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Cardiotonic activity of aqueous extract of heartwood of *Pterocarpus marsupium*.

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The present study was undertaken to evaluate cardiotonic activity of aqueous extract of heartwood of *P. marsupium.* This plant species contains 5,7,2-4 tetrahydroxy isoflavone 6-6 glucoside which are potent antioxidant and are believed to prevent cardiovascular diseases. Cardiotonic effect of aqueous extract of heartwood of *P. marsupium* was studied by using isolated frog heart perfusion technique (IFHP). Calcium free Ringer solution was used as vehicle for administration of aqueous extract of *P. marsupium* as a test extract and digoxin as a standard. A significant increase in height of force of contraction (positive inotropic effect) and decrease in heart rate (negative chronotropic effect) at a very low concentration (0.25 mg/ml) was observed with test extract as compared to the same dose of a standard digoxin. The present results indicated that a significant increase in height of force of contraction with decrease in heart rate was observed as the dose of test extract increased. The test extract produced cardiac arrest at 4 mg/ml, a higher concentration, as compared to standard, digoxin (0.5 mg/ml). Compared to digoxin, a drug with narrow therapeutic window, *P. marsupium* showed wide therapeutic window.

Keywords: IFHP, Digoxin, *P. marsupium*, Therapeutic window

Cardiac disease is an important cause of premature death in industrialized countries. It is estimated that cardiac disease will emerge as single largest contributor to morbidity in India accounting for nearly one third of total deaths in near future. Cardiac glycosides and catecholamines have been used as main therapeutic agent in the treatment of congestive cardiac failure[1]. However, the danger of cardiac glycosides intoxication are well documented[2] and doubts have been expressed about their effectiveness. Despite continuing advancement in understanding the basic pharmacology of cardioactive drugs, cardiac glycosides, intoxication with digitalis a narrow therapeutic index drug remains a common clinical problem. Synthetic catecholamine has been reported to cause a severe oxidative stress in the myocardium through free radical formation[3]. It necessitates research for new drug and with this aim we have chosen *P. marsupium* and evaluated its cardio active potential.

In this modern era a large Indian population still relies on the traditional system of medicine, which is mostly, plant based. One such plant *P. marsupium* is used as astringent, antidiabetic, in diarrhoea and in skin diseases. Kino is the juice obtained from the incision in trunk insissated without artificial treat. The principle constituent of Kino is peculiar tannin, Kino-tannic acid [4,5].

It was reported that aqueous extract of *P. marsupium* possesses anti-inflammatory activity[6]. A new isoflavone C-glycoside macrocarposide isolated from heartwood of *P. marsupium* has been characterized as 5,7, 2-4 tetrahydroxy isoflavone 6-6 glucoside[7]. Flavonol aglycone and their glycosides as main phenolic contents are potent antioxidants[8,9], which are believed to prevent degenerative diseases including cardiovascular diseases. They exhibit a wide range of biochemical effects including vasodilatitary actions[10,11] and inhibition of platelet aggregation[12].

Hence, it was considered to evaluate cardiac activity due to the presence of above constituents in this plant.

Materials and Methods

Drug—The heartwood of *P. marsupium*. (Papillionacae) was purchased from Eminence International, Mumbai. Digoxin was procured from Cadila Healthcare Pvt.Ltd., G.I.D.C., Ahemadabad.

Animals—Frogs of Rana tagrina species from the animal house of Government College of Pharmacy, Karad, were used for studies. The animals were fed with food and water *ad libitum*. The animals were

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maintained as per the norms of CPCSEA and the experiments were cleared by CPCSEA and the local ethics committee.

Preparation of extracts—The dried heartwood (15 g) of *P. marsupium* was kept in beaker containing 100 ml distilled water for 12 hr for extraction. The brown coloured aqueous extract with light blue shade on surface was collected in the morning and concentrated on water bath. The product was dried by using rotary evaporator, finally dried under sunlight and powdered. The Powdered test drug was dissolved in sterile water to obtain appropriate concentration of 0.25, 0.5, 1, 2, and 4mg/ml and the pure sample of digoxin (as reference standard) was dissolved in sterile water to make solutions of concentration 0.25, 0.5, 1 as experimental set up (Table 1).

Isolated frog heart perfusion for study of cardiotonic activity of drugs was followed as per described earlier [13]. The isolated frog heart perfusion technique was used as a model to evaluate the activity of various concentrations of aqueous extract of heartwood of *P. marsupium* and compared with

Table 1—Effects of the aqueous extracts from heartwood of *P. marsupium* on isolated frog heart perfusion.

Expt. set up	Conc. of extract/Digoxin (mg/ml)		Dose (ml)	Conc.at different doses (mg)	HR	HFC (mm)	Change in HFC (%)
1	Extract	0.25	control	—	54	05	—
			0.1	0.025	53	07	40
			0.2	0.050	51	08	60
			0.4	0.1	50	8.5	70
2		0.5	control	—	55	03	—
			0.1	0.05	54	05	66.66
			0.2	0.10	52	07	133.33
			0.4	0.20	50	10	233.33
3		1	control	—	49	06	—
			0.1	0.10	47	11	83.33
			0.2	0.20	45	15	150
			0.4	0.40	41	16	166.66
4		2	control	—	38	07	—
			0.1	0.20	34	13	85.71
			0.2	0.40	28	14	100
			0.4	0.80	22	17	142.85
5		4	control	—	33	12	—
			0.1	0.40	28	22	83.33
			0.2	0.80	26	25	108.33
			0.4	1.6	23	27	125.00
6	Digoxin	0.25	control	—	54	07	—
			0.1	0.025	56	08	11.42
			0.2	0.050	59	09	12.85
			0.4	0.10	61	12	17.12
7		0.5	control	—	51	07	—
			0.1	0.050	53	12	17.12
			0.2	0.10	55	13	18.57
			0.4	0.20	58	—	—
8		1	control	—	50	05	—
			0.1	0.10	58	07	40
			0.2	0.20	61	10	100

HR – heart rate; HFC – height of force of contraction.
The statistical analysis was carried out using Students' *t* test and it was found significant at $P < 0.001$

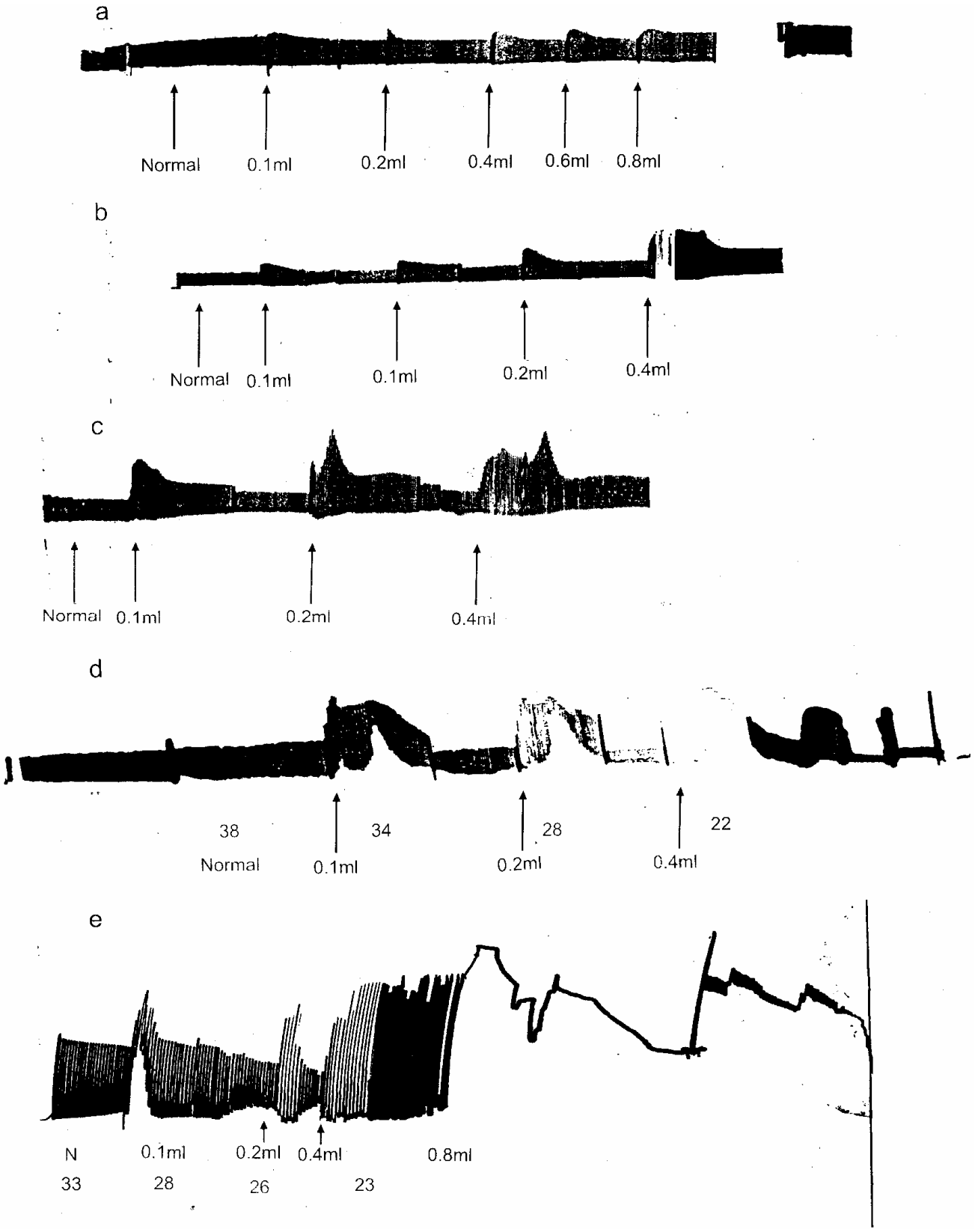

Fig. 1A—(set 1) Effect of aqueous extract of *P. marsupium* at (a) 0.25 mg/ml; (b) 0.5 mg/ml; (c) 1 mg/ml; (d) 2 mg/ml and (e) 4 mg/ml

digoxin. The experiment was carried out by using Ca++ free ringer solution[14]. The basal cardiac contraction was recorded on a kymograph after the administration of calcium free Ringer solution. The average basal heart rate and the contraction amplitude were 48 beats/min and 6.5 mm, respectively. The responses of digoxin and *P. marsupium* at various concentrations were recorded on kymograph and their cardiac activity in terms of heart rate (HR) and height of force of contraction (HFC) was noted and compared. Effects obtained with the drugs (Digoxin) and test extracts of *P. marsupium*) were converted to the respective percentage of basal values as shown in Table 1. The frog heart was washed with Ringer solution after every administration of test extract and reference drug till it was brought to normal state.

Results and Discussion

Effect was studied by using calcium free Ringer solution and isolated frog heart perfusion technique. Incremental dosage of aqueous extract of *P. marsupium* produced positive inotropic and negative chronotropic effect on isolated frog heart, and are dose dependent. The similar concentration of solution of digoxin produced positive inotropic and chronotropic effect. The cardiotonic action was studied by the effects of aqueous extracts at various concentrations given in Table1 and as shown in kymographs (Fig. 1) as well as graphs (Fig. 2).

From the observations, it was revealed that at a very high dilution (0.25 mg/ml) the aqueous extract of *P. marsupium* showed decrease in heart rate (negative chronotropic) and increase in height of force of contraction (positive inotropic) effect. At concentration (0.5 mg/ml) of test drug showed increase in percentage of force of contraction with negative chronotropic effect. At a concentration (4 mg/ml) the test drug showed cardiac arrest at 0.8 ml dose (Fig. 1A). While at a low concentration (0.25 mg/ml, 0.5 mg/ml) digoxin showed increase in height of force of contraction (about 20%) which is 4 times less than height of force of contraction produced by aqueous extract of *P. marsupium* (about 80%). The cardiac arrest shown by digoxin was at

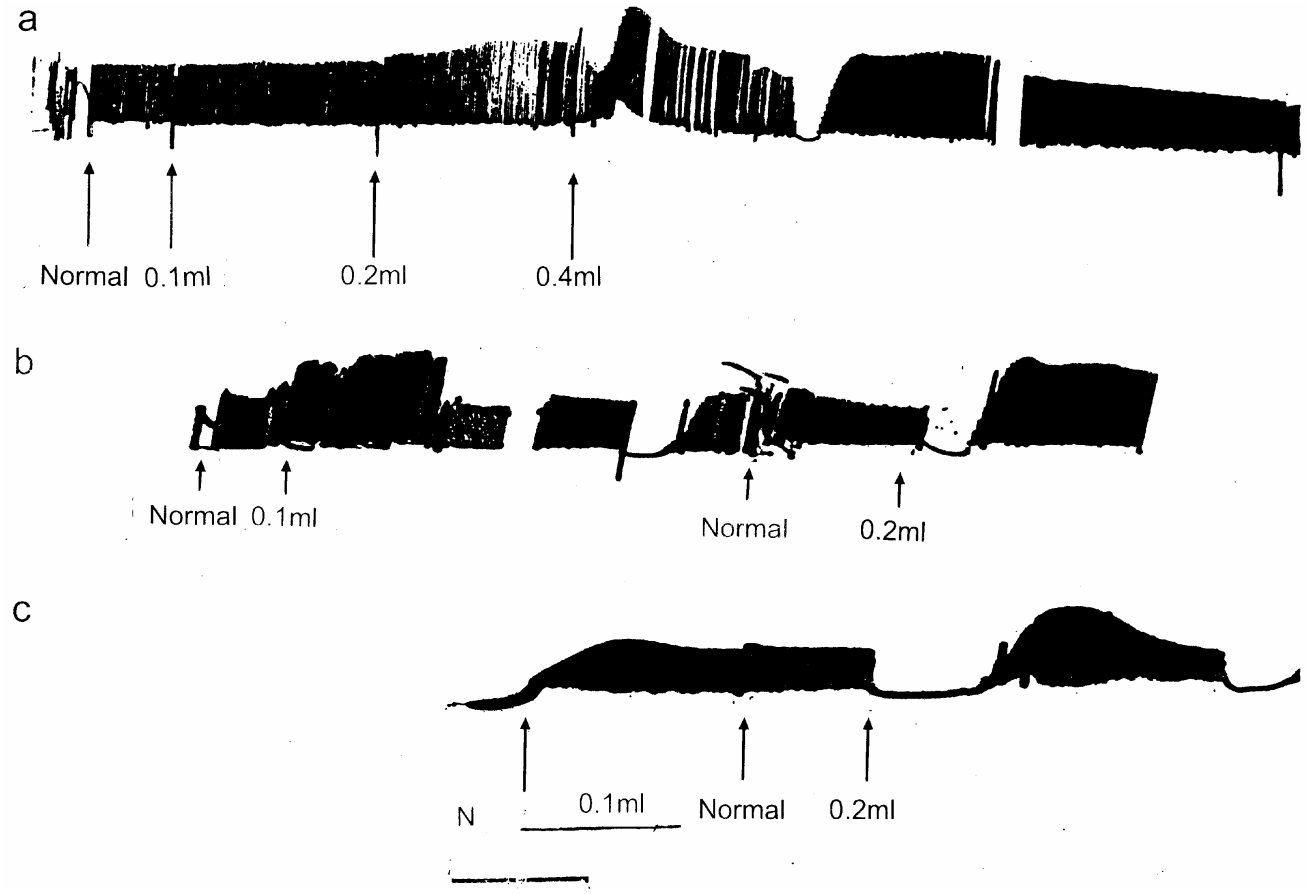

Fig. 1B—(set 6) Effect of aqueous extract of digoxin at (a) 0.25 mg/ml; (b) 0.5 mg/ml; and (c) 1 mg/ml

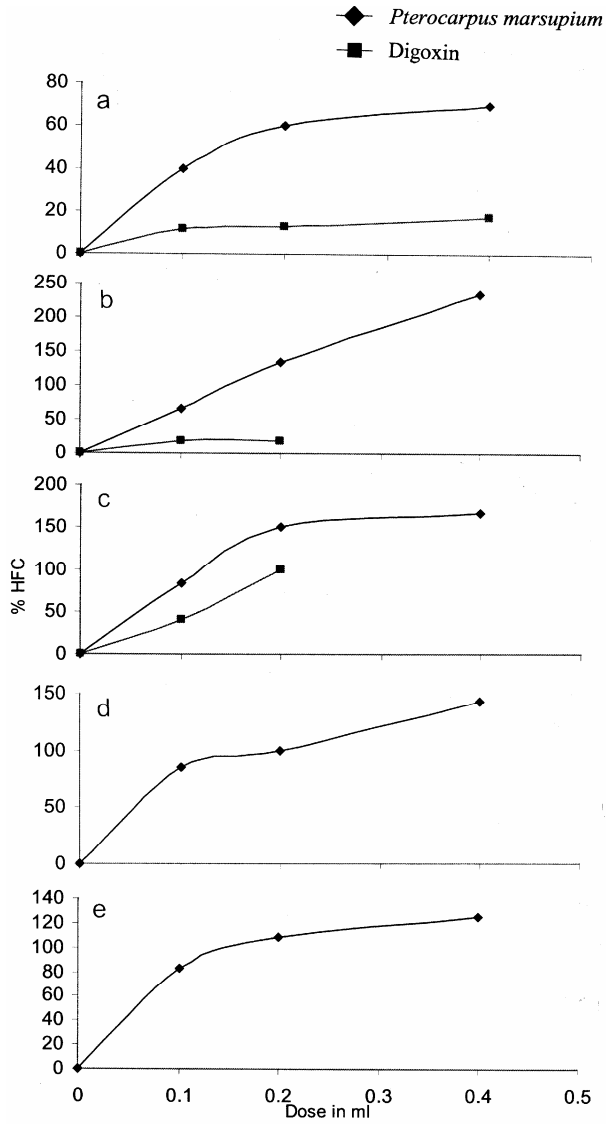

Fig. 2—(set 1) Effect of aqueous extract of *P. marsupium* and digoxin at (a) 0.25 mg/ml; (b) 0.5 mg/ml; (c) 1 mg/ml; (d) 2 mg/ml and (e) 4 mg/ml

1 mg/ml (0.2 mg/0.2 ml dose, Fig. 1B). This indicates that aqueous extract of *P. marsupium* have high therapeutic index and high margin of safety as compared to digoxin.

The kymograph obtained indicates that even at lower doses of extract of *P. marsupium* gives quick and significant increase in height of contraction as compared to digoxin. The dose of digoxin required to produce cardiac arrest was much lower i.e. 0.6 ml of 0.25 mg/ml and 0.4 ml of 0.5 mg/ml digoxin. However the cardiac arrest with aqueous extract of *P. marsupium* was observed at high dose level i.e. 0.8 ml of 4 mg/ml.

From the graphs (Fig. 2), it revealed that both digoxin and *P. marsupium* showed dose dependant activity. However, aqueous extract of *P. marsupium* produced significant (three fold) positive inotropic actions than at the same doses of digoxin.

Heart failure is a common and serious condition associated with high morbidity and mortality[15]. The side effect of currently used therapy for congestive cardiac failure are well documented. Toxicity of Digitalis is high and margin of safety is low. Therapeutic index (1.5-3 mg) and fatalities have occurred occasionally about 25% patients develop one or more toxic effects with digitalis[16].

The results obtained reveal that the therapeutic efficacy of extract of *P. marsupium* is much wider than that of digoxin. Limitation of using digoxin can be overcome by using an aqueous extract of *P. marsupium*, which has been found to posses an excellent cardiotonic activity, having wide margin of safety as compared to digoxin. It may prove an effective and safe alternative to digoxin in the treatment of congestive cardiac failure. The present results indicated that aqueous extract of *P. marsupium* showed high therapeutic index as compared to cardiac glycosides.

Further investigation is necessary for evaluation of traditional uses and phytochemcial nature of those constituents that are responsible for cardiotonic effects.

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References

1 Kitada Y, Narimastu A, Suzuki R, EndoH M & Taira N, Dose the positive ionotropic action of a Novel cardiotonic agent, MCl – 154, involve mechanism other than cyclic Amp ? *J pharmacol Exp Therap*, 243 (1987) 639.

2 Beller G A, Smith T W, Abelmann W H, Harber E & Hood W B Digitalis intoxication A prospective clinical study with serum level correlations, *N Eng J Med*,. 284 (1987) 989.

3 Harada K, Futaka Y, Miwa A, Kaneta S, Rukushima H & Ogawa N, Effect of KRN 2391 a novel vasodilator, on various experimental animals models in rats. *Jpn J Pharmacol*, 63 (1993) 35.

4 Dr. K. M. *Nadkarni's Indian Materia Medica*, 3rd edition (Popular Prakashan, Popular Press., Bombay) 1976, 355.

5 Kirtikar, Basu., *Indian Medicinal plants*, 2nd edition (Bishen Singh, Mahendrapal Singh, Dehradun Jayyed Press, Delhi) 1975, 828.

6 Salunkhe V R, Yadav A V, Shete A S, Kane S R & Kulkarni A S, Anti-Inflammatory Activity of Hydrogels of Extracts of *Petrocarpus marsupium* and *Coccinia indica*, *Indian Drugs*, 42(5) (2005) 319.

7 Rastogi & Mehrotra, *Compendium Indian medicinal plants*, (PID, New Delhi) 1993, 537.

8 Kato Y, Nagao A, Tera J & Osawa T, inhibition of myeloperoxidase catalyzed tyrosylation by phenolic antioxidants *in vitro*, *Biosci Biotechnol Biochem*, 67 (2003) 1136.

9 Roginsky V, Chain breaking antioxident activity of natural polyphenols as determined during the chain oxidation of methyl linoleate in Trioton X-100 micelles, Arch Biochem Biophys, 414 (2003) 261.

10 Duarte J, Vizcaino F P, Utrilla P, Jimenez J, Tamargo J & Zazzuelo A, Vasodilatory effects of flavonoids in rat aortic smooth musceles; Structure-activity relationships, *Biochem Pharmacol*, 24 (1993) 857.

11 Hanaski Y, Ogawa S & Fukui S, The correlation between active oxygen scavengers and antioxidative effects of flavonoids, *Free Rad Biol Med*, 16 (1994) 845.

12 Tzeng S H, Ko W C, Ko F N & Tzeng C M, Inhibition of platelet aggregation by some flavonoids, *Thromb Res.* 64 (1991) 91.

13 Kulkarni S K, Experiments. on isolated preparation, *Handbook of experimental pharmacology* 1st edition (Vallabh Prakashan, Delhi) 1987, 4.

14 Burn J H, *Practical pharmacology* (Blackwell Scientific Publications) 1952, 1

15 Satoskar R S, Bhandarkar S D & Nirmala N Rege, *Handbook of pharmacology & pharamacotherpeutics*, Revised 19th edition (Popular Prakashan Pvt. Ltd. Popular Press Bldg, Mumbai) 2005, 435.

16 Thripathi K D, cardiac glycosides & drugs for CHF, *Essentials of Medical Pharmacology*, 4th edition (Jaypee Brothers Medical Publishers Pvt. Ltd. New Delhi) 2002, 497.