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PHARMACOLOGICAL AND TOXICOLOGICAL SCREENING OF EXTRACTS /ACTIVE PINCIPLES OF THE FRUITS OF SEMECARPUS ANACARDIUM

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ABSTRACT

The fruit of Semecarpus Anacardium are accredited with a number of therapeutic properties in ayurvedic treatments like skin, sexually transmitted diseases, rheumatoid arthritis, piles, asthma, bronchitis, cough and treat joint pains, indolent ulcers, leprosy. All the extracts/principles such as SAPE, SAPE-3, SA-ether, SA-2 and SAF-1 exhibited positive inotropic and positive chronotrophic effect on frog isolated heart and possess cardio tonic activity. SAPE-3 is a potent cardio tonic. All above extracts/principles exhibited Na^+/K^+ ATPase inhibitory activity, anti nociceptive activity, anti-inflammatory activity, antipyretic and anti oxidant activity. These are did not exhibit any behavioral changes and did not cause any mortality at the doses tested. These are did not exhibit any behavioral changes and did not cause any mortality at the doses tested.

Key Words: Semecarpus Anacardium, Skin, Ayurvedic treatments, Screening.

INTRODUCTION

Fruits of semecarpus anacardium as potent as mercury as far as their utility in treating various diseases like skin , sexually transmitted diseases, rheumatoid arthritis , piles, asthma, cough and also used externally to treat joint pains , indolent ulcers, leprosy. What is most important is the claim ayurvedic treatises that these fruits have a rejuvenating property like they would convert grey hair into black hair improve the eye sight make it as sharp, increase longevity. They would bestow the speed of horse, strength of an elephant, bring in the vim and vigor.

After going through these claims one would wonder if they are true or false, in order to know which is correct, a systematic study is necessary. If one only believes the claims, one would be tempted to imagine that they would have beneficial effect on the organs like heart, liver. Different extracts like the petroleum ether (SAPE), ether (SA-ether) ethyl acetate (SA-2) prepared from the.

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fruits of s.annacardium proved to beneficial effect on different organs on the body. A mixture of two alkenyl catechols (SAPE-3) and tetra hydro `amentoflavone (SAF-1) was obtained during the column chromatography of this extract. This extracted material chemically named as biflavine were screened for the effect on frog heart (normal and hypodynamic), Na/K ATP-ase inhibition, Anti oxidant activity, anti nociceptive activity, anti inflammatory activity, effect on liver, acute toxicity [1-17].

MATERIALS AND METHODS

Petroleum ether (SAPE), ether (SA-ether) ethyl acetate (SA-2), Alkenyl catechols (SAPE-3) and tetra hydro amentoflavone (SAF-1). A fraction containing a mixture of alkenyl catechols was prepared by column chromatography of petroleum ether extract.

Animals

Mice (18-25 gms) and rats (120-150gm) of either sex were procured from National Institute of Nutrition, Hyderabad and were housed in animal house, University college of pharmaceutical Sciences, Kaktiya University, Warangal. Animals had free access for feed and water ad

libtum and air conditioned (22 $\pm 1^0$C; 60-80% RH) and illumination controlled (12/ 12h) environment.

Effect on isolated Heart

The isolated heart was connected to a sterling heart lever and stebilised for 15 minutes. The heart was moistened with fresh ringer solution with time to time and moniter the heart rate, cardiac output when they administered standard and test samples [18].

Effect on hypodynamic frog's heart

Experiments conducted by rendering the frog heart hypo dynamic by letting into heart, frog ringers containing half calcium from another reservoir through cymmes cannula. Force contraction was monitored to give half the magnitude of normal force of contraction. The suspensions of SAF-1, SA-2, SAPE, SAPE-3, SA-ether were administered into the heart through cymes cannula with the help of syringe. * up to 5 min Ringers containing ¼ calcium was perfused and from 6th minute ringers containing ¼ calcium and the drug (oubain) or extract.

Perfussion of frog heart

Frog heart pericardium was remove, right arota was tied off and a loose ligature was placed around the left arota ready for cannulation. A Cannula was first tied tied in the inferior vena cava so that blood washed away from the heart soon to avoid clots. A loose ligature was placed around the vena cava, while the heart was held far ward by a swab of moist cotton wool. A marriotte bottle of 1 Litre was connected by the tubular at the base of a length of rubber tubing which was attached to the cannula in the vena cava. The height of the marriotte bottle containing frog ringer 's for perfusion was adjusted so that small amount of fluid escaped from the cannula when placed at he height of the heart.

When the vessel was cut, the fluid washed the blood. The cannula was inserted and tied, an arotic cannula put in away from the heart (Cannula with a right angle blend) i.e tube was supporter at height of 4cm [18].

Marriotte bottle are set at height of 7 cm and filled with frog ringer's containing ¼ th of calcium and then extracted samples were (SAF-1, SA-2, SAPE, SAPE-3, SA-ether) 50µg/ml are added. The outflow is record for 5 min and bottle changed to oubain / sample solution is allowed, graph was plotted between number of drops versus time.

Antinociceptive activity by acetic acid-Induced writhing method in mice

The seigmund technique modified by koster was adopted to assess the antinociceptive activity in prescreened mice. Healthy and adult mice of either sex weighing 18-24 gms fasted for 24 hrs before the test, were divided into groups of six animals each. The control group received blank solution and second group received diclofenac sodium (standard drug) in the dose of 20mg/kg body weight i.p. The compound under test (SAF-1, SA-2, SAPE, SAPE-3, SA-ether) was administered i.P. to other groups of animals in dose of 10 mg/kg, 30kg/kg, and 100 mg/kg body weight was administered was suspension.

After 30minutes, the compounds and blank were administered all mice were given in 1.0% v/v solution of acetic acid i.p., the dose being 0.1 ml/10gm body weight of the mouse. In these mices note the time taken for the onset of writhings (induction time) was noted , counted the writhing for 20 minutes and the number of writhings produced in the treated groups was compared with those in the control group, percentage protection was calculated by using below formula[19-21].

EXPERIMENTAL

$$\% \text{ Protection} = 100 - \frac{\text{EXPERIMENTAL}}{\text{CONTROL}} \times 100$$

Anti Inflammatory Activity by Carrageenin Induced Rat Hind Paw Edema Method

Wistar strain albino rats weighing between 185-250 gm, fasted 24 hrs before the test, were divided into five groups of six animals each for each compound. The volume of the right hind paw was measured by using a plethysmometer, note the initial reading test compounds were tested in three doses i.e. 10 mg/kg, 30mg/kg and 100 mg/kg body weight. Ibuprofen 50mg/kg was used as standard. Control group received suspending agent. The entire test compounds were administered as suspension through intra p. 1 hour before the injection of carrageenin, 0.1 ml of 1.0% w/v carrageenian suspension in normal saline was injected into the plantar region (aponeurosis) of the right hind paw. The swelling produced after injection of the phlogistic agent was measured at hourly interval for 6 hrs. Percentage inhibition of edema was calculated using the formula given below [22, 23].

$$\% \text{ of Inhibition of edema} = \frac{\text{Mean edema of control group} - \text{Mean edema of treated group}}{\text{Mean Edema of control group}} \times 100$$

Antioxidant Activity by Dpph Method

For estimation of antioxidant activity *in vitro* a stable free radical α, α- diphenyl –β-picryl hydrazyl (DPPH) was used at the concentration of 0.2 mM in methanol. In a set of clean and dry set test tubes 0.1 ml different concentrations of drug / extract/ principle solutions were taken (1,3, 10, 30, 100, 300µg and 1 mg). To each of these tubes 2.4 ml of methanol and 0.5 ml DPPH reagent solutions were added with micro pipette and mixed thoroughly. Absorbance of colour in each test tube due to DPPH reagent solution read against blank (only DPPH solution in methanol) at 517 nm using Ultra violet double beam spectrophotometer. Substrate blank readings were also recorded for each concentration. These readings

were deducted from the absorbance obtained in presence of DPPH, using ascorbic acid as a standard [24-27].

(N+, K +) ATP-ase Inhibitory activity

Rana tigrina adults weighing 200-250 gm heart was isolated and washed in ice cold saline. The vertical portion was exercised and homogenized at 0^0 C for 5 min in Tris-HCl buffer (pH 7.4). The homogenate was filtered to remove the coarse particles and 0.2ml aliquots of the whole tissue homogenate were used as the source of ATP-ases. ATP-ase activity was estimated in a reaction, mixture of 1 ml containing 140mM Nacl, 140 mM KCl, 3 mM $MgCl_2$, 0.1 mM EDTA, 20 mM tris –HCl buffer (7.4pH) and 0.1 mM EGTA. SAF-1, SA-2, SAPE, SAPE-3 and SA-ether were suspended in double distilled water on other hand oubain solution was prepared in double distilled water.

The enzyme was pre incubated in the reaction mixture with 10 mM oubain and active principles/ extracts (100 µm, 500 µm, 1000 µm) respectively for 10 min. The reaction was initiated by the addition of 0.05 ml of 30 mM Na-ATP solution and maintained at 31 ± 1 ^{0} C for 30 min with occasional shaking. The reaction was terminated by addition of 1ml of 10% Trichloro acetic acid. Enzyme blanks were run in a similar way but the enzyme protein was added after the addition of tri chloro acetic acid. Substrate blanks were also run in similar way, but Na-ATP was replaced with Tris –HCl buffer. These are centrifuged at 3500 rpm for 20 min and supernatant was separated [28,29].

The difference in the specific activities in the presence and absence of oubain was considered as due to ATP-ase. Activity in the presence of oubain was taken was taken as due to Mg++ ATP ase. The specific activities are expressed as n moles Pi liberated/ mg protein/h.

The enzyme protein was estimated by the biuret method using bovine serum albumin (BSA) as standard. The inorganic Phosphate liberated in the reaction was detemined by the method of Gommori using potassium dihydrogen phosphate as standard [30].

Estimation of Total Proteins

This was carried out by biuret method.

a) TEST: 0.2 ml homogenate was added to 5 ml working biuret solution and made upto volume 7 ml with double distilled water.

b) Standard: 0.2 ml standard sample (BSA) was added to 5 ml working Biuret solution and make up to 7ml double distilled water.

C) Reagent blank 2ml water was added to 5 ml working biuret solution.

All tubes were incubated at 37^0 for 10 min. After cooling to room temperature the absorbance were measured at 555 nm using the reagent blank set to the zero [31].

Phosphate estimation

This was carried out by method of gomorri.

Method: seven tubes were set up containing respectively 1 ml of the supernatant to which 4 ml of TCA 10% was added and mixed. Blank consisted of 5 ml TCA. To each was added 1 ml ammonium molybdate solution and 1 ml metol. The mixture was allowed to stand for 30 min and absorbance read at 680 nm against the blank [32]. Percentage inhibition of ATP-ase was calculated using the formula given below.

$$\% \text{ inhibition of ATP-ase} = \frac{\text{Total enzyme activity} - \text{Test}}{\text{Total enzyme activity}} \times 100$$

Curative effect of extract / active principles in hepatic injury induced by carbon tetrachloride

Wistar strain albino rats weighing between 180-200 gm, fasted 24 hours before the test and divided into ten groups of six animals each.

Group I: Normal control
Group II: CCl_4 treated (5 ml /Kg)
Group III: Solvent control (1% CMC)
Group IV: Solvent control (PEG 200)
Group V: Silymarin (200 mg/ Kg)
Group VI: SAPE (50 mg/kg)
Group VII: SAPE-3 (50 mg/kg)
Group VIII: SAF-1(50 mg/kg)
Group IX: SA-2 (50 mg/kg)
Group X: SA ether (50 mg/kg)

Rats were first treated with 5 ml/kg of CCL_4 in liquid paraffin, orally on days 1, 3 and 5 for all groups, from day 6 to 10 i.e., for 5 days. Silymarin, solvents and extracts/principles were administered orally. All rats were kept on same diet for 10 days water was provided ad libitum and following investigations were made.

Bio chemical: All rats were anaesthetized with ether on day 11, after over night fast.Blood was drawn into glass tubes by cardiac puncture and allowed to clot, the serum was separated and the following estimations were made.

Standard procedures were mentioned in manuals of the kits supplied by the manufactures were followed [33,34].

a) Serum Glutamate Pyruvate Transaminase (SGPT)
b) Serum Glutamate Oxalate Transaminase (SGOT)
c) Serum Alkaline Phosphatase (SALP)
d) Serum Bilirubin

Histopathology

These sections of liver were examined for any changes in histology.

Functional

Pentobarbitone sleeping time was determined on day 11 prior to anaesthesia and exsanguinations. To the 10 groups of animals pentobarbitone was given an i.p. dose of 40 mg/kg. The time was noted when the animals first lost

their reflex. Starting from that time when the animals regain their righting reflex was noted. (Duration of sleep or sleeping time)

Acute Toxicity

The acute toxicity studies were carried out on healthy adult albino mice of ether sex. The dosage was graded as 10, 50, 100, 250, 500 mg/kg body weight. A single dose of each dose level was administered orally to all groups, containing six animals in each and animals were kept under observation for any change in behavior, toxic symptoms and mortality for 24 hours at 15 mins, 30 min, 60 min, 120 min, 4 hour, 24 hour intervals. One group served as solvent control. Toxicity study was carried out for SAPE, SAPE -3, SA- ether and SAF-1 [35].

RESULTS

Effect on Isolated Frog Heart

SAPE elicited a dose dependent increase in force of contraction (positive inotropic effect) in doses of 100 µg and 300µg. At lower doses (10 and 20 g) SAPE did not produce any effect. The positive inotropic effect produced by SAPE was persisting for 10-15 min after administration of a dose. The positive inotropic effect of SAPE could not be blocked by propranolol. There was no change in the heart rate but a nominal increase (0.5 ml) in cardiac output was observed.

SAPE -3 elicited a dose dependent increase in force of contraction (positive inotropic effect) at lower doses i.e.10µg, 30 µg, and 100µg. The positive inotropic effect produced by SAPE-3 was persisting for 15-20 min after administration of a dose (FIG.2). These responses were in two phases, initially small rise with short duration of action followed by a persistant stimulation of heart. The positive inotropic effect of SAPE-3 could not be blocked by propranolol. There was no change in the heart rate but a nominal increase (0.2 ml) in cardiac output was observed.

SA-ether elicited a dose dependent increase in force of contraction (positive inotropic effect) in doses of 100 µg and 300µg. At lower doses (10 and 30 µg) SA-ether failed to produce produce inotropic effect. The positive inotropic effect produced by SA- ether persisted for for 10-15 minutes after the administration of the dose. The positive inotropic effect of SA- ether was not blocked by proponolol. There was no change in the heart rate but a nominal increase (1 ml) in cardiac output was observed.

SA-2 showing the same activity like SA- ether at higher and lower doses. The positive inotropic effect was persisting for 5-10 min after administration of a dose (Fig.4). The positive inotropic effect was not blocked by proponolol. There was no change in the heart rate and a nominal increase in cardiac output.

SA-ether elicited a dose dependent increase in force of contraction (positive inotropic effect) in doses of 300µg, 1 mg and 3 mg. At lower doses (10, 30 and 100 µg) SA-ether failed to produce produce inotropic effect. The positive inotropic effect produced by SA- ether persisted for for 10-15 minutes after the administration of the dose. The positive inotropic effect of SA- ether was not blocked by proponolol. There was no change in the heart rate and cardiac output observed.

Effect on Hypodynamic Heart

SAPE elicited a dose dependent increase in force of contraction (positive inotropic effect) in doses of 100 µg and 300µg. The positive inotropic effect produced by SAPE was persisting for 10-15 min after administration of a dose. There was no change in the heart rate but a nominal increase (2ml) in cardiac output was observed.

SAPE -3 elicited a dose dependent increase in force of contraction (positive inotropic effect) at lower doses i.e.10µg, 30 µg, and 100µg. The positive inotropic effect produced by SAPE-3 was persisting for 15-20 min after administration of a dose. There was no change in the heart rate but there was a nominal increase (0.5-3 ml) in cardiac out.

Table 1. Per se effect of semecarpus anacardium on isolated frog heart preparation

S.No.	Extrtact/ Principle	Spot Dose (µg)	%Increase in force of contraction Normal Heart	Hypodynamic heart	Blockade with propranolol
1	SAPE	100	22.25	13.69	No
		300	50.8	48.33	
2	SAPE-3	10	6.8	47.4	No
		30	22.89	57.89	
		100	32.94	79.16	
3	SA-Ether	100	20.4	41.66	No
		300	31.18	73.85	
4	SA-2	100	---	9.15	No
		300	13.4	12.42	
		1000	26.48	25.81	
		3000	31.11	---	
5	SAF-1	300	2.24	---	No
		1000	24.71	----	
		3000	28.02	---	

Table 2. Perfusion of Frog heart *in situ*

Time (Min)	No. of Drops	
	Oubain (µg/ml)	SAPE-3 (50 µg/ml)
1	70	69
5	66	65
10	85	89
15	100	103
20	74	96
25	30	93
30	8	89
35	2	87
36	0	87

Table 3. Na+/ K+ ATP-ase activity of S. anacardium Linn. (n=3, p<0.001; *ns; **p<0.05)

S.No.	Enzyme	Drug (µg)	Pi nmoles/mg/hr	% inhibition
1	Total Enzyme (TE)	--	947.39±4.35	--
2	Enzyme Blank (EB)	--	945.13±8.26*	--
3	Substrate Blank (SB)	--	849.87±23.53**	--
4	Sovent CMC (1%), PEG-200	--	947.39±4.35*	--
5	Oubain	10	507.34±15.28	46.41±1.78
6	SAPE	100	571.72±7.81	39.64±0.99
		500	544.63±7.82	42.5±0.98
		1000	503.95±17.06	46.8±2.03
7	SAPE-3	100	890.39±7.82	6.01±0.39
		500	827.1± 11.74	12.6±0.83
		1000	709.58±10.33	24.86±0.88
8	SA-Ether	100	580.78±7.82	38.6±1.66
		500	562.71±0.00	40.6±0.27
		1000	558.18±3.91	41.07±0.68
9	SA-2	100	459.87±1.95	51.4±0.37
		500	562.71±0.00	54.47±1.84
		1000	402.25±7.82	57.5±0.63
10	SAF-1	100	503.00±3.96	46.9±0.66
		500	424.85±27.39	55.1±3.02
		1000	399.99±11.73	57.7±0.96

Table 4. Summary of Anti nociceptive activity of different extracts/ principles of semecarpus anacardium

S. No.	Treatment	Dose mg/kg i.p	Induction time (min)	No.of writhings	% Protection
1	SAPE	10	5.8±0.29	35.16±2.13	21.28
		30	9.85±0.4	15.5±2.16	65.3
		100	18.88±1.92	1.83±3.25	95.91
2	SA-ether	10	17.68±1.35	5.5±3.27	87.86
		30	>20	--	100
		100	>20	--	100
3	SA-2	10	4.2±0.12*	43.8±1.4*	1.03
		30	6.1±0.08	34.6±1.8	21.58
		100	8.2±0.05	17.3±3.2	61.26
4	SAF-1	10	5.1±0.06	41.0±3.8*	12.36
		30	8.0±0.04	27.5±3.2	41.68
		100	10.9±0.29	9.3±1.7	80.21
5	SAPE-3	10	7.2±0.05	39.5±1.04	17.12
		30	8.5±0.21	33.0±2.1	30.75
		100	12.7±1.4	8.6±1.7	81.81

Mean ±S.D n=6; p< 0.001; *p>0.05

Table 5. Summary of anti inflammatory activity of extracts/Pprinciples of of Semecarpus anacardium

S.No.	Treatment	Dose mg/kg i.p.	% Inhibition of Paw volume						
			1h	2h	3h	4h	5h	6h	24h
1	SAPE	100	64.6	73.5	75.5	74.0	70.5	70.0	85.3
2	SAPE-3	100	63.2	68.1	72.1	67.8	68.9	69.0	94.7
3	SA-Ether	100	67.6	73.3	75.5	74.0	70.0	70.0	85.3
4	SA-2	100	69.4	64.7	54.9	45.1	33.3	28.5	83.6
5	SAF-1	100	55.5	54.0	47.6	45.4	40.0	38.4	61.0

Table 6. Anti oxidant activity of extracts/Principles of of Semecarpus anacardium by DPPH method (% Inhibition) (n=3)

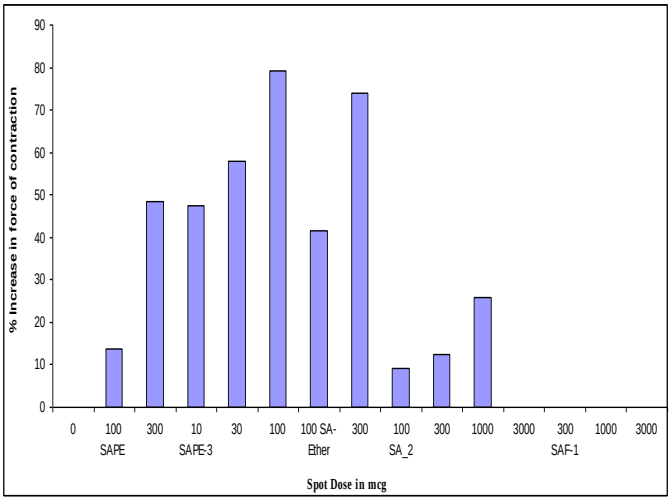
S.No.	Treatment	Dose in µg						
		1	3	10	30	100	300	1000
1	SAPE	6.08±8.3	9.28±1.05	24.09±8.83	48.8±8.21	76.12±9.21	88.85±2.79	96.19±1.04
2	SAPE-3	21.77±3.39	40.78±6.32	73.6±4.54	76.60±4.76	78.61±4.46	80.02±4.86	82.23±5.77
3	SA-Ether	5.32±4.9	17.88±7.5	55.54±8.41	75.28±4.24	77.51±4.7	78.65±4.34	80.14±4.86
4	SA-2	22.23±4.19	34.34±4.98	45.4±5.91	72.95±2.26	88.6±1.2	93.71±1.45	95.04±1.99
5	SAF-1	2.09±0.44	4.32±0.11	7.05±1.17	9.32±0.97	13.21±1.59	18.91±7.47	28.84±9.31
6	Ascorbic acid	11.07±0.39	32.14±0.08	94.1±0.42	100			

Table 7. Curative effect of semecarpus anacardimin hepatic injury induced by carbontetrchloride

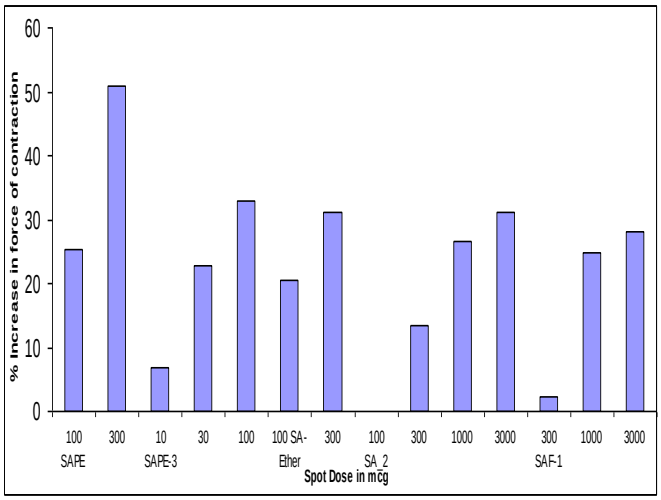
S. No	Group	Dose mg/kg oral	SGOT units/ml	SGPT units/ml	Bilirubin		ALP KA	Sleeping Time
					Total	Direct		
1.	Control	--	21.16±1.94	14.50±3.56	0.42±0.12	0.09±0.06	3.75±0.20	227.33±5.85
2.	CCl_4	5	73.16±2.13	81.00±4.47	0.93±0.18***	0.52±0.04***	14.76±0.28***	242.83±3.31
3.	1% CMC	--	74.08±1.56***	80.66±5.31***	0.93±0.18***	0.52±0.04***	14.76±0.28***	244.40±3.09***
4.	PEG-200	--	71.50±2.58***	81.16±4.62***	0.92±0.13***	0.52±0.12***	14.86±0.18***	244.66±2.94***
5.	Silymaryin	200	29.75±1.08	32.00±7.79	0.59±0.05	0.22±0.04	5.65±0.46	238.83±2.13***
6.	SAPE	50	30.25±1.75	37.50±5.54	0.65±0.06	0.18±0.04	6.63±0.37	238.33±1.36***
7.	SAPE-3	50	34.91±1.42	39.50±4.50	0.60±0.04	0.16±0.04	6.8±0.20	237.66±1.36**
8.	SAF-1	50	47.83±3.48	52.16±5.49	0.73±0.07*	0.21±0.02	9.58±0.58	242±2.09***
9.	SA-2	50	41.66±2.94	50.50±3.61	0.47±0.04	0.11±0.03	7.45±1.84	235.83±3.12*
10	SA-Ether	50	34.33±3.32	32.50±2.73	0.62±0.05	0.11±0.03	7.13±0.33	235.5±3.56**

n=6; p<0.001; *p< 0.05; **p<0.01; p>0.05.

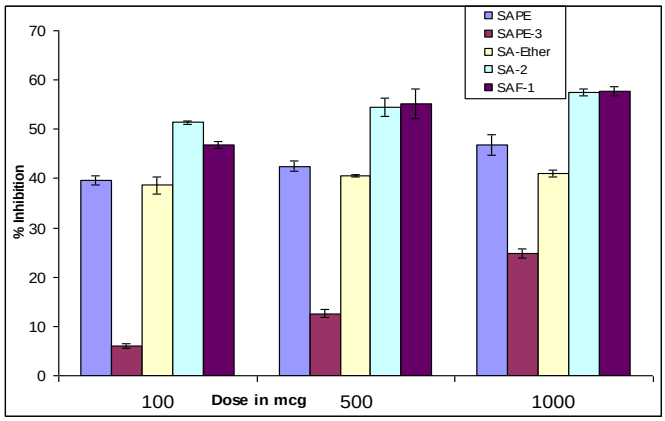
Graph 1. Per se effect of semecarpus anacardium on isolated frog heart (hypodynamic) preparation



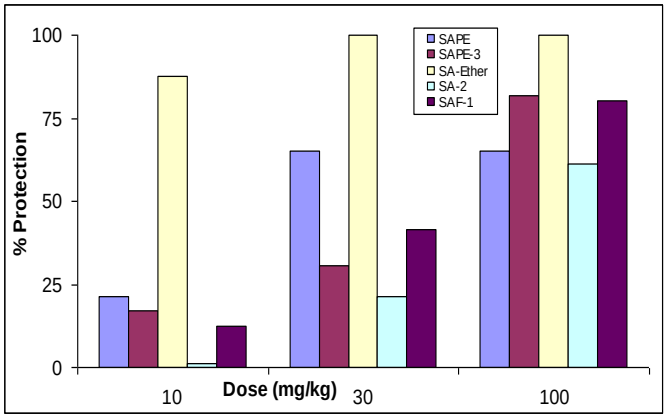
Graph 2. Per se effect of semecarpus anacardium on isolated frog heart (normal) preparation



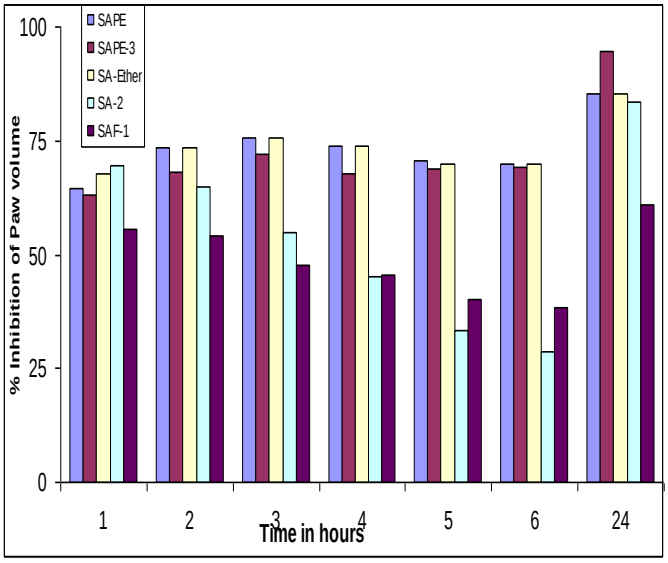
Graph 3. Na+/K+ ATP-ase activity of S. annacardium Linn.



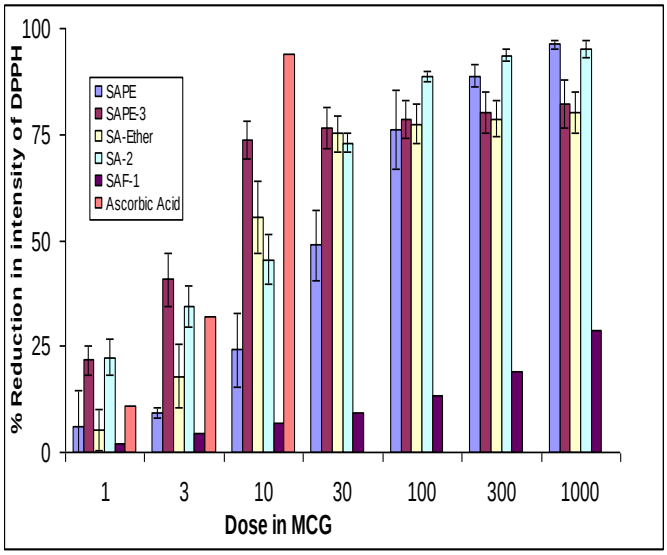
Graph 4. Summary of antinociceptive activity of different extracts/priciples of *semecarpus annacardium.Linn.*



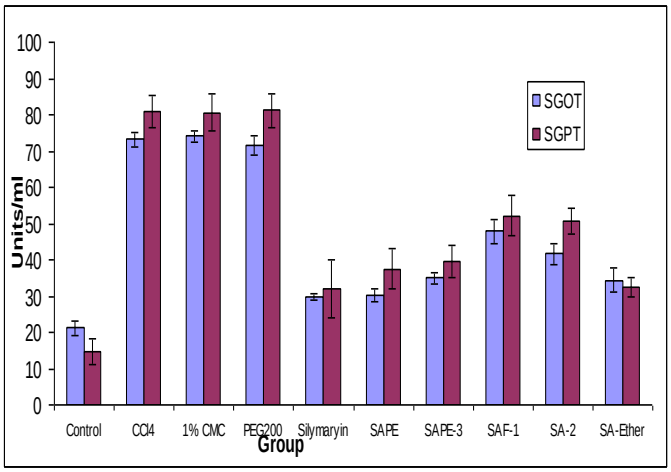
Graph 5. Anti-Inflammatory activity of Samecarpus annacardim Linn.



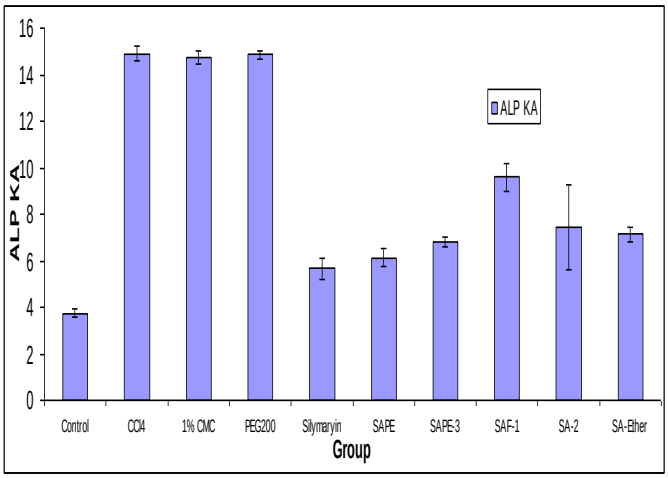
Graph 6. Antioxidant activity of semecarpus annacardium by DPPH method



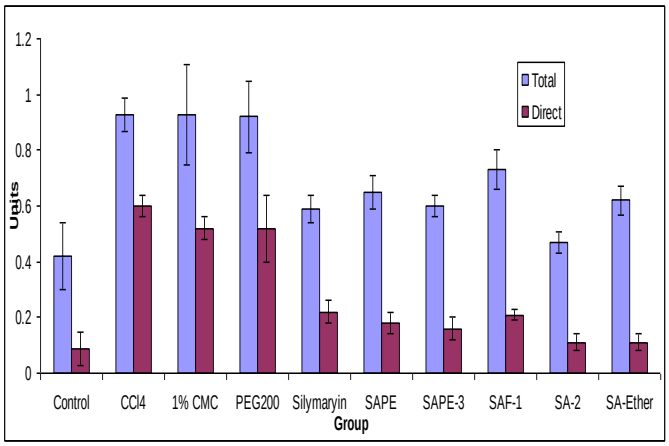
Graph 7. Curative effect of semecarpus annacardium in hepatic injury induced by carbon tetrachloride



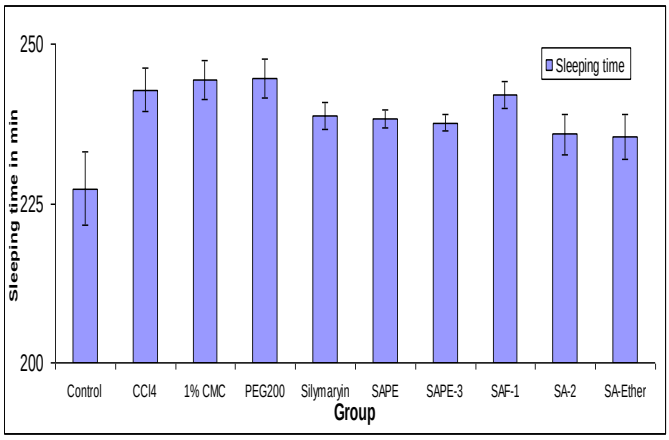
Graph 8. Curative effect of semecarpus annacardium in hepatic injury induced by carbon tetrachloride



Graph 9. Curative effect of semecarpus annacardium in hepatic injury induced by carbon tetrachloride



Graph 10. Curative effect of semecarpus annacardium in hepatic injury induced by carbon tetrachloride



SA-ether elicited a dose dependent increase in force of contraction (positive inotropic effect) in doses of 100 µg and 300µg. there was two peaks initially short peal followed by prominent one. The positive inotropic effect produced by SA-ether was persisting for 10-15 min after administration of a dose. There was no change in the heart rate but there was a considerable increase (4-8 ml) in cardiac output. SA-ether elicited a dose dependent increase in force of contraction (positive inotropic effect) in doses of 100 µg, 300µg and 1 mg. The effect persisted for 5 min after administration of a dose. There was a no change in the heart rate but a nominal increase (0.5-2 ml) in cardiac output was observed. SAF-1 failed to elicit any response on Hypodynamic heart at 100 µg, 300µg and 1 mg. The values are presented in table.1 and increase in force of contraction is shown in graphically in Graph.2.

Preparation

Effect on frog heart *in situ*

SAPE -3 exhibited an increase in number of drops (cardiac output) at 50 µg/ ml and attained a peak after which there was no further increase but a slight decrease in CO. A constant cardiac output was maintained upto 30 minutes. No cardiac toxicity was observed in Table 2.SAPE, SA-ether, SA-2, and SAF-1 failed to increase the number of drops (cardiac output) at 50 µg/ ml. The results tabulated in table.2 and increase in cardiac output is shown graphically (Graph.3). * up to 5 min Ringers containing ¼ calcium was perfused and from 6th minute ringers containing ¼ calcium and the drug (oubain) or extract.

(Na+, K+) Atp-Ase Inhibitory Activity

Protein Estimated by Biuret Method

Protein estimated by using Bovine Serum Albumin (BSA) graph. The enzyme protein present in the test sample was 5.9 mg which was found from the standard graph.

Phosphate Estimation by the method of Gomorri

The extracts/ principles of fruits of semecarpus anacardium exhibited dose dependent NA+, K+ ATP ase inhibitory activity, the doses tested were 100, 500 and 1000 µg. SAF-1 and SA-2 exhibited maximum Na+ K+ ATP ase inhibition when compared to SA-ether , SAPE, SAPE-3 . SA-ether and SAPE exhibited more activity than SAPE-3 which showed least NA+, K+ ATP ase inhibitory activity. The order of NA+, K+ ATP ase inhibitory activity is SAF-1>SA-2> SAPE> SA-ether> SAPE-3. Oubain (10 µg) caused an inhibition of 46.4% in the specific activity of the enzyme under the same experimental conditions. The results presented in table.3 and graph.3.

Antinociceptive Activity

SA-ether, SAPE, SAPE-3, SA-ether, SAF-1 and SAPE exhibited antinociceptive activity against acetic acid induced writhing in mice. SA-ether, SAPE, SAPE-3, SA-2 and SAF-1 caused a dose dependent (10 mg/kg, 30 mg/kg, 100 mg/kg) reduction in number of writhings produced by acetic acid increase in induction time. The activity of the compound at 100 mg/kg was more than that of standard, diclofenac sodium 20 mg/kg. The antinociceptive action of SA –ether, SAPE, SAPE-3 was found to be highly significant ($p< 0.001$) at all the doses tested. These results are summarized in table 4 and graph 4. SA-2 did not exhibit anti nociceptive activity at 10 mg/kg dose ($p> 0.05$). The activity of the compound at 100 mg/kg is less than that of the standard, diclofenac 20 mg/kg. The anti nociceptive action of SA-2 and SAF-1 was found to be highly significant ($p< 0.001$) at 30 mg/kg and 100 mg/kg doses. Results are summarized in table7,9 and g.13,14,17.

Anti Inflammatory Activity

SAPE and SA- ether caused a dose dependent attenuation of carrageenin – induced paw edema. The attenuation of SAPE, SA-ether at 30 mg/kg was more than that of the standard ibuprofen (50 mg/kg) upto 3rd hour but

at 100 mg/kg upto 6th hour. The anti inflammatory action was found to be highly significant (p< 0.001) at all the doses tested. SAPE-3 at did not attenuate carrageenin induced paw edema (p>0.05). SAPE-3 at 100 mg/kg attenuated more than that of the standard at ibuprofen (50mg/kg),anti-inflammatory action was found to be significant (p< 0.001) at 100 mg/kg. SA-2 attenuation of SA-2 at (10 mg/kg, 30 mg/kg) was less than that of the standard ibuprofen (50 mg/kg) but at 100mg/kg it was more. SAF-1 attenuated carrageenian- induced paw edema at 100mg/kg but at 10mg/kg, 30mg/kg only in the first hour.The attenuatin of SAF-1 at 100mg/kg was less than of standard ibuprofen (50mg/kg). The anti-inflammatory action of SA-2 was highly significant (p<0.001) up to 5th hour, at 6th hour (p,0.001) at 100mg/kg doses.

Anioxidant Activity

The principles /extracts of the senecarpus anacardim exhibited antioxidant activity. SAPE, SA-2 and SAF-1 showed dose dependent inhibition of DPPH. SAPE-3 exhibited dose dependent inhibition up to 10 µg but after this particular dose the inhibition was less. SA-ether showed dose dependent inhibition up to 30 µg and thereafter the effect was less. At 1g SAPE-3 and SA-2 are almost equal. At 3 µg SAPE-3 is superior antioxidant activity, at 10 µg also SAPE-3, at 30 µg SA-ether and SA-2 inhibition of DPPH are closer to SAPE-3. But at 100 µg SA-2 is superior to all other extracts/principles and 1000 µg SAPE and SA-2 are almost equal. The anti oxidant activity of ascorbic acid which was used as standard showed 100 % inhibition at 30 µg.

Heoatoprotective Activity

BioChemical

SGOT AND SGPT

The rise in SGOT and SGPT after hepatotoxicity nduced by CCl_4 was significant. The enzyme levels in the control group were within the normal range. Silymarin which was used as a standard caused significant disease in the enzyme levels. The principles /extracts of the senecarpus anacardim also caused a decrease in the enzyme levels and the effect was significant (p< 0.001). SAPE was superior to SAPE-3, SA-ether, SA-2, SAF-1 in causing a decrease in SGOT enzyme level but in the case of SGPT enzyme level SA-ether was superior. SAF-1 caused aonly a decrease up to 47.83 units/ml in SGOT level and 52.16 units/ml in case of SGPT level. SA-2 caused a decrease of a 4.166 units/ml in SGOT level and 50.5 units/ml in SGPT level. (T-23, g-31).

ALP

CCL_4 caused a significant rise in the ALP enzyme levels while the control group maintained the normal level of the enzyme. The principles /extracts of the semecarpus anacardim caused a significant decrease in the enzyme levels (P< 0.001). SAPE caused a decrease of 6.13 units/ml while SAF-1 caused 9.58 units/ml. silymarin, which was the standard, decreased ALP level significantly. (T-23,G-32).

Bilurubin Total

CCL_4 caused a significant rise in the bilurubin and the control groups maintained the normal range. Silymarin caused a decreased in total bilirubin which was significant (P< 0.001). SAPE, SAPE_3, SA-2 and SA-ether caused a significant decrease in the total bilurubin level (P< 0.001) but SAF-1 caused a decrease which was less significant (p< 0.05).

Direct

Bilirubin direct was induced but control groups maintained their normal range. Silymarin caused a significant decrease. The principles /extracts of the senecarpus anacardim caused a significant decrease in bilirubin direct levels (P< 0.001). SA-2 and SA-ether which caused a more decrease than SAPE, SAPE-3 and SAF-1. (T-23,G-33)

Sleeping Time

The sleeping time is the index of metabolic functioning of lever. The increase in the sleeping time following the induction of CCL_4, It indicates improper functioning of the lever as a in disease conditions. Silymarin failed to reduce sleeping time significantly (P> 0.05). SAPE, SAPE-3, SAF-1 also failed to reduce sleeping time, but SA-2 and SA-ether reduced sleeping time which was less significant. Histopathology: Hepatotoxicity was induced by carbon tetrachloride which did not cause significant damage to the liver when found histologically. It was found that, there was no significant variation between the control group and therapeutic groups i.e., silymarin, SAPE, SAPE-3, SA-ether, SA-2 and SAF-1. There was no lymphocytes present, no fatty change and also inflammation in portal areas was less. SAPE, SAPE-3, SA-ether, SA-2 and SAF-1.protected the rats from the fatty changes caused by CCl_4.

Behavioural and acute toxicity study

There was no gross changein the general appearance and behavior of the mice following the administration of SAPE, SAPE-3, SA-ether, SA-2 and SAF-1 at all doses (10,100,250 and 500 mg/kg)intraperitoneally within 24 hours. It was observed that i.p. administration of extracts/ principles in mice did not cause any mortality within 72 hours.

DISUSSION

The effect of different extracts/ principles prepared from the fruits of S. Annacardium on isolated frog heart, found that all materials tested has positive inotropic effect. The increase in cardiac output was nominal , no change in the heart rate.SAPE-3 was found to

be more potent when compared to SAPE, SAF-1, SA-ether, SA-2 due to the positive inotropic effect of these materials could not be blocked by propronolol indicating that their effect is not mediated through β1 –receptors.

Thus, as far as this experiment is concerned, it can be concluded that SAPE, SAPE-3, SA-ether have carditonic activity. They are producing an increase in force of contraction and cardiac out put without causing an increase in heart rate. All materials were tested in an situ experiment on frog. This caused in increase in cardiac output followed by a decrease and finally heart stopped in systole due to the onset of toxicity. SAPE-3 exhibited a cardiac output went on increasing as with oubain as standard rest of the extracts did not show this activity.

The extracts of SAPE, SA-ether, SA_2 exhibited Na/K+ ATP –ase inhibitory activity at all doses tested. The activity was much more than SAPE_3 which showed maximum cardiotonic activity isolated frog heart and *in situ* experiment showed least Na+/K- ATP-ase inhibitory activity. SAF-1 which did not exhibit activity and SA-2 which exhibited least activity showed maximum Na+/K+ ATP-ase inhibitory activity.

Acetic acid induced writhing in mice was the versatile model for detection of analgesic activity by peripheral action. SA-ether exhibited the antinociceptive activity, at 10 mg/kg is afforded 87.8% protection and at 30 mg/kg a 100% protection is giving. The order of antinociceptive activity for different extracts/principles is as follows: Sa-ether,>SAPE>SAPE-3> SASF-1>SA-2.

Biflavanoid binaringenin ,which is the other name for tetrahydroamenotoflavone isolated from clusia columnaris exhibited potent anti nociceptive activity. It was found to exhibit potent and dose related antinocicptive action with ID_{50} values of 22 µmol/kg against the writhing test and 28 µmol/kg against the second phase of the formalin test. Based on a hot plate test, its mechanism of action was found to be unrelated with the opioid receptors. The ID of SAF-1 obtained from SA at 105.1 µmol/kg, as per our results, the ether extract (SA-ether) and petroleum extract (SAPE) were found to be more potent than SAF-1. Thus these extracts contain principles which have greater antinociceptive activity than the biflavonoid.

SAPE, SAPE-3 and SA-ether exhibited better anti-inflammatory activity at 100 mg/kg (maximum at 3rd hour) than SA-2 and SAf-1 (maximum at 2ndhour). The activity decreased with time in the case of SA-2 and SAF-1 considerably less for rest of extracts. The anti inflammatory activity of the extracts/principles in the decreasing order is follows: SAPE> SA-ether> SA-2> SAPE-3> SAF-1.

At 100 µg SA-2 and SAPE was showing the more potent antioxidant activity than SAPE-3, SA-ether and SAF-1. There is no correlation between the antioxidant activity, anti inflammatory activity and anti nociceptive activities of the extracts/ principles of semecarpus anacardium. For example, SA-2 exhibited maximum anti oxidant activity but it has the least anti nociceptive activity.

SA-ether has superior anti inflammatory and anti nociceptive activities when compared to other extracts/principles. Though it shows good antioxidant activity in a dose dependent manner, it is not the most active extract. Thus there is no perfect correlation between the antioxidant activity and anti-nociceptive activities even for this extract.

SAPE has more anti-inflammatory and antinociceptive activities than SAPE-3, SA-2 and SAF-1.When antioxidant property is considered SAPE is more potent than SAPE-3, SA-ether and SAF-1.Thus there is correlation between the anti oxidant, anti inflammatory and anti nociceptive activities as far as this extract is concerned. SAPE may be exhibiting its anti inflammatory and anti nociceptive activities due to its antioxidant property.

SAPE-3 has moderate anti inflammatory activity and moderate anti nociceptive activity at low doses but at 100 mg/kg it was good analgesic. The anti oxidant activity was good at 10 µg and there after the increase in activity was less. Hence here also no correlation can be drawn between anti oxidant , anti inflammatory and anti nociceptive activities.

The extracts/principles of the fruits of semecarpus anacardium have hepatoprotective activity. Elevated levels of enzyme like SGOT, SGPT an ALP were brought back to near normal. They also reduced the total bilirubin and direct bilirubin significantly. But they could not reduce the pentobarbitone induced sleeping time significantly. When SGOT is considered SAPE exhibited a superior activity than SAPE-3, SA-ether, SA_2 and SAF-1. The order of reduction of SGOT is SAPE> SAPE-3>SA-ether> SAF-1> SA-2.

In SGPT level reduction SA-ether exhibited a superior activity than other extracts/principles. The decreasing order of activity for SGPT enzyme level is SA-ether>SAPE>SAPE-3>SA-2>SAF-1.When the ALP enzyme levels are concerned SAPE exhibited better activity than others, the orders of the activity is SAPE>SAPE-3>SA-ether>SA-2> SAF-1. In case of total bilirubin SA-2 showed activity i.e. reduction of the level. The order of activity is SA-ether, SA-2> SAPE-3>SAPE>SAF-1.As the extracts/principles could not protect the liver significantly, the sleeping time was not reduced significantly, but significance of SA-ether ($P<0.001$) and SA-2 ($P<0.05$) was considerable than the other three.

The hepatoxicity induced by CCL_4 could not produce sufficient histopathological changes.Hence, no conclusion can be drawn for the extracts/principles when histopathology is concerned as the toxicant itself has not sufficient damaged the liver to exhibit there hepatoprotective activity. In conclusion SA-ether has a better hepatoprotective activity than other

extracts/principles. SAF-1 has the least hepatoprotective activity.

The extracts/principles of the fruits of semecarpus anacardium did not cause any behavioural changes in mice. They also did not exhibit any acute toxicity in mice when given intraperitoneally. No mortality was observed at all doses tested. LD_{50} was not calculated as the dosage could not be increased further. In conclusion the extracts/principles are safe for acute administration and also do not cause any behavioural changes. However further studies are required to know more about the sub acuteand chronic toxicity profile of the extracts/principles.

CONCLUSION

All the extracts/principles such as SAPE, SAPE-3, SA-ether, SA-2 and SAF-1 exhibited positive inotropic effect on frog isolated heart and possess cardiotonic activity. SAPE-3 is a potent cardiotonic. To know the mechanism of action further investigations should be carried out. All above extracts/principles exhibited Na^+/K^+ ATP ase inhibitory activity, anti nociceptive activity, anti-inflammatory activity and anti oxidant activity. These are did not exhibit any behavioral changes and did not cause any mortality at the doses tested. Further investigation should be carried out to throw more light on the safetiy of extracts/principles.

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