

Original Research Article

Effect of Aqueous Extract of Aegle Marmelos (Bael Leaves) on Perfused Toads Heart

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ABSTRACT

Introduction: Aegle marmelos/Bael leaves commonly used in day to day life of people as Prasad in LORD SHIVA TEMPLE as medicaments in household remedies. Few research has been done eliciting antifungal, antibacterial, antiprotozoal, hypoglycemic properties where as its cardiac effect has not been studied sufficiently till now. So this study is undertaken.

Objective: To evaluate effect of Aqueous extract of leaves on perfused toads heart and to elucidate the possible mechanism of action.

Material and Method: Aqueous extract of bael leaves was prepared and its effects on the perfused heart studied. GDR was studied and compared with known drugs on toads heart, on a smoked drum of kymograph and analysed.

Result: BLE extract in lower doses increases both HR and amplitude of contraction and in higher doses transiently inhibit the heart, followed by further stimulation

Conclusion: Aqueous extract of Aegle marmelos (bael leaves) has a unique stimulatory effect on Toads heart.

Key words: BLE-bael leaf extract, GDR-graded dose response, Kymograph, HR-heart rate.

INTRODUCTION

Indigenous system of medicine-medicine from HERBS and PLANTS taught and used as homemade remedies to disease treatment at VAIDYAS. Recent modern millennium is a fast moving and though the potency of allopathic medicine is undoubtful, as they provide fast result, but the ill-lighted side is their several adverse effects and contraindications. On the other hand the plants are good counterfeit for those medicines because of their less or no adverse events and their ability to alleviate the cause from their root. Herbs and plants are used as main source of ALLOPATHIC medicine even to-day, bael leaves used very commonly in day to day practice as PRASAD in LORD

SIVA TEMPLE, as household remedies in indigestion, acidity. Hypoglycemic effect of Aegle marmelos aqueous leaf extract streptozotocin induced diabetic rats was seen from previous studies. [1-6]

Antihyperlipidemic activity was even evident from few previous studies too. [2]

Successful use of leaf extract showing significant reduction of blood cholesterol levels along with slight lowering of blood glucose in few patients with diabetes mellitus. [3] Aqueous extract of *Aegle marmelos* leaves, was studied to have hypoglycemic and antioxidant effect in alloxan induced diabetes in male albino rats and was proposed to be useful in the long-term management of diabetes. [4-6] Its antifungal activity, [7] antibacterial activity,

[8,9] antiviral activity, [10,11] anti malarial activity, [12] anti-inflammatory (with analgesic and antipyretic), [13] anticancer activity, [14] antiulcer activity [15] and hepatoprotective activity [16] was also clearly evident from different studies. Anti obesity and kid to an infertile woman, abdominal problems, cirrhosis patients are known and heard true legends, by doing full fasting Monday and taking a bael leaf only. Role of fasting or bael leaf or Grace of GOD should be evaluated. C.G. Hema and K. Lalitakumari- 1980-multifactorial benefit and did CVS study using both aqueous and ethanolic extracts. Aqueous extracts showed stimulatory to CVS, smooth muscle relaxant and uterine stimulant where as Alcoholic extracts proved to be cardiac depressant and uterine relaxant. [17]

Aims and Objectives:

- To evaluate the effect of Aqueous extract of fresh bael leaves on perfused toads heart
- To search for active chemical entity responsible for action
- To compare its action with known stimulants and depressants.

MATERIALS AND METHODS

Animals: Six common Indian toads/wt.-100-150 gms was recruited for this study. Approval has been taken from the Institutional Ethics Committee, the Institutional Animal Ethics Committee and all procedures were conducted according to the revised guidelines of CPCSEA Act, 1960 India.

Collection and Identification of Plant: Fresh leaves of Bael plant collected provincially from single source during the month of august were used for the study. Recognition and certification of the leaves of *Aegle marmelos* was done by the senior lecturer and head of department of Botany, Prof. Mrs Mdhusmita Panda at the Royal college of science & technology, Utkal university, Bhubaneswar, Odisha, India. A specimen sample was stored in our

department of pharmacology for future reference.

Plant Material Extraction: The leaves were washed, kept under shade and air dried. Then grounded to paste in pestle and mortar filtered in what man filter paper no 40, fresh juice collected (method of rosenthaller-1930). The prepared juice was diluted with the help of distilled water in different proportion and was labeled in *graded way* as

X1=0.5mg/ml BLE

X2=1mg/ml BLE

X3=1.5mg/ml BLE

X4=1mg/ml BLE+Ach

DRUGS AND DOSES

Perfusion fluid-frog ringer solution and ¼ cacl2 frog ringer soln.

DRUGS-Ach, kcl, Adr, Cacl2, Atropine

Used as follows--

0.1mg(1 drop), 0.2mg(2 drops), 0.4mg(4 drops), 0.8 mg(8drops) respectively (1mg=1ml=10⁻² solution).

INSRUMENTS USED

Student Kymograph (Sherington Rotating Drum, Sterling.s heart Lever) with intact power supply.

Method

ED OF Ach found out, ED OF BLE found out, Drug found to be stimulatory, Antagonism test done, Stimulatory effect of BLE compared with cacl2, Adr.GDR of BLE in ¼ cacl2 and GDR of Digoxin on Hypodynamic Heart.

EVALUATION OF CARDIOTONIC ACTIVITY

The toad 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 Sterling's 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 X1,X2,X3,X4 were administered in different grades doses viz. 0.1mg(1 drop),

0.2mg(2 drops), 0.4mg(4 drops), 0.8 mg(8drops) respectively(1mg=1ml=10⁻² solution). The rate and force of heart contraction were noted as given below in table1.

OBSERVATION

BLE IS STIMULANT, may be unique, may be like Adr, Cacl2, Atropine, Digoxin. But more likely mimicing action like adrenalin(less efficient than adrenalin) ,but mimicing activity like cacl2 which increases the tone and contractility in same manner,as evidenced by graded dose response of both drugs.

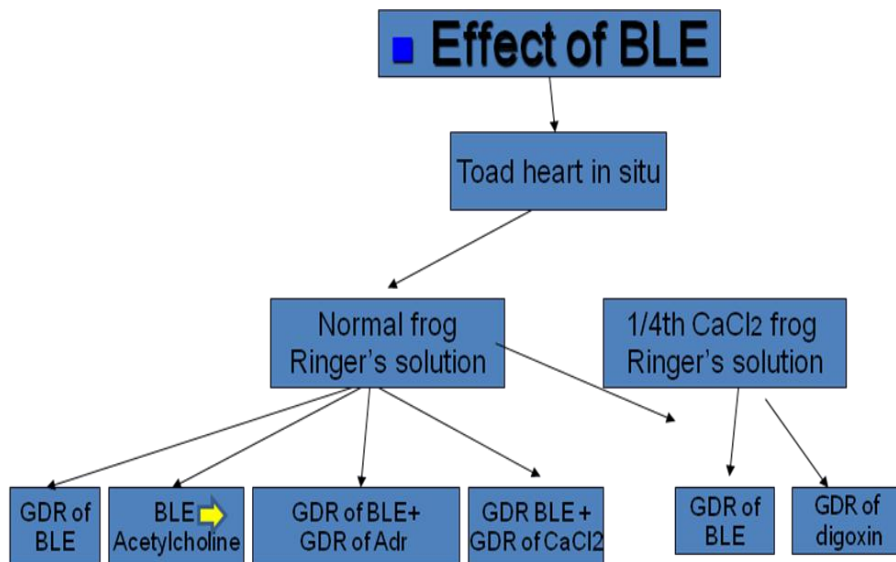


figure1: Plan of the study

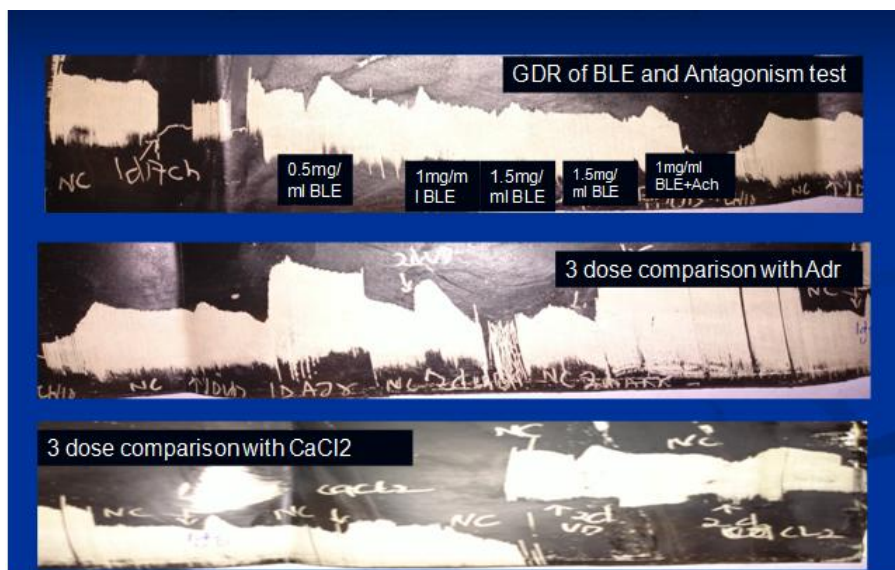


Figure 2: GDR of BLE and its antagonism and its dose comparison with Adr and Cacl2

6.3 Observation

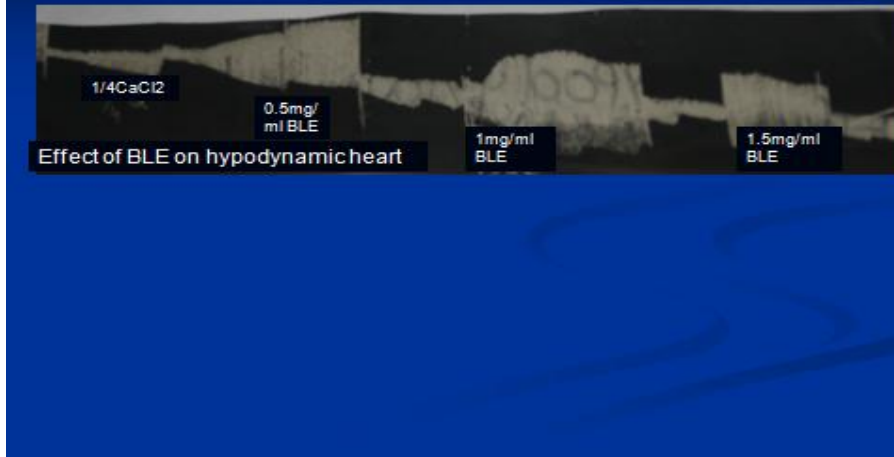


Figure 3: Effect of BLE on hypodynamic heart

Table 1: Rate and force of contraction with different graded administered samples

DRUG AND DOSE	RATE	RHYTHM	AMPLITUDE	EFFECT ON BASELINE	COMMENT
FROG RINGER SOLUTION	N	N	N	ON LINE	NC
ACH	↓	↓	↓	ON LINE	DEPRESSANT
1 DROP UD (Bael Extract)	↑	↑	↑	ABOVE BASELINE	MAY BE STIMULANT
2 DROPS UD	↑↑	↑↑	↑↑	ABOVE BASELINE	E D OF THE STIMULANT
4 DROPS	↑↑	↑↑	↑↑	ABOVE BASELINE	MAY BE STIMULANT
8 DROPS	TRANSIENT ARREST OF THE HEART FOLLOWED BY...				
	↑↑	↑↑	↑↑		

DRUG AND DOSE	RATE	RHYTHM	AMPLITUDE	EFFECT ON BASELINE	COMMENT
ED of UD ED of ACh	↓	↓	↓	ON BASELINE	UD NOT ATROPINE
1 D of UD 1 D OF ADR	↑↑	↑↑	↑↑	ON BASELINE	BOTH ARE STIMULANT
ED of UD ED of ADR	↑↑↑	↑↑↑	↑↑↑	ON BASELINE	BUT ADR IS MORE EFFECTIVE
1 D of UD 1 D of CaCl ₂	↑	↑	↑	ABOVE BASELINE	STIMULANT
2 D of UD 2 D of CaCl ₂	↑↑	↑↑	↑↑	ABOVE BASELINE	BOTH ARE STIMULANT
4 D of UD 4 D of CaCl ₂	↑↑	↑↑	↑↑	ABOVE BASELINE	MIMICS EACH OTHER

RESULTS AND DISCUSSIONS

BLE, a good stimulant of heart in lower doses, increases both HR and force of contraction, both in normal and hypodynamic heart model. In high doses produces transient cardiac arrest, followed by stimulatory activity, not so effective as Adr, mimics with that of CaCl₂, but not crosses base line. Thus Bael leaf may contain unique stimulatory chemical which may act directly or may modify or potentiate action of other stimulants. In Aqueous extract bael leaf may contain plenty of CaCl₂ to show above result, this cannot be also ruled out.

As from the review articles, use of Bael as cardiac depressant and in palpitation (in accordance to our study in higher doses) has also been reported. [18,21-24] The leaf extract of *Aegle marmelos* has preventing effects in isoprenaline induced myocardial infarction in rats. The activity of creatine kinase and lactate dehydrogenase was significantly increased in serum and decrease significantly in heart of isoprenaline-treated rats. The leaf extract contains a compound Marmesnin which has preventing effects in isoprenaline (isoproterenol)-induced myocardial infarction in rats. The activity of creatine kinase and lactate dehydrogenase was significantly increased in serum and decreased significantly in heart of isoprenaline-treated rats. [19,20]

The cardioactive nature of this leaf extract might be due to the two chemical compounds present are Aegelin and Lupeol. [25] A review article also has focussed on property of the leaves of *Aegle marmelos* having cardiotoxic property and thus can be useful in weakness of various heart ailments. [26,27] Though cardiotoxic effect largely depends on the cytosolic calcium, further studies should be in line to find out whether the increased extracts calcium could have either augmented the phytoconstituents cardiotoxic activity or would have supplemented with calcium in producing the activities seen in CaCl₂

supplementation by Ringer in 1889. Cationic constituents points to the presence of more calcium in both aqueous and alcoholic extracts and 4 times more in aqueous as compared to alcoholic extracts and study results showed variation in cationic salts composition in both the extracts but in the same order Ca>Mg>K>Na. [28]

CONCLUSION

Aqueous extract of *Aegle marmelos* (bael leaves) has a unique stimulatory effect on Toads heart. So its action APD, Indigestion, other abdominal problems, antibacterial, antiprotozoal, antidiabetic and other properties as proved by earlier workers proves presence of one to many chemical present in it. Analysis of chemical constituents couldn't be done due to non-availability of HPTLC and AAS. Thus, in future it will be interesting to isolate the active chemical constituents which are responsible for the cardiotoxic activity as well as to determine the possible mechanism of action. Further studies can also confirm the reduced toxicity. Infertile women doing a fasting on Monday, taking a bael leaf as Prasad becomes a mother in spite of many trials of medicine and surgery. Chinese Herbs and plants were experimented and evaluated to be Anticancer; Antimalarials are in rampant use now. When Indian herbs with traditional healing properties are known since Vedic period why we are so silent! I wish my study will put the focus to that direction and will inspire young scientist to probe into HIDDEN TREASURE.

Abbreviations

ED - Effective dose, BLE - Bael leaf extract, UD-Unknown drug, APD - Acid peptic disorder, GDR-Graded dose response, Ach-Acetylcholine, CaCl₂-Calcium chloride, HPTLC-High performance thin liquid chromatography, AAS-Atomic absorption spectrometer

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Conflicts of Interest - Nil

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