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EVALUATION OF CARDIOTONIC ACTIVITY OF *MORINGA OLEIFERA* ROOTS

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ABSTRACT

Moringa oleifera roots possess cardiotonic activity as per Ayurvedic books and literature. Present study was carried out to find better and safer herbal cardiotonic alternate of digoxin by evaluating cardiotonic activity of *Moringa oleifera* roots. The present study was carried out to evaluate positive inotropic effects of various extracts of roots of *Moringa oleifera.* Methanolic extract of authentified sample of *Moringa oleifera* roots was subjected to various experimental models as follow: Isolated rat heart preparation using normal and hypodynamic Ringer Locke solution on Langendorff's assembly, to evaluate cardiotonic activity as well as to find underlying mechanism of action. Biochemical study of 15 days for evaluation of cardiac activity of methanolic extract of *Moringa oleifera* roots on cardiac enzymes and cytosolic Ca^{2+} level was also performed. Methanolic extract of *Moringa oleifera* showed significant *($p<0.001$)* increased in force of contraction and cardiac output in normal and hypodynamic heart preparations. These activities were inhibited by propranolol which indicates its sympathomimetic activity. It significantly increased cytoplasmic Ca^{2+} level after 15 day treatment but didn't alter cardiac enzymes level i.e. didn't alter normal physiology of heart. Present research work emphasizes cardiotonic potential and therapeutic usefulness of roots of *Moringa oleifera* as better alternate of digoxin in management of congestive heart failure.

Key words: *Moringa oleifera* roots, Langendorff's assembly, Hypodynamic heart, Cardiotonic, Congestive heart failure.

INTRODUCTION

Cardiotonics are the agents which tend to increase the efficiency of contraction of heart muscle i.e. increase the tone of cardiac muscles (Rang HP *et al.*, 2007). Cardiotonics are useful therapeutically in congestive heart failure (Goyal RK *et al*., 2000), mitral regurgitation (Uehara Y *et al.*, 1998), persistent pulmonary hypertension in newborn (Abman SH, 2007), cardiac diseases (Thormann J *et al.*, 1983), etc. Cardiac glycosides and catecholamines have been used as main therapeutic agents in the treatment of congestive cardiac failure. However, the danger of cardiac glycosides intoxication is well documented and doubts have been expressed about their effectiveness (Bairi R *et al*., 2011).

Looking at the dire need of a new safe and economical cardiotonic molecule, we resolved to investigate cardiotonic activity of *Moringa oleifera* roots. It is commonly known as "*Sargavo*" belongs to family Moringaceae. According to Nandakarni AK *et al*. and other review articles on *Moringa oleifera* roots suggested that roots of *Moringa oleifera* can increase the sympathetic tone which may potentiate β adrenergic activity on heart muscles and increase the force of contraction, i.e. exerts positive inotropic effect and thus it may prove a good candidate as cardiotonic (Nadkarni KM, 1954).

Synonyms of *Moringa oleifera* are drumstick, sigru, horseradish tree, subhanjana, etc (Mishra G *et al.*, 2011). It is distributed throughout foothills of Himalayas, Pakistan, west Bengal, Bangladesh and widely cultivated in Gujarat (Gupta RK, 2010).

Morphologically roots were swollen, tuberous, white taproot which has a characteristic pungent odor, and very sparse lateral roots. Trees grown from seeds

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developed a deep, stout taproot with a wide-spreading system of thick, tuberous lateral roots.

Roots of *Moringa oleifera* contains pterygospermin – a potent fungicidal and antibiotic agent (Nadkarni KM, 1954), Moringine and Moringinine; Spritochin, Phytosterols, Glucotropaeolin, Benzyl-isothiocyanate (Fahey JW, 2005). Roots also contain 4-(alpha-l-rhamnopyranosyloxy)-benzylglucosinolate and benzyl glucosinolate (Francis JK *et al*., 1991), aurantiamide acetate 4 and 1, 3-dibenzyl urea (Lahjie AM *et al*., 1987), alpha-phellandrene, p-cymene (Bennett RN *et al.*, 2003), Deoxy-Niazimicine (Sashidhara KV *et al*., 2009).

Pharmacologically proved actions of roots of *Moringa oleifera* were antimicrobial (Anderson DM *et al.*, 1986) and anticancer (Nadkarni KM, 1954), anti-inflammatory (Medhi B *et al.*, 1996), diuretic activity (Cáceres A *et al.*, 1991), anti-fertility activity (Shukla S *et al.*, 1988), anti-asthmatic activity (Mehta A *et al*., 2008), etc.

MATERIALS AND METHODS

Collection and authentification of roots of *Moringa oleifera*

The Roots of *Moringa oleifera* were collected from the medicinal garden of R. K. College of Pharmacy, Rajkot. The roots of *Moringa oleifera* were dug out and then cut to small pieces. These roots of *Moringa oleifera* were authentified by Department of Botany, Christ College, Rajkot.

Preparation of methanolic extract of roots of *Moringa oleifera*

The collected roots of *Moringa oleifera* were subjected to dry to brittle material at 60°C in hot air oven to remove moisture. These dried roots were subjected for size reduction using mixer grinder and comminuted to very fine powder. Methanolic extract of *Moringa oleifera* roots was prepared by extracting fine powder using Soxhlet apparatus. Powder was extracted for 4 – 5 siphons of Soxhlet apparatus and at the end of extraction methanol was evaporated to dryness which yielded a semi-solid product, which was further subjected for evaluation of % W/W yield, colour, consistency and pharmacological activities.

Biological evaluation of cardiotonic activity of methanolic extract of *Moringa oleifera* roots by *in vitro* studies

Methanolic extract of *Moringa oleifera* roots was subjected for evaluation of cardiotonic activity in various *in vitro* trials.

Selection of animals

Either sex Wistar albino rats of weighing 220-280 g were used for the study. The animals were procured from animal house, Department of Pharmacology, R. K. College of Pharmacy, Rajkot, India. Animals were housed at a temperature of 24±20˚C and relative humidity of 30 – 70 %. A light and dark cycle was followed. All animals were fed on standard balance diet and provided with water *ad libitum.* All the experimental procedures and protocols used in study were reviewed and approved by the Institutional Animal Ethical Committee (IAEC) of R. K. College of Pharmacy and care of laboratory animals was taken as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

In vitro cardiotonic study of *Moringa oleifera* roots using Langendorff's assembly

Preparations of isolated rat heart – Langendorff's assembly

Wistar rat of either sex, pretreated with heparin (300 IU, i.p.), were anesthetized by diazepam (5 mg/kg, i.m.) followed by ketamine (75 mg/kg, i.p.). Thorax cavity was opened to isolate heart. After isolating, heart was washed in ice cold Ringer Locke solution and immediately mounted on Langendorff's assembly with help of bulldog clamp and perfused with normal Ringer Locke solution.

Recording of cardiac response

The apex of heart was directly attached with isotonic transducer of student's physiograph. Base line cardiac contraction was recorded for few seconds. After that digoxin (50 µg/ml – 0.1 ml) and then methanolic extract of *Moringa oleifera* roots (MEMO – 60 mg/ml – 0.1 ml) were administered. Then the heart was allowed to achieve its normal condition. After that Ringer Locke solution was replaced by Hypodynamic Ringer Locke solution. Same procedure was followed as mentioned in normal conditions. The rat heart was washed with the Ringer Locke solution after every administration of extract and drug till it was brought back to the normal state. Force of contraction and cardiac output were measured after administration of drug/extract.

Recording of cardiac response in presence of various antagonists

Hypodynamic Ringer Locke solution was again replaced with normal RL solution. Then heart was perfused with propranolol (3×10^{-5}M), a β – adrenoceptor inhibitor, for 60 seconds followed by administration of MEMO and the force of contraction was recorded. Then Nifedipine (2.88×10^{-5}M), an L-calcium channel (Lcc) blocker, was perfused for 60 seconds and followed by administration of MEMO and recordings were noted.

Biochemical studies to evaluate cardiac activities of roots of *Moringa oleifera*:

Wistar albino rats were divided into 3 groups of 6 animals each.

Group I: distilled water p.o (control)

Group II: treated with digoxin (100 µg/kg, p.o.) (Standard control)

Group III: treated with methanolic extract of *Moringa oleifera* roots (500 mg/kg, p.o.)

Animals were treated with their respective treatment for 15 days. At the end of treatment, blood samples were collected which was subjected for serum separation. Serum samples were subjected for estimation of bio-chemical parameters CPK-MB, LDH and SGOT. After that rats were killed by appropriate method of euthanasia and heart was isolated and subjected for homogenization which was used for estimation of cytosolic calcium levels. The cytosolic Ca2+ ions level were measured in clear supernant obtained after centrifugation of heart homogenate, using Erba Mannheim calcium Kit.

Statistical analysis: The data were expressed as Mean ± SEM. Statistical analysis was performed Tuckey's test in INSTAT software; $p<0.05$ was considered as significant and $p<0.001$ was considered as highly significant.

RESULTS

Pharmacognostic evaluation of methanolic extract of *Moringa oleifera* roots (MEMO) showed red-brown coloured, semisolid, 5.3% W/W yield.

Digoxin and MEMO showed significant increase in force of contraction and cardiac output ($p<0.001$) compared to control (Table 2, figure 1). The same results were obtained in hypodynamic heart preparation (Table 3, figure 2). The cardiotonic activity of MEMO was antagonized by propranolol, β – adrenoceptor blocker, while nifedipine didn't antagonize those responses (Table 4, figure 3).

Both digoxin and MEMO didn't alter the cardiac enzymes viz., CPK-MB, LDH and SGOT level significantly while both increased the cytosolic Ca^{2+} level significantly ($p<0.001$) when compared with control group (Table 5).

Table 1. Pharmacognostic evaluation of various extracts of roots of *Moringa oleifera*

Type of Extract	% W/W yield	Colour	Consistency
Methanolic	5.3 % W/W	Red-brown	Semisolid

Table 2. Effects of digoxin and MEMO on isolated rat heart – Langendorff's assembly – *in vitro* study (Normal heart)

Group	Force of contraction (mm)	% Force of contraction	Cardiac output (ml)	% Cardiac output
Control	25 ± 0.577	100 ± 0.0	7 ± 0.258	100 ± 0.0
Digoxin	45 ± 0.516***	180.27 ± 2.577***	9.33 ± 0.21***	133.92 ± 4.098***
MEMO	47.16 ± 0.477***	189.27 ± 5.42***	9.16 ± 0.307***	132.53 ± 8.901***
F	*541.24*	*200.72*	*24.729*	*11.51*
df	*17(2,15)*	*17(2,15)*	*17(2,15)*	*17(2,15)*
P	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*

Table 3. Effects of digoxin and MEMO on isolated rat heart – Langendorff's assembly – *in vitro* study (Hypodynamic rat heart)

Group	Force of contraction (mm)	% Force of contraction	Cardiac Output (ml)	% Cardiac Output
Control	19 ± 0.258	100 ± 0	6.33 ± 0.210	100 ± 0
Digoxin	32 ± 0.447 ***	168.43 ± 1.18 ***	8.66 ± 0.210 ***	137.3 ± 4.108 ***
MEMO	37.33 ± 0.42 ***	196.62 ± 2.82 ***	8.33 ± 0.210 ***	132.14 ± 4.671 ***
F	*600.25*	*787.55*	*35.83*	*31.66*
df	*17(2,15)*	*17(2,15)*	*17(2,15)*	*17(2,15)*
p	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*

n=6 and results were shown as mean ± SEM

*** indicate significant difference in the data compared to control group and the level of significance was $p<0.001 \approx$ highly significant.

MEMO – Methanolic Extract of *Moringa oleifera* roots

Table 4 (a). Effects of MEMO on force of contraction of isolated rat heart – Langendorff's assembly – *in vitro* study (in presence of antagonist)

Group	In presence of propranolol		In presence of nifedipine	
	Force of contraction (mm)	% Force of contraction	Force of contraction (mm)	% Force of contraction
Control	3.33 ± 0.21	100 ± 0	3.33 ± 0.21	100 ± 0
MEMO	4.67± 0.22***	131.9 ± 0.21***	5.50 ± 0.21***	168.1 ± 0.21***
F	*11.25*	*13.42*	*49.71*	*32.89*
df	*11 (1, 10)*	*11 (1, 10)*	*11 (1, 10)*	*11 (1, 10)*
p	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*

Table 4 (b). Effects of MEMO on cardiac output of isolated rat heart –Langendorff's assembly – *in vitro* study (in presence of antagonist)

Group	In presence of propranolol		In presence of nifedipine	
	Cardiac output (ml)	% Cardiac output	Cardiac output (ml)	% Cardiac output
Control	6.66 ± 0.21	100 ± 0	6.66 ± 0.21	100 ± 0
MEMO	8.33 ± 0.21***	126.19 ± 6.56*	8.66 ± 0.21***	130.95 ± 7.5***
F	*31.25*	*12.1*	*45*	*22.236*
df	*11 (1, 10)*	*11 (1, 10)*	*11 (1, 10)*	*11 (1, 10)*
p	*< 0.001*	*< 0.05 (0.00593)*	*< 0.001*	*< 0.001*

Table 5. Effects of digoxin and MEMO on membrane bound enzymes and cytosolic Ca^{2+} in heart of rats

Groups	CPK – MB (U/L)	LDH (IU/L)	SGOT (IU/L)	Cytosolic Ca^{2+}(mg/dl)
Control	274.3 ± 1.4	225.733 ± 0.33	27.37 ± 0.357	2.443 ± 0.0217
Digoxin	277.7 ± 0.923	224.23 ± 0.60	26.97 ± 0.281	4.823 ± 0.035***
MEMO	276.97 ± 0.686	224.33 ± 0.30	27.00 ± 0.818	4.235 ± 0.025***
F	2.724579	3.55	0.168653	1992.447
df	17 (2, 15)	17 (2, 15)	17 (2, 15)	17 (2, 15)
p	>0.05	>0.05	>0.05 (0.8463)	<0.001

n=6 and results were shown as mean ± SEM
* indicate significant difference in the data compared to control group and the level of significance was $p<0.05$ ≈ significant.
*** indicate significant difference in the data compared to control group and the level of significance was $p<0.001$ ≈ highly significant.
MEMO – Methanolic Extract of *Moringa oleifera* roots

Figure 1. Effects of digoxin and MEMO on isolated rat heart - Langendorff's assembly in normal condition -an *in-vitro* study

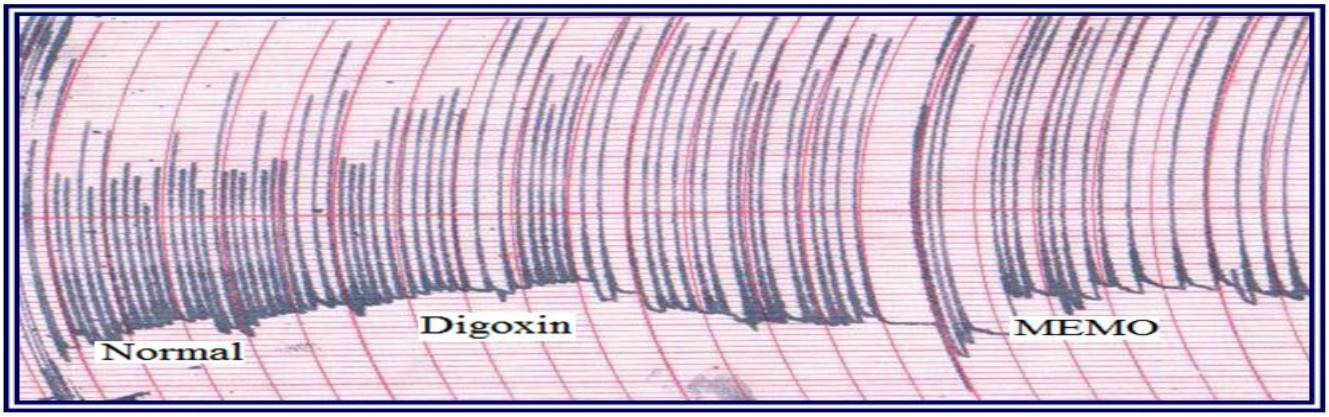


Figure 2. Effects of digoxin and MEMO on isolated rat heart - Langendorff's assembly in hypodynamic condition -an *in-vitro* study

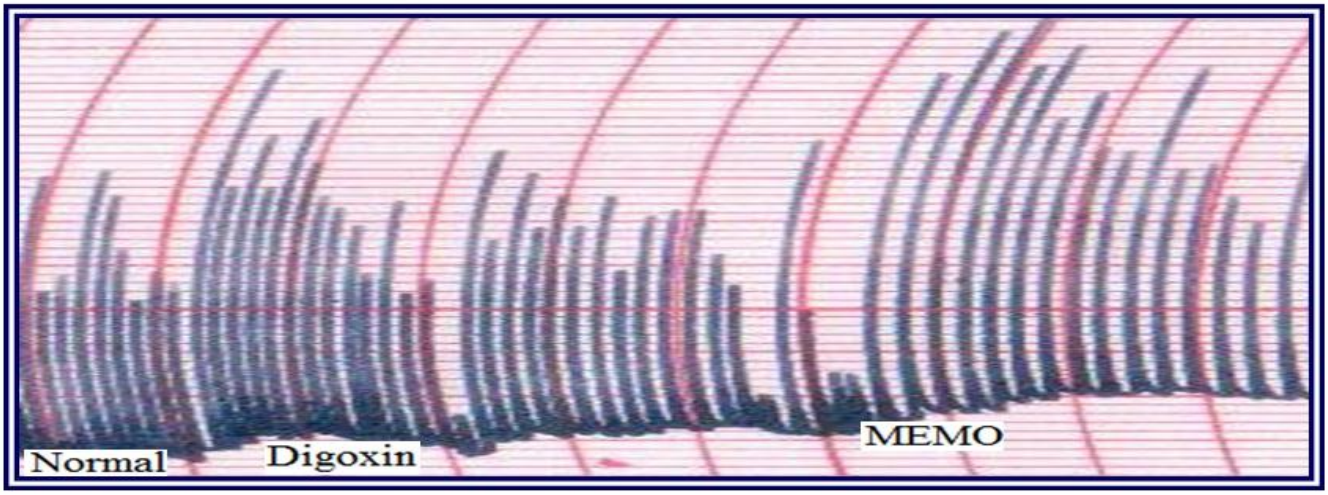
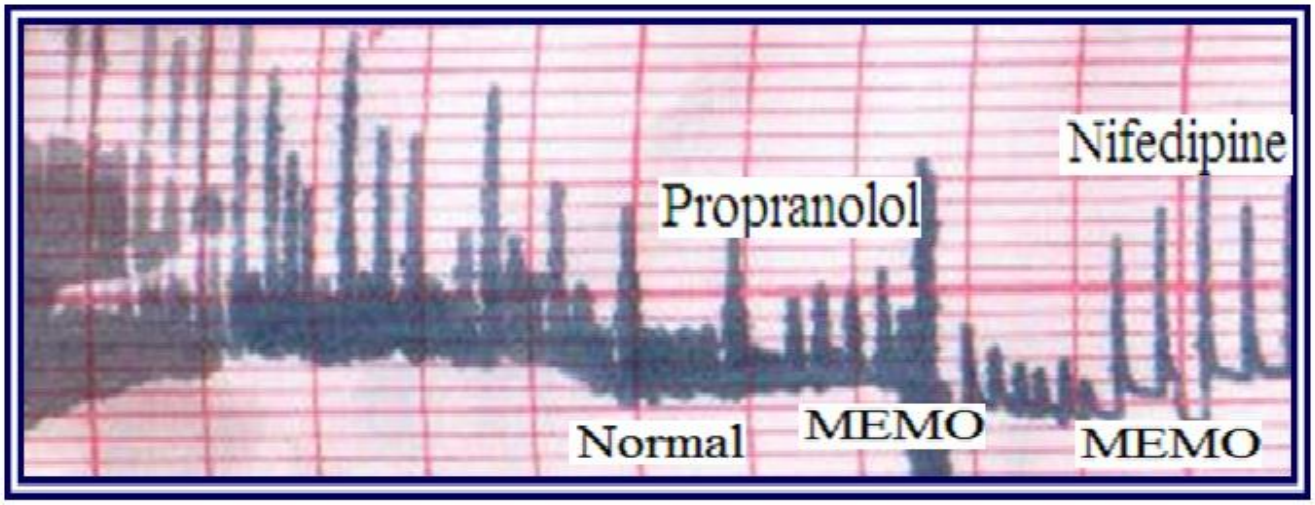


Figure 3. Effects of MEMO on isolated rat heart - Langendorff's assembly in presence of propranolol and nifedipine - an *in-vitro* study



DISCUSSION

Cardiac glycosides and catecholamines have been used as the main therapeutic drugs in the treatment of congestive cardiac failure. However, the dangers of cardiac glycoside intoxication are well documented and doubts have been expressed about their long term effectiveness (Bairi R *et al*., 2011).

In isolated rat heart preparation on Langendorff's assembly, methanolic extract of *Moringa oleifera* (MEMO) roots showed significant increase in force of contraction when compared to normal response. MEMO also increased cardiac output significantly when compared with control group. This indicates that MEMO possesses positive inotropic effect which can reduce the preload in heart.

Hypodynamic isolated rat heart preparation was used to evaluate cardiotonic activity of MEMO in heart failure like condition. In hypodynamic heart preparation, the heart was perfused with hypodynamic Ringer Locke solution, which has comparatively less concentration of Calcium ions. Due to less Ca^{2+} ions, the heart losses its cardiac activity i.e. decreased force of contraction and reduced rapidity of contractions. This condition seems very much similar to congestive heart failure (Pattigadapa HS, 2011; Dama GY, 2011; Kulkarni SK, 2009). This leads to decreased force of contraction and reduced cardiac output. MEMO showed significant increase in force of contraction as well as significantly increased cardiac output compared to normal response in hypodynamic heart. This finding suggests that roots of *Moringa oleifera* are effective in heart failure like condition *in vitro*.

To find out the underlying mechanism of action for cardiotonic activity of roots of *Moringa oleifera*, the

normal heart on Langendorff's was perfused with propranolol and nifedipine in other trails.

Propranolol is a β-adrenoceptors inhibitor which can inhibit the action of drug acting on β-adrenoceptors. Adrenaline like drug act on β-adrenoceptors and drugs acting similar to adrenaline (sympathomimetics) can be antagonized by propranolol, a mixed β-adrenoceptor inhibitor. When the effect of MEMO was observed in presence of propranolol on normal heart preparation, it showed very slight increase in force of contraction. This increase in force of contraction was lesser than that of in absence of propranolol. This finding suggests that MEMO's action was antagonized by propranolol, which suggests that MEMO may act by stimulations of sympathetic system and act in similar manner like sympathomimetics (Bairi R *et al*., 2011; Murlidharan A *et al.*, 2004).

Nifedipine, an voltage gated L-type calcium channel (Lcc) blocker, can antagonize digoxin like drug which increase force of contraction by acting on Lcc (Bairi R *et al*., 2011; Murlidharan A *et al.*, 2004). MEMO showed significant increase in force of contraction and cardiac output, very much similar to those of in absence of antagonist. It suggested that there was no antagonism between nifedipine and MEMO, thus MEMO didn't act through Lcc like digoxin.

In pathological conditions like myocardial infarction, myopathies, etc, the levels of CPK, LDH and SGOT rise significantly due to leaking out from the necrotic heart cells. CK-MB appears to be a sensitive measure of myocardial infarction. LDH has gained much clinical interest recently and measurement of its activity in blood is considered useful in the diagnosis certain cardiovascular disease conditions. Level of AST (SGOT) rises markedly in conditions of extensive damage to muscle especially cardiac muscles. Estimation of this enzyme is widely sought for, to confirm diagnosis of myocardial infarction (Bairi R *et al*., 2011). Levels of CPK-MB, LDH and SGOT in test group were very much similar to those of control group which indicates that MEMO didn't alter normal physiological condition of the heart.

It was observed that treatment with cardiotonic for longer time increases cytosolic calcium ion level (Chen Q *et al.*, 2003). MEMO can significantly increase cytosolic Ca^{2+} level similar to digoxin, which indicates that MEMO can increase the force of contraction by increasing cytosolic Ca^{2+} level.

CONCLUSION

The present study revealed positive inotropic effect of *Moringa oleifera* roots with no cardiac damage or other cardiac toxicity. Thus *Moringa oleifera* roots had been proved a potential cardiotonic herb and could be choice of herb for the better and safer alternate of digitalis (digoxin).

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