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Evaluation of the Bark of *Aegle Marmelos* (Bael) Extracts for Their Effect on Coagulation Profile in Albino Rats

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ABSTRACT

In recent times plant derived substances has received attention and their use as medicines are being tried. *Aegle Marmelos*, one of the medicinal plants with traditional values against various diseases. Therefore, this study intended to investigate its bark extracts for its anti coagulant activity as it contains more coumarin than in any other part of the plant. The bark of *Aegle Marmelos* was dried, powdered and used for preparation of extracts. Two types of extracts were prepared, decoction and alcohol extract. Extracts were fed to male albino rats of 100gm to 200gm body weight which were grouped into 6 groups with 6 animals in each group. Group 1 was a "control" group, Group 2 "standard" and Group 3, 4, 5 & 6 were test rats. Tests were conducted after 1 hour of administering the drug. No animal was reused. The coagulation tests, namely bleeding time, whole blood clotting time, one stage Prothrombin time (PT) and Prothrombin index (PI) were conducted. Mice fed with either decoction or alcohol extract did not show any effect on bleeding time and clotting time. Both decoction and alcohol extract decreased the prothrombin index significantly (<0.05). The decrease was highly significant at larger dosages (200, 500 and 500mg/100gm body weight). *Aegle marmelos* extracts decreases the prothrombin index without affecting the bleeding or clotting time.

Keywords: *Aegle Marmelos*, bleeding time, clotting time, prothrombin index

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INTRODUCTION

In recent times, focus on plant research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems. Over the last few years, researchers have aimed at identifying and validating plant derived substances for the treatment of various diseases. Similarly it has been already proved that various parts of plants such as Leafs, fruits, seeds etc. provide health and nutrition promoting compounds in human diet [1].

Aegle Marmelos belongs to family Rutacae and is also known as Bale fruit tree. It is a moderate sized, slender, aromatic tree, 6.0 -7.5 m in height, and 90 to 120 cm in girth. It is wildly distributed throughout the deciduous forests of India, ascending to an altitude of 1200 meter in the western Himalayas and also occurring in Andaman Island [2]. This is generally considered as sacred tree by the Hindus, as its leaves are offered to Lord Shiva during worship. According to Hindu mythology, the tree is another form of Lord Kailashnath [3]. Leaves, fruit, stem and roots of this tree at all stages of maturity are used as medicine against various human ailments. It has enormous traditional values against various diseases and many bioactive compounds have been isolated from this plant [4,5]. Sharma BR et al have isolated compounds with coumarin like structure from *Aegle Marmelos*.[6] Coumarin compounds have been known for their anti coagulant activity [7]. Therefore, this study intended to investigate its bark extracts for its anti coagulant activity as it contains more coumarin than in any other part of the plant.

MATERIALS AND METHODS

This work was carried out at Government Medical College, Thiruvanthapuram. Ethical clearance obtained from institutional ethical committee. The bark of *Aegle Marmelos* was collected locally and pharmacognostically identified. The bark was dried and powdered and used for preparation of extracts. Two types of extracts were prepared, one is water soluble decoction and the other is alcohol extract.

Preparation of Decoction

The decoction was prepared by the method described by Nadkarni (1954) [8]. 100 Gms of dried powder of aegle marmrlos bark was mixed with 800ml of distilled water and boiled for 1 hour. After cooling it was strained through layers of gauze and the volume was adjusted to 100 ml by further boiling. Now, 1 ml of decoction represents 1 gm of crude drug.

Preparation of Alcohol Extract

Selective extraction was carried out by the method of Rosenthler (1930) [9]. A soxhlet extracter of one litre capacity was used. The loading chamber was filled with 125 gms of the drug powdered bark and was successively extracted with petroleum ether (60 80 degrees) and dehydrated alcohol. The extracts were collected in conical flasks and the solvents evaporated

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and the residues were dried, weighed and calculated in terms of crude drug. The process was repeated according to the amount of the extract necessary for performing the experiment. 250 Gms of crude drug on petroleum ether extraction yielded 1.3 gm (0.52%) of golden yellow substance with an aromatic smell. On alcohol extraction yielded 15gm of brownish black, water insoluble substance, per 250 Gms of crude drug (6%). Alcoholic extract is water insoluble and was used as a suspension in 3% tween 80.

Methods

Male albino rats of 100gm to 200gm were used. They were divided into 6 groups of 6 animals each. Group 1 was treated with "control" solution, Group 2 with standard drug and Group 3, 4, 5 & 6 were treated with test solutions. The control drug, standard drug and their dosages with respect to their tests are shown in the table 1. Tests were conducted after 1 hour of administering the drug. Except heparin which was given by intraperitoneal route, all the controls, standards and test solutions were given orally. No animal was reused. The coagulation tests conducted include Bleeding time (by modified Duke's method), Whole blood clotting time (by Dale's capillary tube method), One stage Prothrombin time (quick's method) & Prothrombin index (PI). PI is the percentage of expression of the prothrombin time as a percentage of the control. Mean and standard deviation of each group was calculated and compared with the control and standard using a non parametric test Friedman rank sum test.

Table 1

Sl No	Test	Group 1 Control	Group 2 Standard	Group 3 Test	Group 4 Test	Group 5 Test	Group 6 Test
1	Bleeding time with decoction	Ditilled water 1ml/100gm	Heparin (IP) 2.5mg/100gm	Decoction 100 mg/100gm	Decoction 200mg/100gm	Decoction 500mg/100gm	Decoction 1000/mg100gm
	Bleeding time with alcohol extract	3% Tween 80 1ml/100gm	Heparin (IP) 2.5mg/100gm	Alcohol extract 100 mg/100gm	Alcohol extract 200mg/100gm	Alcohol extract 500mg/100gm	Alcohol extract 1000/mg100gm
2	Whole blood clotting time with decoction	Ditilled water 1ml/100gm	Heparin (IP) 2.5mg/100gm	Decoction 100 mg/100gm	Decoction 200mg/100gm	Decoction 500mg/100gm	Decoction 1000/mg100gm
	Whole blood clotting time with alcohol extract	3% Tween 80 1ml/100gm	Heparin (IP) 2.5mg/100gm	Alcohol extract 100 mg/100gm	Alcohol extract 200mg/100gm	Alcohol extract 500mg/100gm	Alcohol extract 1000/mg100gm
3	One stage prothrombin time with decoction	Ditilled water 1ml/100gm	Phenindion 80µg/100gm	Decoction 100 mg/100gm	Decoction 200mg/100gm	Decoction 500mg/100gm	Decoction 1000/mg100gm
	One stage prothrombin time with alcohol extract	3% Tween 80 1ml/100gm	Phenindion 80µg/100gm	Alcohol extract 100 mg/100gm	Alcohol extract 200mg/100gm	Alcohol extract 500mg/100gm	Alcohol extract 1000/mg100gm

IP - Intraperitoneal

RESULTS

Mice fed with either decoction or alcohol extract did not show any effect on bleeding time and clotting time. Only standard group (i.e heparin treated) showed significantly high (<0.01) bleeding and clotting times when compared with the control (distilled water fed) and the test groups (Table 2).

Both decoction and alcohol extract decreased the prothrombin index significantly (<0.05). In the standard group, the effect started after 12 hours and lasted for 24 hours. In the

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rats fed with decoction, the fall started after 24 hours and lasted for 72 hours. In the rats fed with Alcohol extract the decrease in prothrombin index started after 18 hours and lasted for 72 hours. The decrease was highly significant (<0.01) at larger dosages (200, 500 and 500mg/100gm body weight). (Table 3)

Table 2

Sl No	Test	Group 1 Control	Group 2 Standard	Group 3 Test	Group 4 Test	Group 5 Test	Group 6 Test
1	Bleeding time with decoction	190±26.46	300±17.32*	150±17.32	165±20	170±10	170±20
	Bleeding time with alcohol extract	170±43.59	300±17.32*	150±17.32	135±26.46	140±36.06	140±26.45
2	Whole blood clotting time with decoction	68.33±13.01	620±40*	63.33±6.01	66.66±12.02	65±5	67.66±4.33
	Whole blood clotting time with alcohol extract	66.66±4.40	600±69.28*	75±8.66	71.33±6.57	60±4.42	63±2.52

Friedman rank sum test, *P value <0.01

Table 3

Sl No	Prothrombin index	After hrs	Group 1 Control	Group 2 Standard	Group 3 Test	Group 4 Test	Group 5 Test	Group 6 Test
1	with decoction	6	100	100.29±6.57	102.72±5.86	103.46±7.62	101.28±5.74	105.04±7.75
		12	100	82.83±4.95*	111.69±9.01	110.37±6.13	112.38±9.57	116.36±13
		18	100	78.07±4.06**	112.72±9.47	100.91±13.68	96±6.33	81.54±18.69
		24	100	49.95±2.23**	62.86±6.00**	74.94±4.48**	60.45±11.07**	56.54±5.14**
		48	100	104.04±3.24	84.37±7.79	71.88±4.02**	71.78±4.22**	66.76±9.84**
		72	100	108.7±5.25	96.81±6.32	89.58±7.32	74.72±9.40*	80.92±5.10**
		96	100	102.68±5.75	108.25±6.41	108.76±11.29	94.49±4.88	101.27±12.84
2	with alcohol extract	6	100	100.29±6.57	110.58±11.91	115.34±20.67	114.33±14.98	100.80±9.40
		12	100	82.83±4.95*	109.94±8.28	103.20±12.17	101.22±8.82	96.32±7.90
		18	100	78.07±4.06**	100.91±13.6	84.37±7.79*	71.88±4.03**	70.15±1.92**
		24	100	49.95±2.23**	79.10±5.47*	74.14±11.76**	50±7.91**	50.25±4.08**
		48	100	104.04±3.24	95.4±2.17	78.51±10.33*	68.65±15.45**	66.77±4.84**
		72	100	108.7±5.25	117.2±5.65	91.38±1.94*	86.20±4.54*	83.12±5.69*
		96	100	102.68±5.75	94.05±5.26	94±5.26	108.39±6.95	115.07±10.26

Friedman rank sum test, **P value <0.01, *P value <0.05

DISCUSSION

Medicinal plants are of recent interest and extractions of active ingredient from these are in vogue. Compounds with coumarin like structure have been isolated from fruits, leaves, root and bark of *Aegle Marmelos* [6]. Coumarins prevent the conversion of Vitamin K epoxide to Vitamin K hydroquinone and thus prevent the coagulation process [7]. Dr Hema while screening the hypoglycemic effect of the extract of this plant observed excess bleeding among test rats [10]. In view of these facts this study was undertaken to investigate the anticoagulant activity of the bark extract. The bark was chosen because it contained more coumarin than in any other part of the plant. A decoction and an alcoholic extract of the bark were used in the present study.

This study showed that both type of extracts namely decoction and alcohol extract decreased the prothrombin index without the increasing the bleeding or clotting time. The rise

was more consistent in the dosages of 200, 500 and 500mg/100gm body weight, indicating the requirement of large doses. When compared with the Phenindion the onset was late but lasted for longer duration. Between the preparations alcohol extract had the early onset and lasted for longer duration, indicating the better pharmacokinetics of alcohol extract.

The prothrombin time (PT) and prothrombin index (PI) are measures of the extrinsic pathway of coagulation. They are used to determine the clotting tendency of blood, in the measure of warfarin dosage, liver damage, and vitamin K status. In the present context, it can be inferred that extracts of *Aegle marmelos* has anticoagulant like action. Also, since PI is an indicator of liver damage, further studies with liver function tests are required.

Currently, oral anticoagulant treatment is based on the prothrombin ratios and because of which warfarin dose is widely varied across the globe and high-dose regimes are associated with more bleeding [11]. In this context extracts of *Aegle marmelos* can be tried on the ratios as it has no effect on clotting or bleeding time only on PI.

CONCLUSION

Aegle marmelos extracts decreases the prohrombin index without affecting the bleeding or clotting time

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