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In Vivo Studies of Anticoagulation Activity Of *Triclisia Dictyophylla* Using Albino Wistar Rats

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ABSTRACT: *Some plants possess anticoagulation property but have not been fully utilized in this regard. Triclisia dictyophylla is one of such plants. This work therefore investigates the effect of the aqueous extract of the root of Triclisia dictyophylla in vivo using albino wistar rats. LD 50 of the extract was established and the rats were grouped into four groups and different concentrations (50, 100, 200 mg/kg) of the extract were administered. Blood samples were collected through ocular puncture and analyzed for some haematological and haemorheological parameters. The LD 50 was established at 547.72 mg/kg. There was no significant difference ($p>0.05$) when group 1 (control) was compared with group 2 that received 50mg/kg of the extract. At 100 mg/kg, the haemoglobin concentration and platelet count were reduced. At 200 mg/kg, the haemoglobin concentration was significantly ($p<0.05$) increased while platelet count was also reduced. The aqueous extract of the root of Triclisia dictyophylla exhibited haematinic property and also anti-platelet activity, this probably justifies its use natively as medicinal plant.*

Keywords: *Anti-platelet activity, haematinic, haematological, haemorheological, Triclisia dictyophylla.*

I. INTRODUCTION

Anticoagulant drugs (in vivo) are used to treat and prevent hyper coagulatory state and thrombotic disorders [1], and to prevent and treat stroke and transient ischaemic attack [2]. They are also given to prevent abnormal blood clotting after major surgery or during haemodialysis. The most commonly used ones are heparins, low-molecular weight heparins and the newer heparin derived-drugs such as tinzaparin, all of which are administered intraparentally. Warfarin, which is taken orally is derived from coumarin and exerts its anticoagulation effect by inhibiting vitamin K reductase which invariably prevents the gamma carboxylation of glutamic acid residue of factors II, VII, IX and X. Heparins exert their anticoagulation activity by catalyzing the penta saccharide residue of anti thrombin III (AT-III) and facilitating its antithrombin effect. Inhibition of protein C and protein S by a natural action of Antithrombin helps to prevent hypercoagulatory state. Deficiency of these proteins has been demonstrated in factor V Leiden and has been shown to be responsible for hypercoagulatory state in this category of patients.

1.1 Ideal Anticoagulant

An ideal anticoagulant should have the following features: effectiveness, safety and lack of serious toxicity, a mechanism of action independent of the metabolic pathway of vitamin K (metabolism independent of the cytochrome p-450 system), a wide therapeutic window, no need for monitoring, oral bioavailability (for long-term use), safety during pregnancy, low cost and a short half-life for drugs used in the acute setting of thrombosis or a long half-life for prophylaxis[3]. However, drugs currently used as anticoagulants have various limitations, thus being far from ideal [4]. All anticoagulants and fibrinolytic drugs have an increased bleeding risk as their principle toxicity [5].

1.2 Anticoagulant From Plants

Some plants have been attributed to possess anticoagulation property [6, 7, 8]but have not been properly utilized. Anticoagulant from plant source should definitely have better safety margin and eliminate monitoring of therapy. Such anticoagulant may present with little or no side effects both for laboratory and clinical use.

Medicinal plants show good anticoagulant therapeutic effect comparable to orthodox drugs and yet exhibit minimal unwanted side effects [9]. There is a need to investigate local medicinal plants with a view to identifying and harnessing their anticoagulant properties both for laboratory and medical practice.

1.3 *Triclisia Dictyophylla*

Triclisia dictyophylla, a member of the family Menispermaceae (Moon seed) is a medicinal plant that is indigenous to Africa and has been shown to possess anticoagulation activity [10, 11]. Earlier works on *Triclisia dictyophylla* led to isolation of morphinan alkaloids [12]. It is indigenous to West Africa and has been used natively as medicinal plant in the treatment of several ailments like oedema, anaemia and spasm [13].

The current mainstay of anticoagulant drugs has unwanted side effects such as haemorrhage, skin lesions, and thrombocytopenia [14]. Existing anticoagulants in medical practice cause so much adverse reactions in pregnancy and ordinary state that to date, no ideal anticoagulant has been found. We therefore set out to determine if the extract of Triclisia *dictyophylla* could be safe and well-tolerated in vivo.

II. MATERIALS AND METHODS

a. Aqueous Extraction

Triclisia dictyophylla plants were uprooted from their natural habitat and allowed to air-dry for 4 days. The roots were chopped into bits and pounded in a wooden mortar and immersed in 2 litres of distilled water and left undisturbed for 48 hours. Filtration was done using Whatman's No 1 filter paper to obtain the brownish filtrate. The filterate was poured into the conical flask of rotary evaporator. The filterate was evaporated into a dry semisolid black-brown residue which was poured into an evaporating dish and put in an incubator at 60^0C for 3 days[11]. Solid extract was obtained, weighed and refrigerated.

2.2 Animal Study

A total of 26 albino Wistar rats of 1 -2 months old were used for this study. The rats were weighed before the study and housed in standard wire mesh cage. The rats were allowed to acclimatize for one week and fed with standard feed and water.

2.3 Acute Toxicity Test (Ld50)

The LD50 of the plant was determined using Lorke method[15]. A total of thirteen rats were used, the test involved two stages. The first stage is the preliminary trial stage using three different groups of three rats each. The first group received 10mg/kg of the extract in water vehicle intraperitoneally. The second group received 100mg/kg while the third group received 1000mg/kg of the extract. The animals were constantly monitored in the first two hours, intermittently for the next 6 hours and then after 24 hours, the number of deaths were noted. From the result of the first stage, the second stage was carried out. Here, they were divided into four groups of one in each group. Group 1 received single intraperitoneal dose of 500mg/kg. Group 2 received 600mg/kg while groups 3 and 4 received 700mg/kg and 800mg/kg respectively. The animals were monitored over a period of 24 hours and the deaths were noted.

2.4 Oral Administration

Another set of rats were weighed and housed in standard wire mesh cage containing four groups: four rats in group1, and three rats in groups 2 – 4. The rats were allowed to acclimatize for one week and fed with standard feed and water.

2.4.1 Placement

Group 1 : The rats in this group served as control. They were administered with 0.4ml of distilled water.
Group 2 : The rats in this group were administered with standard feed, water and 50mg/kg of the extract.
Group 3 : The rats in this group were administered with standard feed, water and 100mg/kg of the extract.
Group 4 : The rats in this group were administered with standard feed, water and 200mg/kg.

2.5 SAMPLE COLLECTION AND ANALYSIS

Blood samples were collected from the rats using ocular puncture under chloroform anaesthesia. The samples were transferred into EDTA and sodium chloride bottles for haematological and haemorheological tests respectively. Samples were analysed within six hours of collection using standard methods [16, 17, 18].

III. RESULTS

Table 1 the Result for Ld50

STAGE 1	**DOSE**	**NUMBER OF DEATHS**
	10mg/kg	**0/3**
	100mg/kg	**0/3**
	1000mg/kg	**3/3**
STAGE 2	**500mg/kg**	**0/1**
	600mg/kg	**1/1**
	700mg/kg	**1/1**
	800mg/kg	**1/1**

LD50 = √axb

Where a = highest dose that does not produce death

b = lowest dose that produced death

= √ 500 x 600

= 547.72 mg/kg

In the stage 1, all the rats in the 1000mg/kg group died while no death was recorded in 10 and 100 mg/kg group while stage 2 recorded death at 600 mg/kg concentration but no death at 500 mg/kg concentration.

Table 2 Shows The Mean ± Sd Of The Haematological And Haemorheological Parameters Of 50mg/Kg Of *T. Dictyophylla* (Test) And The Control (0.4 Ml Of D/Water) In Albino Wistar Rats.

PARAMETERS	GROUP 1 (control)	GROUP 2 (50mg/kg)	P VALUE
PCV (%)	37.00±1.52	36.00±3.08	p>0.05
ESR (mm/hr)	2.00±0.57	2.00±1.15	p>0.05
RPV (m.pas)	1.18±0.22	1.18±0.10	p>0.05
RWBV (m.pas)	1.38±0.05	1.32±0.10	p>0.05
PFC (g/l)	0.77±0.30	0.79±0.17	p>0.05
Hb (g/dl)	13.10±2.87	14.70±4.16	p>0.05
WBC ($x10^9$/l)	3.90±0.61	4.40±0.58	p>0.05
PLT ($x10^9$/l)	344000±23280	285000±16250	p>0.05

Where * is significant at 0.05

PCV – Packed Cell Volume

ESR – Erythrocyte Sedimentation Rate

RPV – Relative Plasma Viscosity

RWBV – Relative Whole blood Viscosity

PFC – Plasma Fibrinogen Concentration

Hb – Haemoglobin Estimation

WBC – White Blood Cell Count

PLT – Platelet Count

Table 3 Shows The Mean ± Sd Of The Haematological And Haemorheological Parameters Of 100mg/Kg Of *T. Dictyophylla* (Test) And The Control (0.4 Ml Of D/Water) In Albino Wistar Rats.

PARAMETERS	GROUP 1 (control)	GROUP 3 (100mg/kg)	P VALUE
PCV (%)	37.00±1.52	35.00±2.12	p>0.05
ESR (mm/hr)	2.00±0.57	2.00±1.15	p>0.05
RPV (m.pas)	1.18±0.22	1.09±0.07	p>0.05
RWBV (m.pas)	1.38±0.05	1.26±0.04	p>0.05
PFC (g/l)	0.77±0.30	0.87±0.04	p>0.05
Hb (g/dl)	13.10±2.87	11.00±0.92*	p<0.05
WBC ($x10^9$/l)	3.90±0.61	3.70±0.28	p>0.05
PLT ($x10^9$/l)	344000±23280	273000±17450*	p<0.05

Where * is significant at 0.05

Table 4 Shows The Mean ± Sd Of The Haematological And Haemorheological Parameters Of 200mg/Kg Of *T. Dictyophylla* (Test) And The Control (0.4 Ml Of D/Water) In Albino Wistar Rats.

PARAMETERS	GROUP 1 (control)	GROUP 4 (200mg/kg)	P VALUE
PCV (%)	37.00±1.52	36.00±1.58	p>0.05
ESR (mm/hr)	2.00±0.57	2.00±0.57	p>0.05
RPV (m.pas)	1.18±0.22	1.17±0.57	p>0.05
RWBV (m.pas)	1.38±0.05	1.39±0.93	p>0.05
PFC (g/l)	0.77±0.30	0.81±0.20	p>0.05
Hb (g/dl)	13.10±2.87	17.90±1.54*	p<0.05
WBC ($x10^9$/l)	3.90±0.61	5.30±0.44	p>0.05
PLT ($x10^9$/l)	344000±23280	275000±10410*	p<0.05

Where * is significant at 0.05

Table 1 is the result for LD 50 of the extract comprising of two stages. In table 2, the control group was compared with group 2 that received 50mg/kg of the extract while table 3 compared the control with group 3 that received 100mg/kg of the extract. Table 4 compared the control with group 4 that received 200mg/kg of the extract.

IV. DISCUSSION AND CONCLUSION

The toxicity of the extract was determined by using albino wistar rats. The extract was administered to the rats at different concentrations of 10mg/kg, 100mg/kg and 1000mg/kg. The 1000mg/kg killed all the rats in that group while the 10mg/kg and 100mg/kg failed to record any death. In the second stage of the test, 500mg/kg, 600mg/kg, 700mg/kg and 800mg/kg were used as shown in Table 1. MLD50 (minimum lethal dose that can kill 50% of the animals) was then calculated by using 600mg/kg (highest dose that does not produce death) and 500mg/kg (lowest dose that produced death). The MLD50 was therefore gotten as 547.72mg/kg. Earlier works recorded 550mg/kg[10].

Albino Wistar rats were used for the *in vivo* studies and were divided into four (4) groups. Group 1 which served as the control were given 0.4ml of normal saline while group 2 were given 50mg/kg of extract of *T. dictyophylla.* When the two groups were compared, there was no significant difference in both the haematological and haemorheological parameters studied. This is an indication that at concentration of 50mg/kg the extract is well-tolerated and hence no changes were recorded. At concentration of 100mg/kg, the packed cell volume (PCV) was reduced (though not statistically significant) while the haemoglobin concentration which was also reduced was statistically significant. The platelet count showed statistically significant reduction. At 100mg/kg the extract exhibited anti-platelet activity. Interestingly, at 200mg/kg the haemoglobin concentration was increased. This probably justifies its use in the treatment of anaemia [13]. The extract also exhibited anti-platelet activity at this concentration. This anti-platelet activity could possibly be the reason why *Triclisia dictyophylla* is used in the treatment of leg oedema and also as muscle relaxant [19, 20]. The aqueous extract of the root of *Triclisia dictyophylla* exhibited haematinic property. It also showed anti-platelet activity which could be responsible for its anticoagulation.

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