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Research Article

Effects of *Farayola Herbal Mixture* on the Anti-Oxidant Status of Phenylhydrazine - Induced Anaemic Rats

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Abstract

This study investigated the anti-anaemic efficacy of Farayola herbal mixture in phenylhydrazine-induced anaemia in rats. Phytochemical screenings were carried out on the aqueous and methanolic extracts of the constituent herbs. Male and female rats (100-160g) were divided into 6 groups of 5 rats each. Group 1 was the control, 2, 3, 4, 5 and 6 were administered phenylhydrazine (8mg/kg) to induce anaemia and treated with 400mg/kg, 800mg/kg, 1200mg/kg and 1600mg/kg of the extract respectively, for 14 days while group 6 was untreated. Red blood cell counts (RBC), packed cell volume (PCV)and haemoglobin concentrations(Hb) were determined before and after induction of anaemia, 7 and 14 days after treatment. Liver and kidney were harvested for histological analysis and assay of reduced glutathione (GSH), superoxide dismutase (SOD), catalase and malondialdehyde (MDA). RBC, PCV and Hb increased ($p \leq 0.05$) in the treated anaemic rats when compared with the control. There was no change in the group adminstered 1600mg/kg dose of the extract. Hepatic MDA, GSH and SOD concentrations in the anaemic rats showed no difference compared with the control. There was significant increase($p \leq 0.05$) in the hepatic and renal catalase activity while renal GSH was significantly lowered in anaemic rats; MDA and SOD activites were unaltered while catalase activity was higher in the extract-treated anaemic groups ($p \leq 0.05$). Groups administered 1200mg/kg and 1600mg/kg dose of the mixture showed pathological abnormalities with decrease in the concentration of MDA. The mixture possesses haematinic and antioxidant properties but may be toxic at high doses.

Keywords: Farayola, herbal mixture, haematology, anti-oxidant, histology

INTRODUCTION

Over the years, medicinal plants have been recognised to be of great importance to the health of individuals and communities. In many developing countries, herbal medicines are assuming greater importance in primary health care and their international trade has increased. However, the markets in these countries are not adequately regulated and many herbal products in circulation are unregistered by national regulatory bodies (WHO, 1996).

Anaemia constitutes a serious health problem in many tropical countries because of the prevalence of malaria and other parasitic infections (Dacie and Lewis, 1994). In anaemia there is decreased level of circulating haemoglobin, less than 13 g/dl in male and 12 g/dl in female (Okochi *et al*., 2003). In the tropics, due to endemicity of malaria, between 10 to 20% of the population presents less than 10 g/dl of haemoglobin concentration (Diallo *et al*., 2008) with children being more vulnerable.

A good number of medicinal plants are traditionally employed to ameliorate deleterious effects of anaemia. Some of these plants include *Telfeira occidentalis*, *Combretum dolichopetalum*, *Psorospermum ferbrifugum*, *Jatropha curcas*, *Flacourtia flavenscens and Brillantasia nitens* (Alada, 2000; Dina *et al*., 2006).

Farayola anti anaemic herbal mixture manufactured by Reverend Farayola in Ogbomosho, Oyo State, Nigeria, is a commercial herbal preparation that recently made its way into the herbal medicine market in Nigeria. The main objective of this study was to investigate the claim by the manufacturer that the product ameliorates and reverses the negative effects of anaemia. The specific objective was to determine the effect of the herbal mixture on the full blood count and antioxidant status of phenylhydrazine - induced anaemic rats.

MATERIALS AND METHODS

Faryola Herbal mixture and the constituent herbs used in its preparation were obtained from Atipo Ventures Limited, Onipanu Village,Iresapa Road, Ogbomoso, Oyo State, Nigeria. The mixture contains *Zanthoxylum zanthoxylores* (32%), *Vicia Faba* (18%), *Alcohornea* (14%), *Cordifolia* (14%), *Alinus glustinesa* (14%) and Caramel (22%).

Preparation of extract

The herbal mixture was concentrated using Telstar Josep freeze dryer (Cryodos model 2009). Aqueous and methanolic extracts of the constituent herbs were prepared by soaking it for a period of 48 hours. The filtrate was then concentrated with a freeze dryer.

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Phytochemical screening
Phytochemical screening was done on the dried sample, aqueous extract and methanolic extract using standard procedures as described by Sofowara (1993), Evans (1989), and Harborne (1973).

Animal studies
Thirty (30) Wistar albino rats (weighing 100-160g) were purchased from the animal house of the College of Medicine, University of Lagos, Idi-Araba, Lagos. The rats were kept in polypropylene cages and were allowed to acclimatize for two weeks with access to clean water and rat chow obtained from Livestock feed Nigeria Plc. A cycle of light and dark (12 hours each) and a temperature of 27±2 ^{0}C were maintained in the cages. The protocol was conducted in line with the internationally accepted guidelines on use of animals in research. The rats were divided into 6 groups of 5 rats each. Group 1 was the control, 2, 3, 4, 5 and 6 were administered 8mg/kg of phenylhydrazine to induce anaemia and treated with 400mg/kg, 800mg/kg, 1200mg/kg and 1600mg/kg of the mixture (Peter *et al*., 2009), respectively for 14 days while group 6 was not treated.

Haematological parameters
Blood samples were collected by ocular puncture into EDTA bottles to determine the full blood count before and after induction of anaemia, 7 and 14 days after treatment. Haematological parameters were determined with the use of Mindray BC – 3200 Auto Haematology Analyzer in the Central Research Laboratory, Lagos University Teaching Hospital (LUTH).

Biochemical analysis
The liver and kidney were washed and homogenised in 0.1M phosphate buffer (7.2), the concentration of reduced Glutathione (GSH) was determined by the method of Jiang *et al*., (1992); super-oxide dismutase (SOD) by the method of Nishikimi *et al*. (1972); catalase (CAT) by modified method of Aebi, (1984)and malondialdehyde (MDA) by the method of Sedlak and Lindsay (1968)

Histological Analysis
The rats' liver and kidney were excised and preserved in 10% formal saline, processed and stained with Haematoxylin and Eosin (H & E) stain. The slides were examined at a magnification of 100. All photomicrographs were taken in the Department of Anatomy and Molecular Pathology, LUTH, using Olympus microscope, Tokyo, Japan.

Statistical analysis
All values were expressed as mean ± standard error of mean and the statistical significance between treated and control groups were analysed by one way analysis of variance (ANOVA) and Tukey's *post-hoc* test using SPSS statistical software (version 20.0). $p<0.05$ was considered significant.

RESULTS

Phytochemical screening
The preliminary phytochemical screening of the aqueous and methanolic extracts of the constituent herbs show that they contain saponins, alkaloids, flavonoids, tannins, phlobatannins, steroids and reducing sugars. Tables 1a-k, Figures 1-6 and Plates 1-12 show the results obtained from the haematological, anti-oxidant enzymes and histological analysis respectively.

Table 1a:
Haematological parameters in experimental animals administered Farayola *herbal mixture* for 14 days

Day	Treatment groups	Red Blood Cell Count of rats(Mean± SD) mm^3	Hemoglobin Concentration of rats (Mean ± SD) g/dl	Pack Cell Volume of rats (Mean ± SD) %	White blood cell count of rats (Mean ± SD) mm^3	Platelet count of rats (Mean ± SD) mL
7	Positive Control	7.71±0.62	12.90±1.14	41.20±3.89^{d}	12.53±1.72^{d}	643.67±62.40^{c}
	400mg/kg extract	10.99±0.97	11.77±1.10	41.33±2.68^{ad}	11.57±5.36^{ad}	790.00±192.20^{ac}
	800mg/kg extract	10.09±0.81	11.63±3.27	40.17±10.00^{ad}	20.70±7.18^{ad}	684.67±162.80^{ac}
	1200mg/kg extract	10.03±0.30	10.87±0.21	38.57±1.43^{ad}	10.83±1.88^{ad}	658.00± 217.12^{ac}
	1600mg/kg extract	9.07±0.25	10.13±0.31	32.10±2.00a^{ac}	20.53±6.81^{ad}	718.00± 55.34^{ac}
	Negative Control	3.97±0.19^{b}	10.97±1.33	20.57±1.76^{b}	50.10± 8.23^{b}	814.00± 59.03^{a}
14	Positive Control	7.69±0.38$^{1,2,3,4,5\,*}$	14.37±0.49^{c}	45.03±1.99^{d}	11.47±0.95^{d}	775.33±161.40^{c}
	400mg/kg extract	11.11±1.21^{1}	12.20±1.92^{ac}	43.13±4.61^{ad}	15.77±6.78^{ad}	581.33± 80.75^{ac}
	800mg/kg extract	10.98±0.55^{2}	9.57±2.74^{ac}	37.97±12.38^{ad}	17.57±9.23^{ad}	666.67±159.00^{ac}
	1200mg/kg extract	10.91±0.12^{3}	11.17±0.48^{ac}	40.40±3.40^{ad}	12.00±1.71^{ad}	470.33± 137.66^{ac}
	1600mg/kg extract	3.12±1.82^{4}	8.50±2.05^{ac}	32.77±2.63^{ac}	9.43±2.53^{ad}	572.67± 91.84^{ac}
	Negative Control	3.72±0.35^{5}	9.40±1.05^{a}	21.73±2.21^{b}	53.33±5.41^{b}	741.33± 37.74^{a}

Values are means ±S.D of 3 measurements a,b,c,d indicate significant differences at $p\leq0.05$ between tests and control

Table 1b :
Haematological parameters in experimental animals administered *Farayola herbal mixture* for 14 days

Day	*Treatment Groups*	*Mean corpuscular volume of rats (Mean ± SD) %*	*Mean corpuscular haemoglobin (Mean ± SD) %*	*Mean corpuscular haemoglobin concentration (Mean ± SD)*	*Neutrophil count of rats (Mean ± SD) mm^3*	*Percentage neutrophil of rats (Mean ± SD) %*	*Lymphocyte count of rats (Mean ± SD)mm^3*
7	Positive control	55.93±4.01^{c}	31.57±1.46^{d}	54.63±2.02^{d}	5.05±0.14^{d}	40.93±6.56^{c}	48.10±7.23^{c}
	400mg/kg extract	75.17±7.66^{ac}	21.30±2.42^{ad}	28.47±0.85^{ad}	2.69±0.94^{ac}	28.03±7.54^{ac}	64.47±8.02^{ac}
	800mg/kg extract	78.20±7.90^{ac}	22.53±3.24^{ad}	28.70±1.41^{ad}	4.67±4.47^{ac}	41.30±19.79^{ac}	49.13±21.53^{ac}
	1200mg/kg extract	76.87±2.63^{ac}	21.60±1.21^{ad}	28.13±1.19^{ad}	1.82±0.29^{ac}	23.50±9.82^{ac}	68.33±11.88^{ac}
	1600mg/kg extract	71.33±0.95^{ac}	23.57±0.95^{bc}	30.97±2.40^{bd}	8.13±1.56^{ac}	26.83±5.35^{ac}	68.17±3.21^{ac}
	Negative Control	58.83±2.41^{a}	17.30±0.63^{b}	31.77±0.65^{b}	12.00±0.66^{b}	29.30±3.70^{a}	69.20±1.57^{a}
14	Positive control	58.53±0.60^{d}	30.30±1.27^{d}	59.60±9.15^{ac}	3.73±0.15^{d}	32.67±2.49^{c}	57.33±4.97^{c}
	400mg/kg extract	72.27±14.43^{ac}	20.13±2.50^{ad}	28.17±2.17^{ad}	5.20±3.56^{ad}	30.80 ±7.50^{ac}	59.60±9.15^{ac}
	800mg/kg extract	83.00±19.42^{ac}	20.63±2.97^{bd}	25.37±2.84^{ad}	5.57±3.45^{ad}	31.10±11.69^{ac}	58.17±11.98^{ac}
	1200 mg/kg extract	68.37±4.64^{ac}	18.87±1.16^{ad}	27.77±2.75^{ad}	4.35±0.58^{ad}	36.43±4.45^{ac}	53.83±4.52^{ac}
	1600 mg/kg extract	65.60±0.46^{ad}	29.10±1.40^{bd}	39.80±4.90^{ad}	6.35±2.22^{ac}	27.00±2.46^{ac}	68.93±5.62^{ac}
	Negative Control	58.57±2.11^{b}	18.63±0.65^{b}	31.86±0.80^{b}	10.30±0.10^{b}	23.17±6.04^{a}	71.77±3.23^{a}

Values are means ±S.D of 3 measurements a,b,c,d indicate significant differences at p≤0.05 between tests and controls

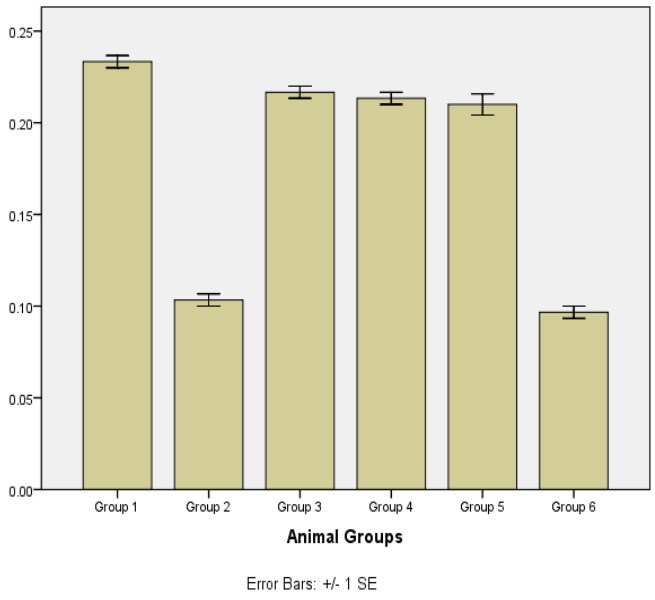


Figure 1:
Mean reduced glutathione (mmol/L/mg protein) in the liver of rats administered phenylhydrazine and treated with the extract at different concentrations when compared with the control group ($p \leq 0.05$). All data are expressed in triplicates of Mean ± SEM.

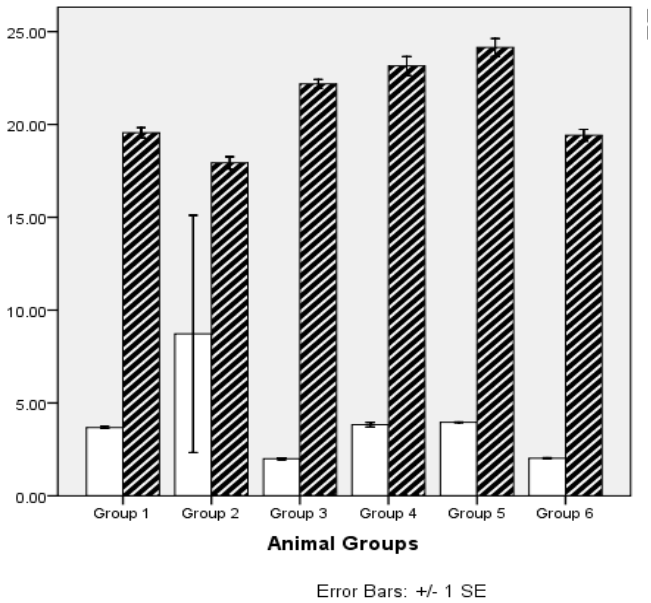


Figure 2:
Mean of super-oxide dismutase and catalase (Units/mg protein) in the liver of rats administered phenylhydrazine and treated with the extract at different concentrations when compared with the control group ($p \leq 0.05$). All data are expressed in triplicates of Mean ± SEM.

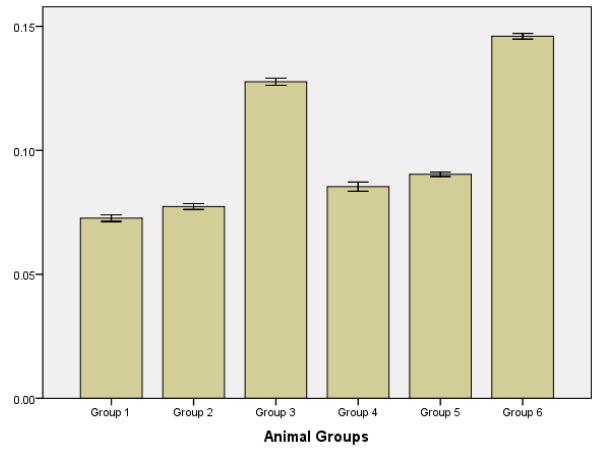


Figure 3:
Mean of Malondialdehyde (mmol/L/mg protein) in the liver of rats administered phenylhydrazine and treated with the extract at different concentrations when compared with the control group ($p \leq 0.05$). All data are expressed in triplicates of Mean ± SEM

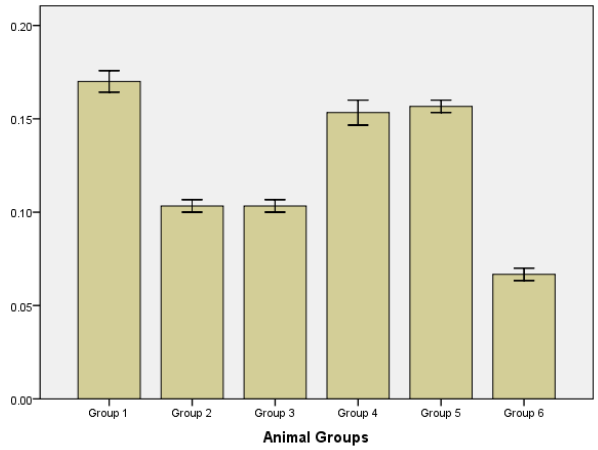


Figure 4:
Mean of reduced glutathione (mmol/L/mg protein) in the kidney of rats administered phenylhydrazine and treated with the extract at different concentrations when compared with the control group ($p \leq 0.05$). All data are expressed in triplicates of Mean ± SEM

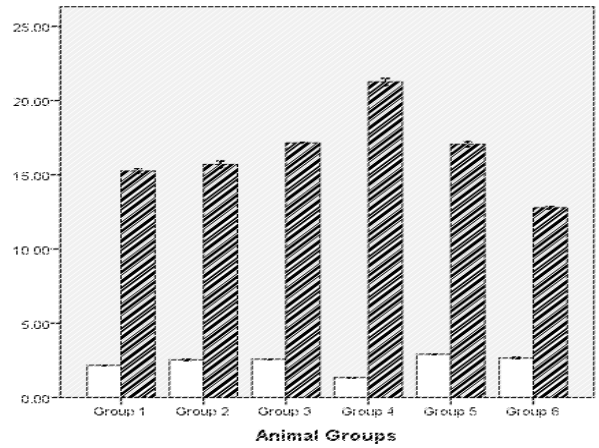


Figure 5:
Figure Mean of Super-oxide dismutase and Catalase (Units/mg protein) of the kidney of rats administered phenylhydrazine and treated with the extract at different concentrations when compared with the control group ($p \leq 0.05$). All data are expressed in triplicates of Mean ± SEM.

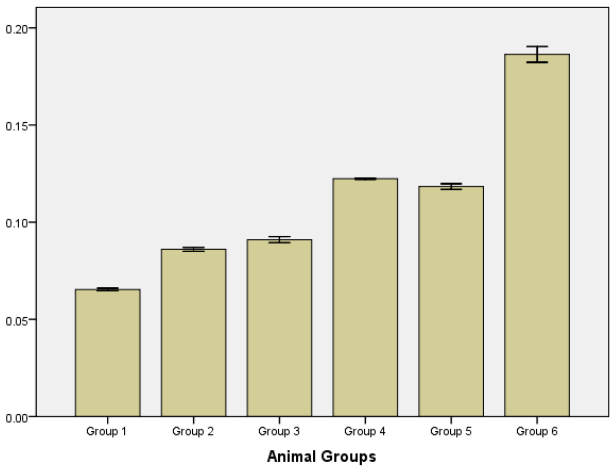
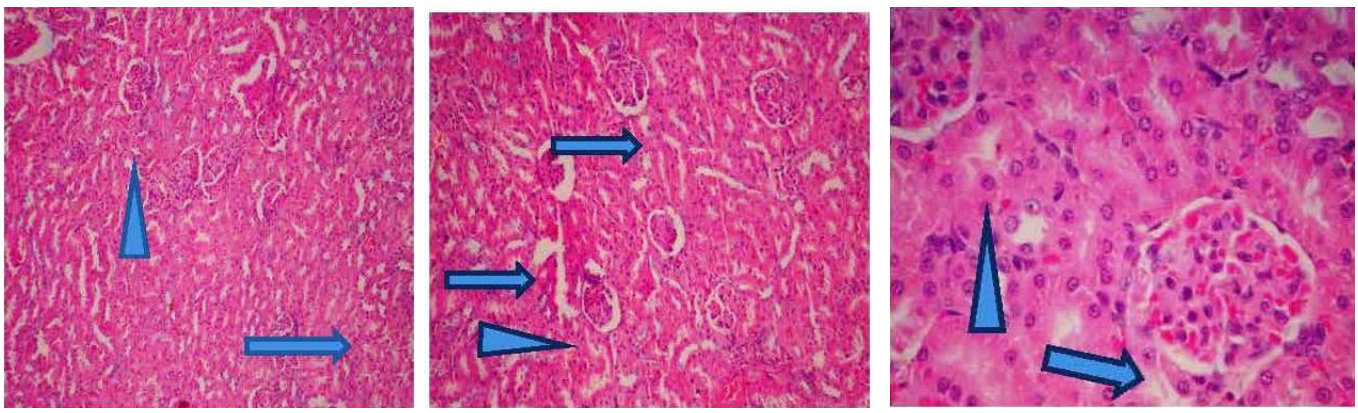
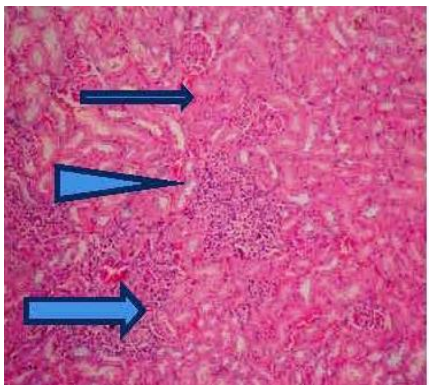


Figure 6:
Mean values of malondialdehyde (mmol/L/mg protein) in the kidney of rats administered phenylhydrazine and treated with the extract at different concentrations when compared with the control group ($p \leq 0.05$). All data are expressed in triplicates of Mean ± SEM



A B C

Plate A: Photomicrograph of normal kidney from rat administered water for 17 day showing normal glomeruli (thick arrow) surrounded by normal tubules (arrow head)
Plate B: Photomicrograph of normal kidney from a rat administered 400mg/kg of the extract for 14 days showing normal glomeruli (thick arrow) surrounded by normal tubules (arrow head)
Plate C: Photomicrograph of normal kidney from a rat administered 800mg/kg of the extract for 14 days showing normal glomeruli (thick arrow) surrounded by normal tubules (arrow head)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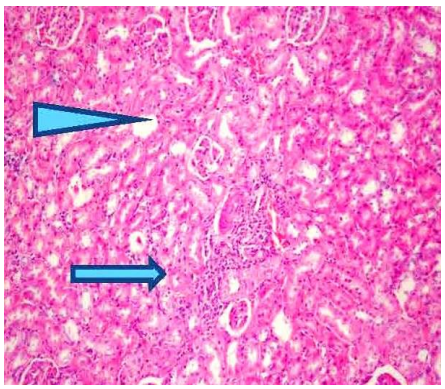
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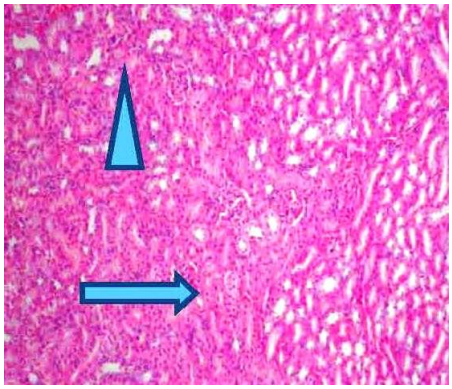
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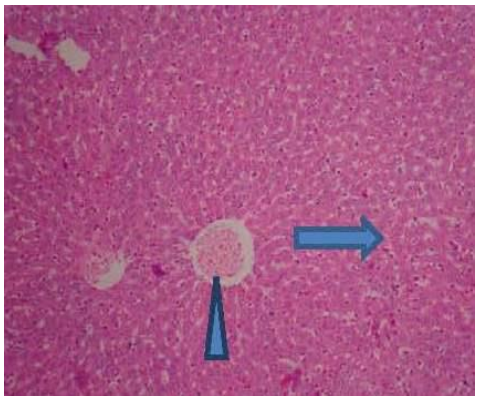
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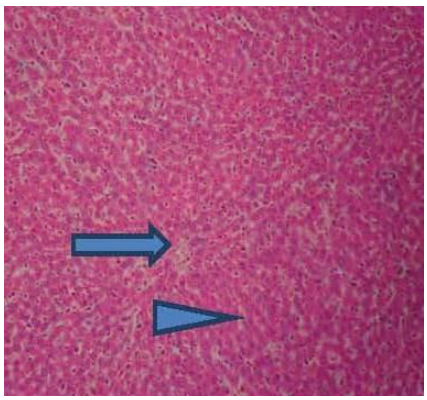
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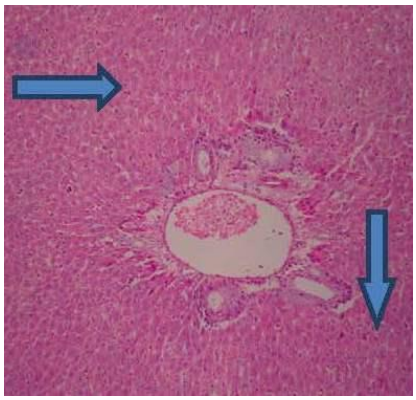
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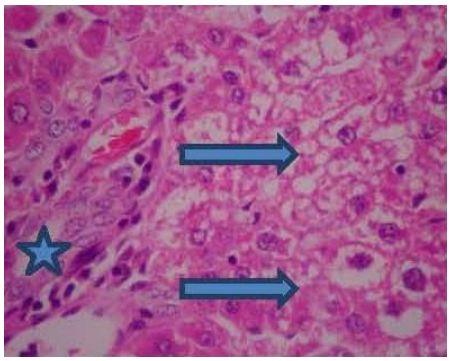
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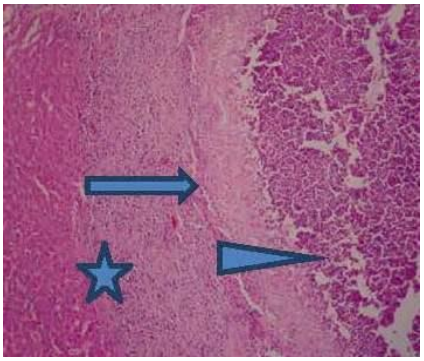
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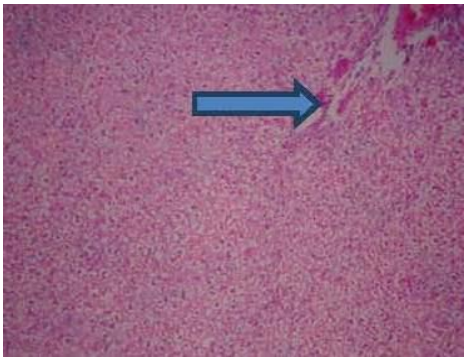
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Plate D: Photomicrograph of kidney from a rat administered 1200mg/kg of extract for 14 days showing normal glomeruli (thin arrow), normal tubules (arrow head) and focal area of interstitial inflammation characterised by infiltration by neutrophils (thick arrow)

Plate E: Photomicrograph of the kidney of a rat fed administered 1600mg of extract for 14 days shows focal interstitial inflammation (thick arrow) and normal glomeruli (arrow head) and tubules (thin arrow). X100

Plate F: Photomicrograph of normal kidney from a rat administered 8mg/ml of phenylhydrazine but not treated for 14 days showing normal glomeruli (thick arrow) surrounded by normal tubules (arrow head) x100

Plate G: Photomicrograph of normal liver of a rat administered water for 17 days showing normal hepatocytes (arrow) and normal sinusoids between the hepatocytes. A central vein (arrow head) is present.

Plate H: Photomicrograph of normal liver of rat administered with 400mg/kg of the extract for 14days; shows normal hepatocytes (arrow) and normal sinusoids between the hepatocytes. A central vein (arrow head) is present.

Plate I: Photomicrograph of normal liver of rat administered 800mg/kg of extract for 14 days showing normal hepatocytes (arrow) and normal sinusoids between the hepatocytes

Plate J: Photomicrograph of the liver from a rat administered 1200mg/kg of extract shows hepatocytes with intracytoplasmic inclusions (arrows) giving the hepatocytes a pale staining cytoplasm. A portal tract (star) is seen. X100

Figure K: Photomicrograph of liver of rat administered 1600mg/kg of extract for 14 days; showing an area of necrosis (arrow head) surrounded by fibrosis (thick arrow) and area of damaged hepatocytes (star). A rim of residual normal hepatocytes (A) is seen on the left side of the field. X100

Plate L: Photomicrograph of a liver of rat administered 8mg/kg of phenylhydrazine only and not treated for 14days; showing diffuse hepatocyte with intracytoplasmic vascularization (arrow). X 100

DISCUSSION

From the result there was a significant decrease in RBC, PCV and haemoglobin concentration after 3 days of administration of 8mg/ml of phenylhydrazine. This was in agreement with previous works of O'Riordan *et al* (1995) and Criswell *et al* (2002). Likewise there was an increase in the concentration of WBC and lymphocytes after the

induction of anaemia by phenylhydrazine. However, these parameters were restored to normal range after treatment with *Farayola Herbal mixture* suggesting its haematinic effect.

In this study, the haematological parameters became normal mainly after 7 days of treatment with the herbal mixture extract. Nevertheless, the group that received the highest dose (1600mg/kg) of the herbal mixture revealed a significant decrease in the RBC, PCV and Haemoglobin concentration. This could be due to the presence of some anti-nutritive such as flavonoids containing polyphenolic compounds including tannins, detected during pytochemical analysis of the mixture. MDA concentration shows no significant difference in the hepatic and renal tissues of the groups treated with the herbal mixture when compared with the control group ($p < 0.05$) However, it was higher in the untreated groups. This suggests that the presence of phenols and flavonoids could be responsible for its preventive role of lipid peroxidation.

In the liver, GSH and SOD showed no significant difference in most of the groups when compared with the control. However, there was an increase in the concentration of catalase activity in group 3, 4 and 5 when compared with the control group. In the kidney, there was no significant difference in concentration of reduced GSH in group 4 and 5 while there was slight decrease in group 2 and 3 when compared with the control. There was also a very significant decrease in the untreated anaemic groups. No significant difference was also observed in the SOD and CAT activities of the herbal mixture treated, anaemic groups when compared to the control. Although there was a significant difference in the group receiving 1200mg/kg dose of the mixture

From the histological analysis of the liver and kidney, the groups that received 400mg/kg and 800mg/kg of the extract were found to be normal. However, the groups that received 1200mg/kg and 1600mg/kg of the extract showed some pathological conditions. Hence, from this study *Farayola herbal mixture* is considered toxic at higher doses. The manufacturer of *Farayola herbal mixture* also made the following claims about the drug: prevention of painful crisis in sickle cell anaemic patients, increase appetite and regaining vitality and health. These claims about the drug are also worth investigating. Finally, it must be pointed out that the main constituent of *Farayola herbal mixture* is *Zanthoxylum zanthoxyloides* (32%), which is the same as *Fagara zanthoxyloides (orin ata)* that has been recognized as an antisickling agent for many years (Sofowora *et al* 1971, Sofowora *et al* 1975 and Ouattara *et al*., 2009).

In conclusion, the findings from this study confirm the claim by the manufacturer of *Farayola Herbal mixture* that it improves haematological indices during anaemic condition. The herbal formulation may help maintain red blood cell integrity, stimulate its production and enhance PCV thus ameliorating painful crisis in sicklers. However, further studies should be carried out on this mixture by treating anaemic conditions using a lower dose but for a longer duration.

Acknowlegdement

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References

Aebi, H., 1984. Catalase in vitro. *Methods of Enzymol.,* 105: 121-126.

Alada, A.R.A 2000. The hematological effect of *Telfelria occidentalis* diet preparation. *Afr. J. Biomed Res.* 3(3): 185-186.

Criswell, K., A.Sulhanen., A.F.Hochbaum, and M.B. Leavens. 2002. Effect of PHZ or phlebotomy on peripheral blood, bone marrow and erythropoietin in Wistar rats. *J. Appl. Toxicol.* 20: 25-29.

Dacie, I.V, and S.M. Lewis. 1994. *Practical Haematology*, 8th edn. Churchill Livingstone, London, pp. 49-59.

Diallo, A., M. Gbeassor ., A.Vovor ., K. Eklu-Gadegbeku., K.Aklikokou., A. Agbonon., A.A. Abena., C. de Souza., and K. Akpagana. 2008. Effect of *Tectona grandis* on phenylhydrazine induced anaemia in rats. Fitoterapia 79: 332-336

Dina, O.A, A.A. Adedapo., O.P Oyinloye ., and A.B. Saba. 2006. Effect of *Telfairia occidentalis* extract on experimentally induced anaemia in domestic rabbits. *Afr. J. Biomed Res.* 3: 181-183.

Evans, C.W 1989. Trease and Evans, Mexican medicinal plants. *J. Ethnopharmacol, pharmacognosy,* 13th Edn. Bailli ere Tindall, London.

Harborne, J.B. 1973. Phytochemical methods: *A guide to modern techniques of plant analysis*. 3rd edition. Chapman and Hall pub. London, U.K., 49: 188.

Harborne, J.B. 1996. The flavonoids: *Advances in Research* since1986. Chapman and Hall, New York.

Jiang, Z.Y.,J.Y Hunt, and S.P. Woulf. 1992. Determination of lipid hydroperoxides using the ferrous oxidation- xylenol orange method. *Ann. Biochem.,* 202: 384-389.

Nishikimi, M., N.A. Roa and K. Yogi, 1972. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. *Bioph. Res. Common.,* 46: 849-854

Okochi, Y.I, J.Okpuzor, and L.A. Alli 2003. Comparison of an African herbal formula with commercially available haematinics. *Afr. J. Biotechnol.* 2(8): 237-240.

Peter, A. A., E. Christian E. Okolo and C. Adaobi ,C. Ezike. 2009. The haematinic activity of the methanol leaf extract of *Brillantasia nitens* Lindau (Acanthaceae) in rats *Afr J. Biotech* 8 (10), pp. 2389-2393

Ouattara, B, O.Jansen., L. Angenot., I.P. Guissou 2009. Antisickling properties of divanilloyquinic acids isolated from *Fagara zanthoxyloides Lam* (Rutaceae) . *Phytomedicine* 16: 125-129.

Riordan, O.D.K, P.Sharp., R.M.Sykes., O. Epstein., and E.S..Debnam. 1995. Response to PHZ-induced haemolytic anaemia. *Eur. Clin. Invest*. 25: 722.

Sedlak, J, and R.H.L. Lindsay.1968. Estimation of total and non-protein sulfhydryl groups in tissue with Ellman's reagent. *Anal. Biochem.,* 25: 192-205.

Sofowora, E.A, W.A. Isaac-Sodeye., and L.O. Ogunkoya.1975. Isolation and characterization of an antisickling agent from *Fagara zanthoxyloides root. Lioydia* 38: 169–171

Sofowora, E.A, and W.A. Isaacs. 1971. Reversal of sickling and crenation in erythrocytes by the root extract of *Fagara zanthoxyloides. Lloydia* 34: 383–385

Sofowora, A.1993. Screening Plants for Bioactive Agents. In: Medicinal Plants and Traditional Medicine in Africa. 2nd ed. Spectrum Books Ltd., Sunshine House, Ibadan. pp. 81-93, 134-156.

World Health Organisation 1996. Traditional Medicine. Fact Sheet N134. *WHO*, Geneva.

Yeshoda, K.M 1942. Phenylhydrazine anaemia in rats. *Curr. Sci.* 11: 360-363.