Effect of selected yoruba medicinal formulations on certain haematological parameters of wistar albino rats

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Abstract

Yoruba medicinal formulations derived from plant extracts are fast becoming one of the most widely consumed medicinal formulation in the rural areas of Nigeria. The aim of this study was to screen and evaluate the effect of these medicinal formulations on cetain haematological parameters of wistar albino rats. Sixty-four rats irrespective of sex were used in this study. They were divided into six groups (control and Groups A-E); with groups A-E split into three groups for testing at week 2, 4 and 6. Each sub group contained four rats and was administered 2.9µl/Kg B.W of each medicinal formulation. Cage side examination was done daily to observe overt signs of toxicity (salivation, lacrimation, yellowing of fur etc). The formulation resulted in a significant increase then reduction in the body weight. The haematological parameters surveyed increased significantly at $p < 0.05$, most especially the WBC count. The increase in WBC also affirmed the presence of toxins in this formulation. The histological findings of the liver sections indicated gross cirrhosis, degeneration, inflammation and apoptosis in the experimented groups as compared to the control group. These findings suggest that, despite the reported and acclaimed benefits of this formulation, irrespective of the source, its use as a medicinal formulation should be with extreme caution. Regulation of dose and frequency of consumption of this formulation may reduce its toxic side effects.

Keywords: Yoruba, haematological parameters

1. Introduction

Medicinal plants are plants which contain substances that could be used for therapeutic purposes or which are precursors for the synthesis of useful drugs (Abolaji *et al.,* 2006). Medicinal plants, since time immemorial have been used in virtually all cultures as a source of medicine. Over 5000 plants are known to be used for medicinal purposes in Africa, but only a few have been described or studied (Taylor *et al.,* 2001). Natural products from plants can be another potent source for the discovery of excellent biological activities, that is: anticancer and antioxidant activities (Adebayo *et al.,* 2010).

It is generally known that the consumption of a variety of local herbs and vegetables by man contributes significantly to the improvement of human health, in terms of prevention, and or cure of diseases because plants have long served as a useful and natural source of the therapeutic agents (Chevelier, 1996). Moreover, traditional medicine is greatly relied upon especially by rural dwellers, for the treatment of various ailments: traditional doctors or healers are the dispensers of such concoctions. Herbal medicinal products are unlikely to pose a significant threat to human health; nonetheless, it is important to validate their safety. The confidence in herbal medicines is backed by their long term usage. Validation of their safety is necessary because crude herbal medicines are given in most cases without accurate dosage and over ingestion can result in toxicity. It is also possible for the plant to have silent toxic effect that may not be evident within a short time (MAC, 2002). The use of herbal medicinal products may present potential risk to human health (De Smet PAGM, 1995), but some toxic herbal medicines have been proven to have beneficial effects at very low doses.

Herbal remedies can either be prepared from dry plant "ingredients" or freshly collected samples from the field. Respondents however affirmed that either plant material is efficient depending on accessibility to plant species as some plants are not easily seen within the locality (kadiri, 2008). Hence, they are collected fresh or bought and preserved dry. In rural communities, it is common practice for dwellers to prepare herbal remedies in local clay pots. This is strongly preferred to aluminium pots (Olowokudejo & kadiri, 2008).When remedies consisted of more than 2 plant parts and recipes, seeds, fruits and stem barks were placed at the bottom of the cooking pots followed by the fragile part like leaves on the top.

2. Materials and methods

Formulations were purchased from several local traditional healers peddling them at Omoko in Aluu, choba, Alakahia, Rumuosi and Rumuokoro communities in Rivers state Nigeria. These formulations were those used mainly in treatment of malaria, fever,

body and waist pains, typhoid, pile (Jedi-Jedi) and several miscellaneous illnesses as confirmed by several respondents in the rural population.

These were prepared from various herbs and plant materials as well as other additives such as pine apples, lemon, local gin, honey and red potash; which may exert therapeutic activity and also used as adjutants. The extracts are prepared by various extraction methods and solvents. A higher percentage of those interviewed showed preference to aqueous extract from fermented maize (98%) followed by water (90%) and alcohol (20%). They stated that alcohol is only used for the preparation of remedies consisting mainly hardy plant parts like stem bark, root and seed.

These herbs for herbal therapy were made from a combination of more than one plant (table 1). The combination of these different plants is what's acclaimed to cure several ailments and dysfunctions associated with the body. According to the practitioners, the dead leaves are usually brown and richer in some active agents than the green leaves. They also assert that the plant would have passed into the dying leaves, certain unwanted metabolites which are required for the medicine.

2.1 Traditional extraction methods

Two main methods in extraction used by these herbal dispensers were; boiling in water or aqueous extract from fermented maize (called ekan ogi or omidun) and soaking in the solvents alcohol. With more preference given to boiling than soaking. Boiling is usually done using either water or aqueous extract from fermented maize starch but more preference is given to aqueous extract from fermented maize as this is believed to be more efficient. Alcohol as solvent was never used when boiling herbal "ingredients". Duration of boiling ranged from 1-2 hours on burning fire wood or cooking stove till a change in color of the solvent is observed indicating "full dissolution of active ingredient into the solvents". Soaking, the second choice of preparation is given a far lesser preference unlike boiling. This method is preferred by few herbal dispersers as they believe that the ingredients will be extracted without the "ingredients" from the plant been exposed to heat which they believe may affect the efficacy of the herbal recipes. Plant part are cut into small piece and soaked in corked bottles or containers for 2-3 days.

Fig. 1 Photos of several medicinal formulations purchased.

The constituent plants for each formulation were identified by the department of Plant science and biotechnology and confirmed by the University of Port-Harcourt Greenhouse staff.

2.2 Experimental animals

Sixty-four healthy wistar albino rats weighing between 125-175g were purchased from the University of Nigeria Enugu campus, animal house regardless of their sex. They were placed in cages (eleven in number) kept in a well aerated room at a temperature of 28-31ºC and humidity of 50-55%. They were then allowed to acclimatize to new housing conditions for twelve days prior to experimentation. They were feed normal feed (purchased from Top feed shop, Choba) and distilled water *Ad libitum*. The cages were cleaned of waste once daily. All animals were treated in a manner that complied with the National Institutes of Health (NIH) Guidelines for the Care and Use of Laboratory Animals (NIH publication, 1985).

2.3 Animal treatment

The rats were divided into six (6) groups (Group A-E and control) based on the number of formulations. Groups A-E was further sub-grouped into three (3) sub-groups each (based on duration of administration) with four (4) rats per sub-group, housed in griffin and George modular cage system. The control group was fed normal feed and distilled water *ad libitum*. Groups A-E were fed normal

feed and distilled water *ad libitum* and also received the different medicinal formulation (labeled A-E) at 2.9µl/kg B.W (based on the estimated traditional human dosage of 200ml for an average man of 70kg). The experimental and control groups ran for forty-two days, while analysis was carried out every two weeks and weights measured also. At the end of week 4 of experimentation, administration of the formulation was discontinued while the experimentation continued for an extra 2 weeks to serve as a second control, to see the effect after withdrawal.

Table 1. *Major plant constituents in each formulation*

Source Of Formulation	Plant Part Used	Botanical Name Of Constituents	Local Name
Omoko, Aluu	Stem barks and leaves	Enantia chloranta Petivera allicea Achyranthes aspera Diospyros mespiliformis	Awopa Aboro,Abora Igi dudu
Rumuchakara, choba.	Stem, bark and Leaves	Alstonia boonei Chrysophyllum albidum Alstomia congensis Abutilon mauritianum	Ahun, Awun Furu,kawo Agbalumo
Alakahia	Stem and bark	Khaya grandifoliola Khaya ivorensis Aframomum melegueta Capsicum frutescens	Oganwo Ata-ire,atare Ata-ijosi
Rumuosi/Rumuekini	Leaves and roots	Bryophyllum pinnatum Allium sativum Cymbopogon citrates Ocimum basilicum Citrus aurantifolia	Ayuu,Garlic Osan wewe
Rumuokoro	Rhizome	Zingiber officinale Tithonia diversifolia Morinda lucida Lawsonia guineensis Azadiractha indica	Ginger Jogbo Agbale Oruwo Dongoyaro

NOTE: This table depicts the major constituents of each formulation and not the only constituents.

2.4 Sample collection

2.4.1 Blood

Each animal was sacrificed by anaesthetizing with chloroform first, then the jugular veins were slit with the aid of a surgical blade. Blood samples for the haematological assay were placed in EDTA bottles.

2.4.2 Organs

Each animal was laid on a flat hard surface with the fore and hind limbs, pinned to the surface. Samples organs were collected after dissection of the underlying skin of the abdominal and thoracic cavities. The samples organs were collected by hand to prevent scaring of the liver tissues by other materials and weighed. Liver was stored in 10% formalin in preparation for histological examination. Samples were analyzed at the University of Port- Harcourt teaching hospital (U.P.T.H) haematological laboratories.

3. Results

The results of assay of heamatological parameters are shown in table 2-5. Mean serum estimation of RBC, WBC and Platelet counts were seen to increase from week 2. While the PCV was slightly altered in all five groups.

Table 2. *Effect of formulations A-E on the mean PCV (%) at weeks 2, 4 and 6.*

Formulation	Week 2	Week 4	Week 6
Control	5.55±0.13		
GROUP A	41.75±1.26b	44.62±1.88a	41.50±1.29b
GROUP B	41.25±0.95b	45.50±1.29a	41.00±0.82b
GROUP C	40.00±0.82b	43.25±1.50b	40.00±0.82b
GROUP D	41.00±0.82b	45.25±0.96a	40.25±1.71b
GROUP E	41.00±0.82b	43.75±0.96a,	40.50±0.58b
Values are expressed as Mean ± Standard deviation. Superscript a indicates significant difference between experimental and control groups, b No a indicates significant difference between experimental and control groups.			

Table 3. *Effect of formulations A-E on the mean WBC count (x 109/L) at weeks 2, 4 and 6.*

Formulation	Week 2	Week 4	Week 6
Control	5.55±0.13		
GROUP A	7.53±0.17a	11.00±0.16a	9.00±0.65a
GROUP B	7.40±0.07a	8.54±0.04a	7.03±0.05a
GROUP C	7.28±0.10a	8.93±0.10a	7.49±0.93a
GROUP D	7.80±0.18a	10.30±0.21a	8.15±0.13a
GROUP E	7.50±0.08a	9.85±0.13a	8.19±0.16a
Values are expressed as Mean ± Standard deviation. Superscript a indicates significant difference between experimental and control groups, b No a indicates significant difference between experimental and control groups.			

Table 4. *Effect of formulations A-E on the mean RBC count (x 109/L) at weeks 2, 4 and 6.*

Formulation	Week 2	Week 4	Week 6
Control	4.50±0.09		
GROUP A	4.67±0.12b	5.65±0.05a	4.48±0.02b
GROUP B	4.60±0.05b	5.25±0.26a	4.51±0.02a
GROUP C	4.38±0.07b	5.63±0.09a	4.52±0.04b
GROUP D	4.59±0.13b	5.54±0.03a	4.51±0.01b
GROUP E	4.31±0.01b	5.46±0.37a	4.44±0.01b
Values are expressed as Mean ± Standard deviation. Superscript a indicates significant difference between experimental and control groups, b No a indicates significant difference between experimental and control groups.			

Table 5. *Effect of formulations A-E on the mean Platelet count (x 109/L) at weeks 2, 4 and 6.*

Formulation	Week 2	Week 4	Week 6
Control	5.55±0.13		
GROUP A	250.50±0.58a	302.50±2.08a	256.50±1.29a
GROUP B	254.50±2.38a	288.25±1.71a	257.50±1.29a
GROUP C	270.00±0.81a	301.25±2.99a	278.25±1.50a
GROUP D	240.00±1.63b	280.00±5.10a	250.75±0.96a
GROUP E	230.00±0.82a	276.00±2.58a	256.75±1.50a
Values are expressed as Mean ± Standard deviation. Superscript a indicates significant difference between experimental and control groups, b No a indicates significant difference between experimental and control groups.			

4. Discussion

The various haematological parameters investigated in this study are useful indices of evaluating the toxicity of plant formulations in animals (Yakubu *et al.,* 2008). Assessment of haematological parameters can not only be used to determine the extent of deleterious effect of formulation on the blood of an animal, but it can also be used to explain blood relating functions of a plant extract or its products (Yakubu *et al.,* 2007). Analysis of blood parameters is relevant in risk evaluation as changes in the haematological system have higher predictive value for human toxicity when the data are translated from animal studies (Olson *et al.,* 2000). It was also observed that the serum count of White blood cells, Platelets and Red blood cells were averagely increased during each period of testing at weeks 2, 4 and 6. This increase was greatest in sub-groups administered 5.5µl/Kg B.W.

Although an increase in white blood cell count is normally indicative of infection (viral or bacterial), this is not the case as animals were kept in sterile cages as proposed by NIH guidelines. From the observed significant values of WBC, it is clear that an increase in the number of WBC is a normal reaction of rats to foreign substances, which alter their normal physiological processes. The increase observed in the indicates a stimulation of the immune system which protects the rats against infection. This increase in WBC, which may be directly proportional to the severity of the causative stress condition and can also, be attributed to an increase in leukocyte mobilization (Celik & Suzek, 2008). Also, when there is a significant increase in the white blood cell counts of the treated albino rats, this could signify toxicity of the drug on the bone marrow of the mice.

The effect of the formulation on the RBC may be an indication that the balance between the rate of production (erythropoiesis) and destruction of the blood corpuscles was not severely altered at 2.9µl/Kg B.W. For sub groups administered 5.5µl/Kg B.W, there was an increase in the RBC counts when compared to the control groups. This can be researched further for anaemic cases. Although the specific mechanism(s) through which the formulation facilitated the increase in these hematological indices was not ascertained in this study, this action is assumed to be a direct effect of the formulation on the haematopoietic systems.

It is possible that the extract contains such constituent(s) that can interact and stimulate the formation and secretion of erythropoietin, hematopoietic growth factors/committed stem cells. Specifically, stimulations of hematopoietic growth factors and erythropoietin systems have been reported to enhance rapid synthesis of blood cells (Murray, 2000).

5. References

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