

The Effects of Crude Neem Leaf Acetone-Water Extract on Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT) in Albino Wistar Rats.

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ABSTRACT

Background

Despite the numerous and long-term use of *neem* leaf as a medicinal plant worldwide, there is scanty literature on its coagulative properties. This study was aimed at investigating the effects of fractionated neem leaf extract on Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT).

Methods

Thirty (30) male albino wistar rats weighing 198-250g were allocated to 5 groups (A-E) of 6 rats each, with similar average body weight. The test groups (A-D) were fed orally by gavage for 4 weeks with graded doses of acetone-water fractionated *neem* leaf extract, while the 5th group (E - control), was given 0.1ml of 2.5% Dimethylsulphoxide (DMSO) for the same study period. At the end of the 4th week, 2mls of venous blood was collected. PT and APTT were estimated using standard haematological methods.

Results

The results showed a statistically significant increase ($P < 0.001$) in the weight of the test rats in groups B, C and D, and a statistically significant decrease ($P < 0.05$) in group (A), when compared with the control group (E). There was also a statistically significant increase ($P < 0.001$) in the PT and APTT mean values of groups (A-D) when compared with the control group (E). Analysis of variance of PT and APTT values of the test group with the control group revealed a statistically significant increase ($P < 0.001$) at the end of the study.

Conclusion

This study has demonstrated a dose-related prolongation in PT and APTT values by fractionated acetone-water *neem* leaf extract.

Key Words: Neem leaf extract, coagulation screening.

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a well acknowledged and established viable profession. This is particularly so in rural areas where the services of modern hospitals may be limited and also in certain circumstances where traditional medicines seem to be preferred. Medicinal plants are therefore an important resource for self-reliance in tropical countries¹.

Furthermore, an estimated 80 percent or more of the world's population depends primarily on traditional medicine for the treatment of ailments. Thus, this dependence on medicines derived from indigenous plants is especially predominant in developing countries where modern western medicine is often unavoidable or is simply too expensive.^{1,2}

In many areas, knowledge of the species used and of the methods of preparation and administering the medication resides mainly with the traditional healer. Secrecy and superstition surrounds the use of these medicines with the healer often reluctant to hand on their knowledge to anyone other than trusted relatives. As the younger generation becomes more mobile and these potential trustees move on, there is a danger that this knowledge will disappear¹.

The neem tree (*Azadirachta indica* A. juss), also called dogon yaro in some Nigerian tribes, has provided various medicinal preparation in many parts of the world for centuries. Preliminary structural analysis indicates neem is composed of carbon, hydrogen and oxygen with one mole equivalent of sodium and probable molecular weight normally 250 Daltons. Antimalarial preparations remain one of the earliest and most widely used in Africa. Udeinya³ reported that an acetone-water extract of neem was heat stable and caused complete cessation of morphological development of malaria parasites, hence preventing maturation beyond the ring stage. Adhesion of infected erythrocytes to the endothelium is a key factor in the pathogenesis of severe *Plasmodium falciparum*⁴. Cytoadhesion also plays a major role in the pathogenicity of other diseases including cancer metastasis and bacterial and viral infections⁵⁻⁸. Blood coagulation is a host-defense system that maintains the integrity of the high-pressure closed circulatory system. After tissue injury, alterations in the capillary bed and laceration of venules and arterioles lead to extravasations of blood into soft tissues or external bleeding. To prevent excessive blood loss, the hemostatic system which include platelets, vascular endothelial cells, and plasma coagulation proteins come into play⁹. To generate clot, both the intrinsic and

INTRODUCTION

It is estimated that more than 40 per cent of prescribed medicines in the western world even today came directly or indirectly from plants. In developing countries of Africa such as Nigeria, Kenya, Zimbabwe and Malawi, the application of medicinal plant especially in traditional medicines is currently

extrinsic pathways components are required¹⁰. In vitro, the generation of thrombin and the formation of a fibrin clot propagate through two separate pathways, the extrinsic pathway and the intrinsic pathway. To generate a clot through the intrinsic pathway, components intrinsic to whole blood are required, while to generate a clot through the extrinsic pathway components extrinsic to blood are required together with the intrinsic components¹⁰. Despite the numerous and long-term use of neem leaf as a medicinal plant in arid zones of Asia, Africa and Central America, there is scanty literature studies on its effect(s) on coagulation. Hence this present study was aimed at investigating the sub-acute effects of an acetone-water neem leaf crude extract on PT and APTT in growing adult male albino wistar rats.

MATERIALS AND METHOD

Thirty (30) male rats of the wistar strain weighing 198–250g were randomly allocated to 5 groups (A–E) of 6 rats each with similar average body weight. The experimental animals were obtained from the Animal House, College of Medicine, University of Nigeria, Enugu Campus, Enugu State, Nigeria. They were allowed two weeks of acclimatization. The test groups (A–D) were fed orally by gavage for 4 weeks with graded doses (0.02, 0.03, 0.1 and 0.2mg/100g body weight of rat/day respectively) of acetone-water fractionated neem leaf extract, while the 5th group (E), served as the control and was gavaged with 2.5% Dimethylsulphoxide (DMSO) at 0.1ml body weight for the same study period. The animal room was adequately ventilated, and kept at room temperature (30±2°C) and relative humidity 30-50% with 12hours natural light dark cycle. Both test and control groups consumed clean tap water and rat chow ad libitum. Good hygiene was maintained by constant cleaning and removal of

feaces and spilled feed from the stainless steel cages daily. The experiment was conducted between the hours of 8-10a.m daily. All animals were weighed before the commencement of treatment. At the end of treatment, all the experimental animals were re-weighed and suffocated with chloroform anesthesia. Two (2)mls of venous blood was aseptically collected from the retro-bulbar plexus of the medial canthus of the eye into 0.25ml tri-sodium citrate anticoagulant bottle and mixed by gentle inversion. PT and APTT were determined using plasma within two hours of sample collection. PT and APTT were estimated using standard haematological methods as described by Dacie and Lewis¹¹.

Data Analysis: Data was analyzed for mean, standard error and standard deviation. Comparison for significance between the control and experimental group were analyzed using analysis of variance (ANOVA) with Scheffe's Post hoc test. The level of significance for all experiment was $p < 0.05$.

RESULTS:

The result showed a statistically significant increase ($P < 0.001$) in the weight of the test rats in groups B, C and D and a statistically significant decrease ($P < 0.05$) in group (A) when compared with the control group (E) (Figures 1 and 2).

There was also a statistically significant increase ($P < 0.001$) in the PT and APTT mean values of groups (A–D) when compared with the control group (E) (Table 1).

Analysis of the variance of PT and APTT values of the test group with the control group revealed a statistically significant increase ($P < 0.001$) at the end of the study (Table 2).

Table I: Comparison of the Mean±SEM PT and APTT of the test with the control groups.

Parameter	Group A	Group B	Group C	Group D	Group E	Key:	SEM	Standard Error of Mean
	Mean±SEM (N=6)	Mean±SEM (N=6)	Mean±SEM (N=6)	Mean±SEM (N=6)	Mean±SEM (N=6)			
PT (secs)	31.73±0.97***	33.47±1.01***	19.07±0.50***	31.77±0.94***	13.37±0.48***	*	Secs	Seconds
						**		$p < 0.05$
						***		$p < 0.001$
APTT (secs)	46.887±0.50	66.57±1.35***	52.23±1.13	56.15±0.82***	46.13±0.53***			$p < 0.0001$

Table II: One-way analysis of variance of the PT and APTT in the test groups.

Parameter	Group A	Group B	Group C	Group D	Key:	SEM	Standard Error of Mean
	Mean±SEM (N=6)	Mean±SEM (N=6)	Mean±SEM (N=6)	Mean±SEM (N=6)			
PT (secs)	31.73±0.97	33.47±1.01	19.07±0.50	31.77±0.94	*	Secs	Seconds
					**		$p < 0.05$
					***		$p < 0.001$
APTT (secs)	46.887±0.50	66.57±1.35	52.23±1.13	56.15±0.82			$p < 0.0001$

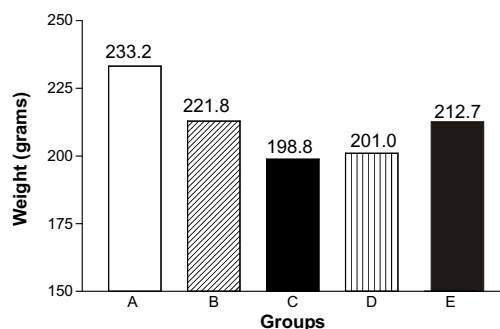


Figure 1: Mean weight before administration of the extract.

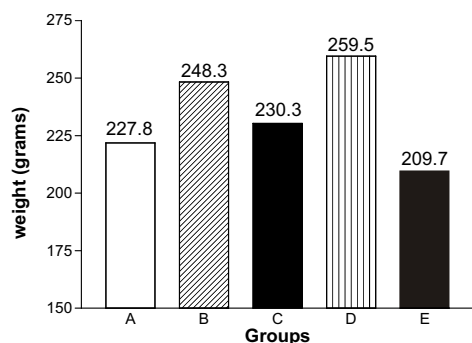


Figure 2: Mean weight after administration of the extract.

DISCUSSION: Fractioned acetone-water neem leaves extract (also known as IRAB) is a complex molecule (202 Daltons) with functional groups that include sodium salt of carboxylic acid and nonaromatic dialcohol³. In this study, the weights of the test groups B, C and D were found to increase after the administration of the extract and demonstrated a statistically significant difference ($p < 0.0001$), while the weights of the test group A and the control group E were found to have a mild or no increase after the administration of the extract, hence showed no statistical significance ($p > 0.050$ when the pre and post weights were compared (figure 1 and 2). The variation in weights gain might be due to the different concentration of the crude extract administered to the test animals.

The Prothrombin Time (PT) values in test groups (A-D) when compared with the control group (E) were found to be high and showed a statistical significant difference ($p < 0.0001$) (table 1). PT values are used to assess the extrinsic system of coagulation which include factors II, V, VII and X⁹. This is done by measuring the time taken for factor II (prothrombin) to be converted to thrombin which acts on fibrinogen initiating the clotting mechanism¹². The higher value in the PT can be attributed to the reduction in the production of these clotting factors. This is inline with the result of an earlier study by Loetitia¹³. The prolonged mean (S.E) PT values might also be suggestive of impaired liver function (though not investigated) which could directly or indirectly affect the synthesis of coagulation factors. This delay in coagulation could further be attributed to a reduction in the function of platelet in circulation (though not determined).

The Activated Partial Thromboplastin Time (APTT) mean (SE) values in test groups (A-D) when compared with the control group E was higher and revealed a statistically significant difference ($p < 0.001$) (table 1). This increase may be attributed to a deficiency in the coagulation factors needed in this pathway- factors XII, XI, VII, fibrinogen and VWF

¹⁴. The above findings were further supported by analysis of the variance of PT and APTT values of the test group with the control group which revealed a statistically significant increase in PT ($p < 0.0001$) and APTT ($p < 0.0001$) at the end of the study (table 2).

In conclusion, the present study have demonstrated the dose-related prolongation of PT and APTT value by oral administration of crude neem leaf acetone-water extract previously shown to have antimalarial³ and antiretroviral⁸ activity. Herb research programme should ensure extensive study on other coagulation test such as platelets count; other platelet function tests should also be investigated. Also liver function screening test should be carried out to confirm the probable cause(s) of prolonged PT and APTT in this study using decreased concentration of the extract and larger number of animals.

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