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Toxicological Evaluation of Five Herbal Drugs Hawked In Minna, Niger State

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ABSTRACT

The medicinal and cultural acceptance of herbal drugs has been established since ancient time but often without any toxicological assessment. In the present study the toxicological assessment of five herbal medicinal concoctions sold in Minna, Nigeria was carried out in mice. Parameters determined included weight variations, packed cell volume (PCV), total serum protein, glucose and triacylglycerides which were compared to control groups that were administered 20ml/kg body weight of normal saline. Phytochemical analysis revealed the presence of alkaloids, tannins, glycosides and flavonoids in most of the drugs. Safe doses of the drugs in the rodents were determined to range between 150 – 800mg/Kg body weight while LD_{50} were in the range of 800 – 2500mg/Kg body weight. Serum glucose, total proteins and triglycerides were each significantly ($p<0.05$) elevated in at least three of the five drug treatments at the end of the five weeks study period. There were however consistent decline in total body weights and packed cell volumes of the experimental animal during the same period. These results constitute early indices to the potential adverse physiological effects of repeated usage of the concoctions analysed.

INTRODUCTION

The search and use of drugs and dietary supplements derived from plants have accelerated in recent years. Pharmacologists, microbiologists, botanists, and natural-product chemists are combing the earth for phytochemicals and leads that could be developed for the treatment of various diseases. In fact, according to the World Health Organization (WHO, 2002), approximately 25% of modern drugs used in the United States of America have been derived from plants. A review of studies into medicinal plants used to treat various diseases across some ethnic and cultural groups in Nigeria showed that there were more than 110 plant varieties used to treat malaria alone (Jigam, 2008). Herbal preparations are freely hawked in Minna, Niger state as is prevalent in other Afican societies. Such drugs are readily available and affordable. These herbal medicinal preparations are used to treat various healthconditions resulting to an increase in the number of people who resort to these cheap resources for their immediate health needs. Studies on liquid herbal drugs have also revealed examples of the following combinations: 'Agbo iba' for treatment of malaria, which contains *Hippocratea indica*, *Nauclea latifolia*, *Enantia spp*. lime, and the bark of *Mangifera indica* (mango); 'Agbo jedijedi' for treatment of fistula containing *Tetrapleura tetraptera*, *Ancistrophyllum secundifolum*, *Eugenia caryophyllus* (cloves), and *Parinari*; 'Agbo giri' for treatment of convulsions in children consisting of *Ocimum gratissimum* and black alum and 'Agbo narun' for treatment of skin rashes consisting of *Lophira alata*, *Ceiba pentandra*, and *Pergu lariadaemia*. (Adebayo and Krettlia, 2011 and Mike *et al*., 2011). Such herbal drugs investigated were found to contain a mixture of three or four of the medicinal plants listed above. The rational for the combination could not be explained by the hawkers as it was a practise that has been found to be effective 'to the best of their knowledge'. Reports of the patients experiencing negative health consequences caused by the use of herbal medicines are also on the rise (Ewu, 2010). The need to analyse the physiological effects of such concoctions is hence imperative.

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MATERIALS AND METHODS

Herbal Concortions.

About one litre each of the already prepared herbal drug extracts were purchased. The mother solution was filtered with muslin cloth and the solvent evaporated over a water bath at 40^0C. The semi-solid residue was weighed, placed in labelled sample bottles and stored in the refrigerator at 4^0C until required for analysis. All samples collected were analyzed in the laboratories of the Department of Biochemistry Federal University of Technology, Minna, Niger State and the National Institute of Pharmaceutical Research and Development (NIPRID), Abuja, Nigeria.

Laboratory Test Animals

Healthy Swiss albino mice (Sprague Dawley strain) with average weights in the range of 20 – 30g were purchased from the National Institute of Pharmaceutical Research and Development (NIPRID), Abuja and Niger State Veterinary Center, Minna. The mice were maintained in plastic cages with wire mesh in the biochemistry laboratory and were allowed free access to pelletized broiler finisher feed (Grand Cereals and Oil mills, Jos) and water. The experiments were conducted in strict compliance with internationally accepted principles for laboratory animal (CCAC, 1997).

Phytochemical Screening of Herbal Drugs

This consisted of simple chemical test to detect the presence of the following phytochemicals: alkaloids, tannins, saponins, cardiac glycosides, anthraquinones and phlobatannins. Extracts were suction filtered until colourless filtrates were obtained necessary for colour reactions characteristic of these tests. The methods described by Harborne (1998) were used to ascertain the presence of alkaloids, cardiac glycosides and phlobatannins. The presence of saponins was detected using the screening procedures of Sofowora (1993), while tannins and anthraquinones were screened for using the method of Trease and Evans (1985).

Safe dose (pre – LD_{50}) Determination

Preliminary acute toxicity of the drug was tested in swiss albino mice using different doses. Six groups of four animals each, were given different doses of the crude extract at a range of 100 – 1400 mg/kg body weight respectively. The drugs were suspended in water and given intraperitoneally (ip) using sterile apyrogenic disposable syringes. A control group was given normal saline (0.9% w/v NaCl) at 20ml/kg body weight. The mice were observed over 72 hours (Gamaniel, 2000) for clinical signs and mortality. Suitable doses were hence identified for each herbal drug and used in subsequent analyses.

Toxicology of Herbal Drugs

Mice were kept in two groups consisting of twenty (20) animals: Group A – test, were gavaged with herbal drug using the pre-determined doses on alternate days. Group B – control, were given 20ml/kg body weight normal saline daily. Both groups were maintained on a normal diet. Four mice were randomly selected from each group at the end of each week and analysed for the different parameters over five weeks (Day 7, Day 14, Day 21, Day 28 and Day 35 respectively).

Weight Determination

Weights (g) of mice were taken using an Avery Balance (W and T Avery Ltd, Birmingham, U.K.) at intervals of seven days for five weeks.

Blood Sample Collection

The animals were sacrificed and blood samples were collected using tuberculin syringes and transferred into specimen bottles containing fluoride oxalate as anticoagulant. Samples were collected at intervals of seven (7) days over a period of thirty-five (35) days from both the test and the control mice. Blood samples were later centrifuged at 3,000rpm for 10 minutes using a MSE centrifuge (Centaur 2). The plasma was collected and dispensed into serum bottles.

Determination of Packed Cell Volume (PCV)

The microhaematocrit method of Gamaniel (2000) was used in the determination of packed cell volume. An uncalibrated capillary tube was filled to two- third (2/3) of its volume with blood by capillary action and one end sealed with a crystal seal (Cat. No.1503; Hawkley and Sons Limited, lancing Sussex.) The tubes were transfered to the haematocrit centrifuge and allowed to spin at 12,000 rpm for 5minutes. The packed cell volume was determined using the haematocrit measuring guage.

Determination of Serum Biochemical Parameters

The determination of blood glucose is adapted from the Glucose oxidase method of Tietz (1995)**,** total proteins were determined using Randox Standard manual for in vitro quantitative plasma/serum total protein diagnostic kit (Cat. No. TP 245-Randox laboratories, Crumlin, U.K) while triacylglycerides were determined using the AGAPPE Triglyceride kit (Cat. Nos. 1121500 – 11215004 – 'Agappe Hills', kerala India). The tests were carried out using the manufacturer's instructions.

RESULTS

The results of the phytochemical tests are in Table 1. It indicates the presence of a variety of plant secondary metabolite, the most prevalent of which are alkaloids, glycosides, steroid neucleus, tannins and flavonoids. Caffeine, cyanophoric glycosides and volatile oils were each detected in only a single concortion.

Results of acute toxicity studies (Table 2) indicate that safe doses and LD_{50} ranged between 300 – 800 mg/Kg body weight and 800 – 2500 mg/Kg mice body weights respectively for the different drugs. Table 3 presents the results of the serum biochemical parameters tested. Serum glucose level for test mice

administered with samples a and c were significantly ($p<0.05$) elevated by weeks 1 and 3 respectively but a recovery was made in mice administered with sample c by week 3. Results of serum total proteins (Table 4) indicate elevated levels from week 1 in sample c and d and in week 2 for sample a but remained elevated in samples a and c while a recovery was made in sample e. Triacylglyceride (TAGS) level (Table 5) was elevated in samples a, b and c throughout the test period. Weight variation (figure 1) indicates a gradual weight loss over the period of 5 weeks but sample c indicated a sharp decrease between weeks 2 and 3 followed by a recovery between weeks 3 and 5. Packed cell volumes (PVC) (figure 2) indicated an initial increase for sample c followed by a decline by week 2. Other samples resulted in a decrease in PVC but the effect was more pronounced with sample e.

Table. 1: Results of phytochemical analysis of herbal drugs.

PHYTOCHEMICALS	OBSERVATIONS				
	Sample A	Sample B	Sample C	Sample D	Sample E
Alkaloids	+++	++	+++	+	+++
Caffeine	-	+	-	-	-
Glycosides	++	+++	++	+	++
Anthraquinones	++	++	++	+	+
Cardiac glycosides	-	-	++	+	-
Cyanophoric glycosides	-	-	-	-	+
Steroidal nucleus	++	++	+	++	++
Tannins	+++	++	+++	++	+++
Hydrolysable tannins	++	++	+	-	++
Saponins	-	++	+++	+	+
Flavonoids	++	+++	+++	+	++
Volatile Oils	-	-	+	-	-
Resins	++	-	+	-	-
Balsams	-	+	-	+	+

Sample A = Agbo typhoid; Sample B= Agbo Jedi jedi; Sample C= Agbo iba; Sample D= Agbo muhu; Sample E = Agbo Arariro; + = Slightly present;++= Moderately present;+++= Highly present;-= Not detected

Table 2: Safe dose and LD_{50} of the different herbal drugs in mice.

Drug	safe dose(ug/Kgbw)	LD_{50} (mg/Kgbw)
Sample a	300	1000
Sample b	400	1500
Sample c	150	800
Sample d	800	2500
Sample e	400	1500

Sample A = Agbo typhoid;Sample B= Agbo jedi jedi; SampleC= Agbo iba; Sample D= Agbo muhu; Sample E=Agbo Arariro

Table 3: Serum glucose level in mice administered with different herbal.

Glc[mg/dl]	Week 1	Week 2	Week 3	Week 4	Week 5
Control	96.03 ±2.11	98.11±1.00	95.24 ±1.00	98.05 ±3.42	102.08 ±4.60
a	95.14 ±1.20	98.04 ±1.20	*105.00 ±1.04	*115.08 ±2.16	*116.41 ±1.00
b	94.15 ±1.11	96.0 ±2.00	96.05 ±1.25	96.03 ±2.11	*110.38±2.00
c	100.24 ±2.1	102.67 ±4.00	96.55 ±2.38	96.03 ±2.11	98.15 ±3.04
d	98.15 ±3.88	98.30±1.80	97.53 ±3.68	96.03 ±2.11	100.22 ±3.57
e	95.55 ±2.52	97.10±2.00	97.00 ±4.90	94.17 ±1.86	*115.02 ±3.26

Mean ± SEM, (n=20)*p<0.05 vs control group

Table 4: Serum Total Protein level in mice administered with different herbal.

Glc[mg/dl]	Week 1	Week 2	Week 3	Week 4	Week 5
Control	5.24 ±1.13	4.89 ±1.10	6.30 ±2.07	5.08 ±1.15	6.86 ±1.19
a	6.88 ±2.06	*7.54 ±1.08	*9.86± 0.47	*9.74 ±0.63	*9.66 ±1.28
b	5.63 ±0.44	4.67 ±0.21	7.83±0.16	7.88±0.32	7.59 ±1.29
c	*8.25 ±1.50	*8.55 ±0.20	*9.12±1.67	*9.86 ±1.28	*9.99 ±1.71
d	6.11±0.43	5.79±0.82	6.19±0.24	6.88±0.73	*8.18 ±1.10
e	7.22±1.35	*7.82±0.60	6.15±1.290	6.00±0.47	6.13 ±1.55

Mean ± SEM, (n=20)*p<0.05 vs control group

Table. 5: Serum Triacylglycerides (TAGS) levels in mice administered with different herbal.

Glc[mg/dl]	Week 1	Week 2	Week 3	Week 4	Week 5
Control	145.25±4.44	155.11 ±3.42	155.22±4.11	158.36±5.28	156.08±4.00
a	*160.97±2.38	*170.31 ±4.00	*175.56±3.56	*173.14±3.57	*175.00±3.66
b	150.0±12.14	*168.80 ±3.80	*165.13±2.58	*180.50±5.60	*178.11±3.99
C	154.35±1.25	149.66 ±0.25	*170.131.29	*175.553.33	*176.124.74
d	140.2±20.60	142.13 ±0.11	140.00±1.56	145.98±1.26	146.07±1.15
e	149.30±1.00	158.00 ±1.30	162.22±3.47	*168.88±2.15	*177.38±5.20

Mean ± SEM, (n=20)*p<0.05 vs control group

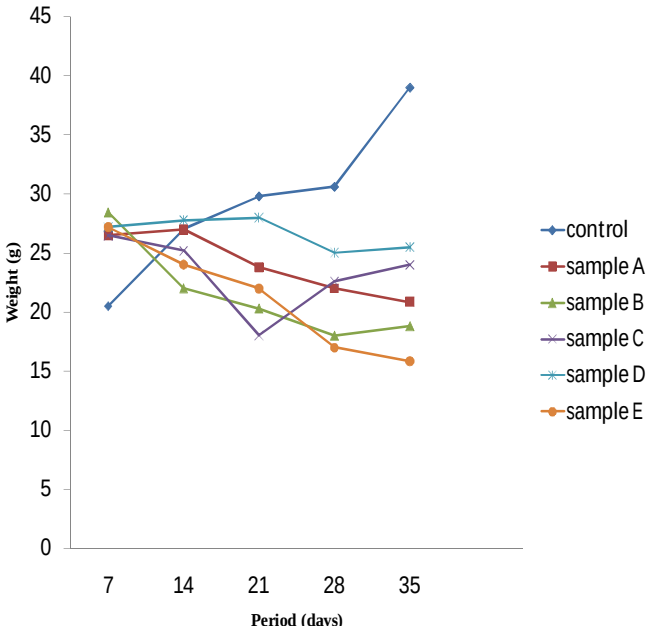

Fig. 1: weight variation in mice dosed with the different herbal drugs .

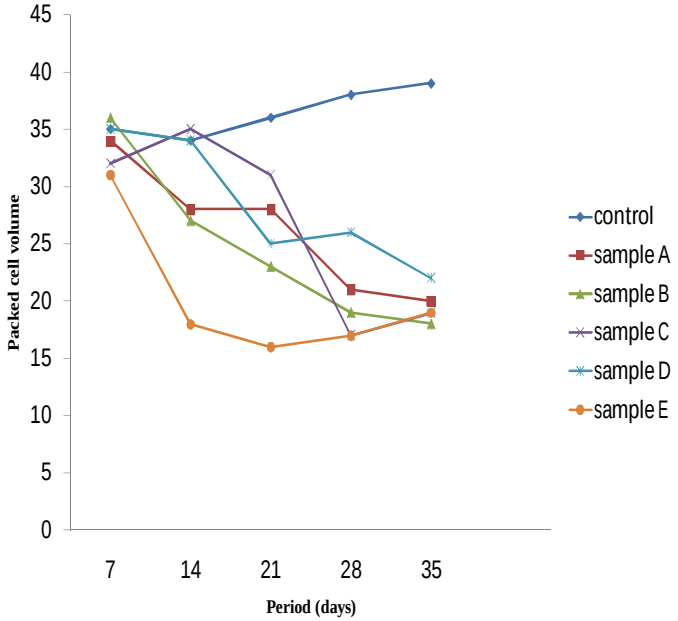

Fig. 2: packed cell volume (PCV) variation in mice dosed with herbal drugs.

DISCUSSION

Alkaloids as secondary metabolites contained in these drugs have been reported to possess broad spectrum antibacterial activity and are also used as analgesics and narcotics for pain relief. This supports earlier findings (Sofowora, 1979) which reported the anti-inflammatory action of neem (*Azadirachta indica*) used in treating malaria. Flavonoids, glycosides and cardiac glycosides found in the extracts are suggestive of their antioxidant properties and use of these compounds as anti-inflammatory agents in treatment of certain conditions have been reported (Iwu, 1993).

It is well known that herbal medicinal products usually contain more than one plant or active constituents and their therapeutic efficacy is not provided by a single group of compounds. Some of these compounds act synergistically to modify the bioavailability and efficacy of the active constituent. Moreover the composition of each drug is determined by the practitioner and therefore differences in composition may exist. Although it is the normal practice to determine the LD_{50} value, it is acceptable to limit the study with an acute toxicity test using several doses including reasonably high doses of the drugs. In the present study, acute toxicity was tested up to a high concentration of 1400mg/kg body weight (three times more than the safe dose). But as the dose increased signs of acute toxicity became more obvious recording one death. Since the main purpose of the preliminary acute toxicity study is to get some idea on conspicuous behavioural changes and death, if any, only 4 animals were used for each group considering the Institute's (NIPRID) Animal Ethics Committee views to minimize the use of animals.

In the present study, the LD_{50} was greater than 2000mg/Kg body weight for a single drug. The extracts could therefore be classified as being mostly safe following the Organization for Economic Cooperation and Development (OECD) (2005), guideline for acute oral toxicity - an LD_{50} less than 2000mg/Kg body weight was considered to be safe.

Significant ($p<0.05$) elevation by any given sample in serum glucose levels reflects an effect on the pancreatic islets of langerhans which are responsible for insulin production (Gad, 2001). A consistent hypoglycaemic effect was however noted for a particular drug. This enforces the wide spectrum of pharmacological action.

The total protein level in some of the treated animals showed appreciable increase over five weeks. Absolute alterations in total serum protein are suggestive that the functions of the kidney may have been compromised (Gad, 2001).

The elevated levels of triacylglycerides in the test animals over the control in the first week for sample a, second week in sample B and third week in sample c and e, is suggestive of the presence of hyperlipidemic agent. There was no significant ($p<0.05$) for test groups fed with sample d. Triacylglycerides are alternative sources of metabolic energy and are readily mobilized when the need arises (Martin *et al.*, 1975) such as the stressed state of experiments.

The effect of the extract on total body weight of the animals during the sub-chronic administration of the drug may have resulted from the reduced food and water intake. It could also be attributed to the presence of anti-nutritional substances such as tannins and saponins. Tannins inhibit growth by decreasing the digestive coefficients of proteins (El-Sayyad and Ross, 1983; Sotohy *et al.*, 1997). These substances have been reported to cause nutrient malabsorption (Kahnut *et* al., 1995). The presence of such anti-nutritional substances in herbal medicinal products have been reported (Puschner, 2000). Remarkable howerver is the

observation that animals dosed with sample c were able to regain weight after the initial decline. The decreased levels of packed cell volume (PCV) in the test animals may also be due to the presence of anti-nutritive substances (Tannins).

Anti-nutritive factors e.g. oxalates and phyrates chelate minerals e.g. iron and adversely affect the bioavailability of vitamins required for hemopoiesis (Shermer, 1967; Jigam *et al.,* 2011).

CONCLUSION

The analysed drugs should be used with caution especially in long term administration. Minerals and vitamins supplementation is advised where the use of these drugs is necessary.

RECOMMENDATIONS

The government should, through the appropriate agencies, take adequate control measures to set specific standards for quality and dosage for traditional herbal medicines.

In Nigeria, traditional medicinal practice is a main source of livelihood for a significant number of the population who depend on it as their main source of income. There is, therefore, the need for constant monitoring and quality control of herbal medicinal products processed, advertised, sold and used in Minna and Nigeria in general.

It is vital that the average person has access to accurate information on herbal therapies so they can make informed decisions about their health.

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