine thread attached to the hook; it was tied to the free limb of the starling heart lever which was fixed to a stand. A proper tension was adjusted by altering the height of the lever. The normal heart rate was noted. All test samples that is MK1, MK2, MK3, MK4, S1 and S2 were administered in different doses i.e. 0.1, 0.2, 0.3 ml respectively. The rate and force of heart contraction were noted. [9-10]

3.RESULTS:

Table 1: Effect of 5µg/ml, 10µg/ml and 15µg/ml of Digoxin S1 (25µg/ml) on heart rate and change in force of contraction of Hypodynamic heart.

S.No.	Drug	Dose in ml	Beats/min	Change in Force
1	-----	Normal	30	Normal
2	S1	0.1	25	Increase
3	S1	0.2	24	Slight Increase
4	S1	0.3	26	Slight Increase

Table 2:Effect of 5µg/ml, 10µg/ml and 15µg/ml of Digoxin S2 (50µg/ml) on heart rate and change in force of contraction of Hypodynamic heart

S.No.	Drug	Dose(in ml)	Beats/min	Change in Force
1	-----	Normal	32	Normal
2	S2	0.1	28	Increase
3	S2	0.2	25	Slight Increase
4	S2	0.3	22	Sudden Cardiac Block

Table 3: Effect of 5µg/ml, 10µg/ml and 15µg/ml of MK1- Undiluted filtrate on heart rate and change in force of contraction of Hypodynamic heart

S.No.	Drug	Dose(in ml)	Beats/min	Change in Force
1	-----	Normal	37	Normal
2	MK1	0.1	33	Rapid Increase
3	MK1	0.2	29	Increase
4	MK1	0.3	27	Increase

Table 4: Effect of 5µg/ml, 10µg/ml and 15µg/ml of MK2- 1:1 (filtrate: distilled water) on heart rate and change in force of contraction of Hypodynamic heart

S.No.	Drug	Dose(in ml)	Beats/min	Change in Force
1	-----	Normal	37	Normal
2	MK2	0.1	37	Slight Increase
3	MK2	0.2	31	Slight Increase
	MK2	0.3	32	Increase

Table 5: Effect of 5µg/ml, 10µg/ml and 15µg/ml of MK3- 1:2 (filtrate: distilled water) on heart rate and change in force of contraction of Hypodynamic heart

S.No.	Drug	Dose(in ml)	Beats/min	Change in Force
1	-----	Normal	35	Normal
2	MK3	0.1	31	Rapid Increase
3	MK3	0.2	29	Increase
4	MK3	0.3	29	Slight Increase

Table 6: Effect of 5µg/ml, 10µg/ml and 15µg/ml of MK4- 1:4 (filtrate: distilled water) on heart rate and change in force of contraction of Hypodynamic heart

S.No.	Drug	Dose(in ml)	Beats/min	Change in Force
1	-----	Normal	33	Normal
2	MK4	0.1	34	Slight Increase
3	MK4	0.2	29	Slight Increase
4	MK4	0.3	28	No change

4.DISCUSSION:

Kymograph obtained indicates that even lower doses of test extract give a significant increase in height of contraction. The dose at which digoxin showed cardiac arrest was 0.2mg and test extract showed a therapeutic effect in the 0.25-2mg without any cardiac arrest. Hence, as compared to digoxin, test extract showed wide therapeutic index. We all know the adverse effects shown by digoxin and difficulty in its dose adjustments. Also, in the market, there is still no safer alternative for digoxin and it is considered as a sole drug for the treatment of congestive cardiac failure. From the above shown observations, the limitation of using digoxin can be overcome by using the alcoholic leaf extract of *Moringa oleifera* which has been found to have excellent cardio tonic activity with the wide therapeutic index as compared to digoxin. Hence, test extract can be a safe alternative to digoxin in congestive cardiac failure. Free radicals play a main role in the prognosis of cardiovascular diseases, e.g. Free radicals cause endothelial dysfunction and activation of macrophages leading to atherosclerosis. *Moringa oleifera* leaf alcoholic extract was reported to have free radical scavenging activity and hence, the plant poses itself as a substance for the prevention of cardiovascular diseases.

5.CONCLUSION:

The incremental dosage of isolated cardiac glycoside from the alcoholic leaf extract of *Moringa oleifera* produced positive inotropic and negative chronotropic effects. The dose at which digoxin showed cardiac arrest was 0.2mg and test extract showed a therapeutic effect in the range of 0.25-2mg without any cardiac arrest. Hence as compared to digoxin, test extract showed its promising effect on the blocked and failed heart without ringer washings. Further, study is needed to characterize the isolated cardiac glycoside in accordance.

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