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Cardiotoxic Effect of Aqueous and Alcoholic Crude Extract of some Indigenous Plants on Rana Tigrina (*Hoplobatrachus Tigerinus*)

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Abstract:- The therapeutic effects like antimicrobial, insecticidal, larvicidal, Piscicidal, molluscicidal etc of various indigenous plants have been described. In present study, the effects of 10 % aqueous and alcoholic crude extracts of some indigenous plants on heart rate of *Rana tigrina* were examined in laboratory conditions. However, both aqueous and alcoholic extracts of used plants viz., *Acacia concinna, Acorus calamus, Balanites roxburghii, Cestrum nocturnum, Nicotiana tabecum* and *Sapindus trifoliatus* were found to be highly significant as Cardiotoxic plants than remaining plants. Secondly, alcoholic extract was more potent to reduce in heart rate than aqueous extract of same plants. These results therefore seems to support the claim that most of used plants contain saponin and glycosides as an active ingredient in them, they might be cause to decrease rate of heart contraction by inhibiting the cellular Sodium-Potassium ATPase pump.

Keyword:- *Therapeutic, indigenous, antimicrobial, crude extract, Na^+-K^+ATPase pump.*

I. INTRODUCTION

Many indigenous plant species have over the years constituted indispensable tools for research and development of new drugs and are used in folk medicine to manage hypertension due to their hypotensive properties (Caius, 1986; Salahdeen et al., 2004; Abudullah, 2012). The medicinal use of plant extracts date back to ancient times and now listed officially as herbal drugs in pharmacopoeias in many countries (Chang et al., 2002). There are thousands of plant species worldwide, but only very few were tested and used medicinally to treat cardiovascular disease (Bahorun et al., 2003). Medicinal plants have over the years constituted indispensable tools for research and development of new drugs (Bonati, 1980). Coupled with the fact that there are still many plants whose medicinal values have not been exploited, it is responsible to describe the plant kingdom as a sleeping giant for potential drug development (Abdullah, 2012).

These studies showed that some of indigenous species increases contraction of the heart dilates peripheral blood vessel and improve blood supply to the heart, thereby help in treating heart disease and mitigating symptoms in early stage of heart failure (Rigelsky and Sweet, 2002). The plant Cardiac glycoside is a organic compound that increase the output force of the heart and decrease its rate of contraction by inhibiting the cellular Na^+ - K^+ ATPase pump (Bekalu et al., 2021), its action include both beneficial and toxic effect on heart. It also used as heart tonic. It has much therapeutic value in congestive heart failure and those with positive inotropic effect. It is believed to increase the force of cardiac muscle contraction by binding to and inhibiting the action of a membrane enzyme that extrude Sodium ions from the cell interior (Suarez et al., 1997; Afolabi and Ebenezer, 2014). The plant alkaloids are used as cardio-protective agent. A steroid in high doses seems to increase the risk of heart disease including heart attack, heart failure and stroke (Gyas Khan et al., 2014; Jayasinghe et al., 2020). Saponin, is an another plant glycoside, it is very toxic, producing immediate death in animals when given intravenously or less harmful producing mild irritation when applied directly to the mucous membrane (Roy and Chatterjee, 1968). These drugs also enhance the release of Calcium from internal store, resulting in a rise in intracellular calcium.

Most of plants used in present investigation are growing naturally during rainy and winter season while some are cultivated. Their extracts have been reported to have various pharmacological effects like piscidial (Patole and Mahajan, 2006), larvicidal (Patole and Mahajan, 2007), insecticidal (Pavela, 2008) and antimicrobial (Patole et al., 2010). Traditionally, the local people as well as *Vaidus* use these plant parts as treatment to reduce blood pressure, blood sugar, cancer and sexual weakness etc (Abdullah, 2012). In extensive search of the literature, there are no much published reports on the hypotensive effect of these plants as far as many of us are aware. As a part of screening for a suitable natural drug and in view of the dearth of information in the literature evaluating the hypotensive property of plants. To the best of my knowledge, no single evaluation study of aqueous and alcoholic crude extracts of some indigenous plants on cardiovascular system has been reported prior to this report either in *vivo* or in *vitro* study. Therefore, the aim of present study is to evaluate the effect of aqueous and alcoholic crude extracts of some indigenous plants by investigating its effect on the force of contraction especially heart rate in *Rana tigrina* to elucidate its mechanism of action.

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II. MATERIALS AND METHODS

A. Preparation of extract

The used plant material was locally collected in and around city and some is purchased from local Ayurvedic shop.The plant material was identified, dried at room temperature, and extracted in Central Research Laboratory, Mooljee Jaitha College, Jalgaon (M.S.). The dried plant material was ground to a powder and extracted by maceration using distilled water for aqueous and 95 % ethanol for alcoholic crude extract (1kg/ 1L, w/v) for 3 days at 37 ^{0}C as per described by Abdul et al (2009). The extract was filtered and evaporated under reduced pressure in a rotary evaporator. The resulting residues were stored at 4 ^{0}C. The residues were dissolved in usual solvent to obtain 10 % (100 mg/ ml) solution used in this study.

B. Experimental animal

The adult healthy frogs, *Rana tigrina* species weighing 150-200 g were obtained from animal house (M. J. College, Jalgaon). They were maintained and fed with standard food. The healthy frog of irrespective sex were selected for experimental group of 4 kept for both extracts of each plant extract and same number (4) of frog was kept as control.

C. Experimental procedure – Frog heart in situ preparation

The frogs were pithed and the heart was exposed. The inferior vena cava was cannulated for perfusing the heart with the frog's Ringer solution (50 ml Ringer contains, 60 mg Sodium chloride, 31 mg Sodium lactate, 3 mg Potassium chloride and 2 mg Calcium chloride (pH = 6.5)). The control cardiac contraction was recorded on a smoked Kymograph drum after administration of frog Ringer solution. The speed of Kymogram was kept constant and it was 2.5 mm/sec. The Ringer and crude extracts were poured drop by drop on exposed heart with pipette. The average control heart rate and contraction amplitude were ranging from 60 to 64 beats/ min and 18 mm respectively. The 10 % aqueous and alcoholic crude extract responses were recorded. The frog heart was washed with the Ringer solution after every administration of extracts to bring back to normal state. There was same frog used for control as well as both extracts of each plant. The frog Ringer solution was administered for 1 minute followed by extracts and the recording were noted.

The results are presented as mean standard error of the mean (SEM). The difference of means values were assessed for statistical significance by using students 't-test' value of 'p' equal or less than 0.05 were taken to imply statistical significance.

III. RESULTS AND DISCUSSION

The Cardiotoxic effect of 10 % aqueous and % alcoholic crude extract of some indigenous plants is presented in table-1. This table also shows part used and its active ingredients. The heartbeat of control frog was counted to be within range of 60 to 65 beats /minutes. Whereas decreases in heart beats were found in experimental animals those exposed to both crude extracts. The most of plants used in present investigation contains saponin, alkaloid, glycoside etc as an active ingredient, which might be responsible for reduction in heartbeats. Among the plant used *Acacia concinna, Acorus calamus, Balanites roxburghii, Cestrum nocturnum, Nicotiana tabecum, Sapindus trifoliatus* etc shows higher Cardiotoxic activity, causes significant reduction in heart rate. The highest Cardiotoxic activity was found in frog exposed to crude extract of *Balanites roxburghii* plant. It was followed by animal exposed to extract of *Cestrum nocturnum, Nicotiana tabecum, Sapindus trifoliatus, Acorus calamus* and so on. These results are corroborated with earlier workers viz., Roy and Chatterjee, 1968; Murlidharan and Dhananjayan, 2004; Jaysinghe et al., 2020;).

The rhythm of the frog heart appears to be purely neurogenic and amplitude of the heart is influenced by nervous stimuli. The rate of heart beat is greatly affected by temperature, activity and physiological state of the animal (Richard and Devis, 1979). In addition to these many factors affect the heart beat under experimental conditions). They include light, the secretion of various tissue extracts and pharmacological active substances (Jones, 1974). The plant used in present investigation contains saponin and glycosides, which has Cardiotoxic and cardiotonic effect (Roy and Chatterjee, 1968). These significantly reduce the heart rate as compared to control value (Parsaee et al., 2006). It might be due to change in amplitude causes coronary flows of the heart were depressed (Roy and Chatterjee, 1968). In this study, higher extract concentrations (100 mg/ml) caused ballooning of the heart, stopping of perfusion solution circulation and stopping of heart beating. One explanation for such effect at these doses could be due to the high viscosity of the extracts, which caused a blockage in the small coronary vessels with subsequent prevention of the perfusion solution from circulating through its normal pathway and instead flowing back through aortic valve into the left ventricle and thus resulting in ballooning of the left ventricle. The results of present investigation showed that the aqueous and alcoholic extracts of used indigenous plants lowers heart rate in frog. These produced significant positive ionotropic and negative chronotropic action on frog heart. The positive ionotropic effects were selectively inhibited by plant ingredient. These are further responsible for significant decrease in membrane Na^+/ K^+ ATPase the basis of Cardiotoxic effect. This observation agrees with the earlier reports of Abdul and Amin (1997); Murlidharan and Dhananjayan, 2004; Salahdeen et al., 2004; Bekalu et al (2021). The heart beats of frog exposed to crude aqueous and alcoholic extracts seems to have a noticeable response to the concentration used in this study.

It is concluded that, the results of present study seem to support the traditional claim of indigenous plants which lowers blood pressure and this is probably mediated through activation of muscarinic cholinergic receptors.

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Sr. No.	Plant name	Part used	Active ingredients	Heart beats per minute			
				Aqueous extract		Alcoholic extract	
				Control (Saline)	Treated with 10 %	Control (Saline)	Treated with 10 %
01	*Acacia concinna* D. C.	F	Saponin, Alkaloid	62 ± 2.0	52 ± 1.5 (16.12) **	63 ± 2.0	50 ± 30 (20.63) **
02	*Acorus calamus* Linn	R	Acorin, Glycoside, Essential oil	61 ± 1.5	50 ± 2.0 (18.03) **	62 ± 1.5	50 ± 2.5 (19.35) **
03	*Anagallis arvensis* Linn	WP	Saponin, Glycoside	64 ± 1.0	59 ± 2.5 (7.81) *	62 ± 1.0	57 ± 2.5 (8.06) *
04	*Azadirachta indica* A. Juss	L	Azadirachtin, Salanin, Meliantriol	63± 0.25	60 ± 2.25 (4.76) NS	61 ± 2.5	58 ± 1.5 (4.91) NS
05	*Balanites roxburghii* Pla.	F	Saponin, Steroid and Sapogenin	63 ± 1.5	48 ± 2.5 (23.81) ***	64 ± 1.5	45 ± 3.5 (29.68) ***
06	*Cestrum nocturnum* Linn	L	Saponin, Glycoside	65 ± 2.0	51 ± 2.0 (21.54) **	63 ± 2.5	48 ± 2.0 (23.81) ***
07	*Citrullus colocynthis* S.	F	Saponin, Colocynthine	60 ± 2.0	58 ± 1.5 (3.33) NS	63 ± 2.2	60 ± 2.5 (4.76) NS
08	*Duranta rapens* Linn	L	Saponin, Alkaloid	62 ± 1.0	56 ± 3.0 (9.68) *	62 ± 2.3	58 ± 1.0 (6.45) *
09	*Nicotiana tabecum* Linn	L	Alkaloid, Nicotine, Anabasine	61 ± 2.5	50 ± 2.5 (18.03) **	61 ± 1.3	48 ± 1.25 (21.31) **
10	*Sapindus trifoliatus* Linn	F	Saponin	62 ± 1.25	47 ± 3.0 (24.19) ***	62 ± 1.2	49 ± 2.0 (20.97) **
11	*Sphaeranthus indicus* Linn	WP	Sphaeranthine, Glucoside	62 ± 1.5	56 ± 2.3 (9.68) *	61 ± 2.0	55 ± 0.5 (20.63) **
12	*Tephrosia purpurea* Pers	R	Glycoside, Rotenone	63 ± 2.0	56 ± 2.0 (11.11) *	61 ± 2.5	57 ± 2.5 (9.83) *

Table 1: Effect of aqueous and alcoholic crude extracts of some Indigenous plants on heart of frog (*Rana tigrina*).

Part used - F= Fruit, WP= Whole plant, L= Leaves, R = Root

Values represent mean ± (n=4); NS = Non significant, *p < 0.05, ** p < 0.01.
Values in the parenthesis are per cent change over control.