# Effect of selected yoruba medicinal formulations on certain biochemical parameters in 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 evaluate the effect of this medicinal formulation on cetain biochemical 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. Markers of hepatocyte injury; alanine transaminase and aspartate transaminase, were initially reduced but increased at week 4. Same was observed for the cholestasis marker alkaline phosphatase. The levels of serum creatinine, urea and blood urea nitrogen steadily reduced over time. The histological findings of the liver sections indicated gross cirrhosis, degeneration, inflammation and apoptosis in the experimented groups as compared to the control group.

**Keywords:** Yoruba,biochemical 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 and 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 where purchased from several local traditional healers peddling them at Omoko in Aluu, choba, Alakahia, Rumuosi and Rumuokoro communities in Rivers state. 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. 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 setup

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.

## 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 enzyme analysis were collected in plane wash bottles, while blood samples for 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) chemical pathology laboratories.

## 3. Results

The results of assay of biochemical parameters are shown in table 2- 8. Significant decreases were observed in the mean serum activities of AST, ALT and ALP at week 2, while increases were observed at weeks 4 and 6 in all the experimental groups when compared to the control group. Decreases were also observed in the mean serum concentration of total creatinine and urea from week 2. The serum concentration of bilirubin was observed to build up as seen in table 5.

**Table 2.** *Effect of formulations A-E on the mean serum AST activity (IU/L) at weeks 2, 4 and 6.*

| Formulation | Week 2 | Week 4 | Week 6 |
|---|---|---|---|
| Control | 120.00±1.63 | | |
| Group a | 112.00±1.63a | 122.00±1.41b | 130.00±1.63a |
| Group b | 100.25±2.5a | 121.00±1.83b | 129.00±0.82a |
| Group c | 112.25±2.22a | 122.00±1.41b | 130.00±1.83a |
| Group d | 115.00±0.82a | 125.00±0.82a | 132.00±0.82a |
| Group e | 113.00±1.63a | 130.00±1.63a | 135.25±2.06a |
| 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 serum ALT activity (IU/L) at weeks 2, 4 and 6.*

| Formulation | Week 2 | Week 4 | Week 6 |
|---|---|---|---|
| Control | 30.00±0.82 | | |
| Group a | 24.25±0.96a | 31.00±0.82a | 36.75±3.18a |
| Group b | 23.25±0.96a | 30.75±0.96a | 42.00±4.83a |
| Group c | 23.00±0.82a | 29.25±0.96a | 36.75±0.96a |
| Group d | 22.50±1.29a | 31.25±1.26a | 38.25±0.96a |
| Group e | 24.75±0.96a | 32.75±0.96a | 39.00±0.82a |
| 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 serum ALP activity (IU/L) at weeks 2, 4 and 6.*

| Formulation | Week 2 | Week 4 | Week 6 |
|---|---|---|---|
| Control | 320.00±1.00 | | |
| Group a | 331.75±1.25a | 356.25±0.96a | 370.75±0.96a |
| Group b | 326.25±1.70a | 361.00±0.82a | 371.50±1.29a |
| Group c | 316.75±1.25b | 342.75±2.21a | 363.50±3.11b |
| Group d | 319.00±0.82b | 369.25±0.96a | 380.25±0.50b |
| Group e | 302.75±2.21b | 353.50±3.11a | 361.00±1.41a |
| 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 serum Bilirubin conc. (µmol/L) activity at weeks 2, 4 and 6.*

| Formulation | Week 2 | Week 4 | Week 6 |
|---|---|---|---|
| Control | 5.91±0.11 | | |
| Group a | 7.13±0.16a | 7.96±0.30a | 8.17±0.19a |
| Group b | 7.26±0.25a | 7.48±0.38a | 8.21±0.51a |
| Group c | 6.46±0.26b | 7.09±0.11a | 8.10±0.15a |
| Group d | 6.40±0.32b | 7.21±0.17a | 8.15±0.13a |
| Group e | 6.68±0.46a | 7.08±0.09a | 8.17±0.13a |
| 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 6.** *Effect of formulations A-E on the mean serum Creatinine conc. (µmol/L) at weeks 2, 4 and 6.*

| Formulation | Week 2 | Week 4 | Week 6 |
|---|---|---|---|
| Control | 87.45±0.76 | | |
| Group a | 85.78±1.48a | 71.53±1.04a | 68.74±0.31a |
| Group b | 81.06±0.77a | 76.59±0.46a | 71.29±0.83a |
| Group c | 87.80±0.91b | 69.43±0.68a | 64.77±0.40a |
| Group d | 85.30±0.50a | 71.38±1.11a | 68.18±0.62a |
| Group e | 86.75±0.95b | 69.00±0.81a | 65.33±1.04a |
| 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 7.** *Effect of formulations A-E on the mean serum Urea conc. (mmol/L) at weeks 2, 4 and 6.*

| Formulation | Week 2 | Week 4 | Week 6 |
|---|---|---|---|
| Control | 5.80±0.005 | | |
| Group a | 5.42±0.24a | 4.92±0.01a | 4.73±0.30a |
| Group b | 5.42±0.02a | 5.15±0.04a | 5.11±0.02a |
| Group c | 6.08±0.10a | 5.13±0.01a | 4.92±0.01a |
| Group d | 6.21±0.01a | 5.54±0.04a | 4.26±0.01a |
| Group e | 5.25±0.58a | 4.96±0.06a | 4.27±0.17a |
| 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 8.** *Effect of formulations A-E on the mean Blood urea nitrogen conc. (mg)*

| Formulation | Week 2 | Week 4 | Week 6 |
|---|---|---|---|
| Control | 2.71±0.00 | | |
| Group a | 2.53±0.14a | 2.30±0.01a | 2.21±0.02a |
| Group b | 2.53±0.01a | 2.41±0.02a | 2.39±0.01a |
| Group c | 2.84±0.05a | 2.30±0.01a | 2.45±0.02a |
| Group d | 2.90±0.00a | 2.59±0.02a | 1.99±0.01a |
| Group e | 2.46±0.29a | 2.31±0.29a | 1.99±0.12a |
| 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 biochemical 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).

The liver plays an important role in many metabolic processes; any disturbance in the liver would affect the normal level of measurable biochemical parameters in this organ. AST, ALT, and ALP are marker enzymes present in high concentrations in the liver, when liver cells are inflamed or damaged, these enzymes leak into the blood stream leading to a rise in the plasma level of these enzymes (David, 1985; Nkosi *et al.,* 2005). ALT is selectively a liver parenchymal enzyme than AST and a sensitive indicator of acute liver damage (Shah *et al.,* 2002). Thus elevation of these enzymes in our study indicates inflammation or damage of the liver cells.

It was observed that at week 4, the level of liver enzymes (AST, ALT and ALP) has increased which is indicative of liver damage. At week 6 there was a significant increase in the serum concentration of liver enzymes when compared to the treatment groups at weeks 2 and 4 and also with the control groups. This effect proved irreversible at 5.5µl/Kg B.W dosage as the level of these liver enzymes still increased despite termination of formulation administration.

Nerbert in his publication also reported that damaged liver cells lose the ability to conjugate bilirubin or remove unconjugated bilirubin from the blood thus an increase in unconjugated bilirubin in the serum. The fact that the direct bilirubin is high in this study indicates liver damage. The low levels of albumin, total protein and creatinine in the serum may also explain damage to the liver and diminished synthetic function of the liver.

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