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Research Article

The Normalizing Efficacy of Roselle (*Hibiscus sabdariffa*), Moringa (*Moringa oleifera*), Ginger (*Zingiber officinale*) and 'Ugwu' (*Telfairia occidentalis*) in the Liver Enzymes of wild rats (*Rattus rattus*) living around a Cement Plant

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Abstract

The normalizing efficacy of roselle, moringa, ginger and 'ugwu' in the liver enzymes of wild rats living within the vicinity of a cement factory was evaluated. The rats were placed into seven groups, comprising 15 rats per group. Group one was tagged control 1, and rats in this group were obtained from a cement dust-free zone. Group two was tagged control 2 and, together with groups three through seven, consisted of rats collected from the cement factory. All the rats in the seven groups were kept in the cement dust-free zone. Before commencing the experiment, the ALT, AST and ALP levels in the rats were measured using standard protocols. The rats in the test groups were thereafter treated with 400 mg kg^{-1} ethanolic extracts of roselle, moringa, ginger, 'ugwu' and a mixture of the plant extracts, respectively, while the control 1 and 2 rats received only distilled water (10 ml/day). The activities of the liver enzymes were monitored for a period of 180 days. At the end of the treatment, the percentage decrease in the liver enzymes of the test rats were significantly ($p<0.05$) higher than the percentage decrease in the liver enzymes of rats in control 1 and 2. This finding suggests the normalizing efficacy of the plant extracts. It also shows that the food plants could be used to normalize or reverse health problems caused by cement dust exposure.
Keywords: cement dust, liver enzymes, wild rat, plant extract, pollutant.

1. Introduction

The Cement industry is important to the economy because it employs a large number of people and provides cement required for housing and infrastructural development. However, in spite of its desirability, the dust generated from its production pollutes the environment and poses health hazard to exposed persons. Cement dust exposures have been shown to cause haematological diseases, multiorgan injuries, eyes and skin defects, weight loss, genetic problems and respiratory problems[1,2,3,4]. Despite the health hazards of cement dust, cement remains indispensable in the building industry attributable to its superiority over other materials[5]. Cement is durable, reliable, affordable and available compared to previous building materials[6]. Hence, there is a need to ameliorate the health effects of cement dust exposure in order to sustain the growth of cement industry.

Conventional pollution prevention and control strategies in the cement industry often fail due to lack of funds, strategies technicality, weak environmental protection laws, ignorance and non-disclosure attitudes of some cement manufacturers[5]. Consequently, pollution from cement plants remains a persistent problem with attendant health risks. Since

plant-based nutrition is one of the measures to solve health challenges, the normalizing and therapeutic efficacy of roselle, moringa, ginger and 'ugwu' plants on the liver enzymes of wild rats living around a cement plant were evaluated. The selected food plants are vegetables and spices found in West-Africa and many tropics of the world[7,8].

2. Materials

2.1 Animal Husbandry

Eighty-five wild rats (85) weighing between 230 and 280 g were collected from the vicinity of the cement factory and another twenty (20) rats of the same species weighing between 208 and 274 g were collected from a cement dust-free zone, about 30 km from the cement factory[9]. The rats were identified and authenticated by Mr. Nnamdi Amaeze in the Zoology Department, University of Lagos. The rats were left for about seven days in cages to acclimatize to the ambient temperature and humidity, and subjected to 12 hour light/dark cycle before commencing the research. Pellet feeds from the F. A Feeds industry, Lagos and water were given to the rats *ad libitum.*

2.2 Source of the Plant Materials

The plant materials- roselle (*Hibiscus sabdariffa* L.), moringa (*Moringa oleifera* L.), ginger (*Zingiber officinale* R.), and 'ugwu' (*Telfairia occidentalis* H.) were purchased from Ketu in Lagos metropolis. They were identified by a curator, Mr. Odewo T. Kolawole, in the Department of Botany, University of Lagos and the voucher numbers of authenticated materials are LUH 4394, LUH 4558, LUH 4396 and LUH 4395 for roselle, moringa, ginger, and 'ugwu', respectively.

2.3 Preparation of the Plant Materials

Fresh leaves of the plant materials were washed gently to remove impurities and air-dried under shade for one week. The dried leaves were milled into a powder using laboratory mill, Norris Limited, Poole, England at the Department of Pharmacognosy, University of Lagos. Besides the powder of individual plant materials produced, a mixture of the plant materials was also formed by mixing the four parts each of the ground plant materials in the ratio 1:1:1:1. The ground plant materials were then stored in desiccators before use.

2.4 Preparation of the Plant Extracts

The bioactive compounds were extracted from the plant materials using the method of Okigbo and Ogbonnaya (2006)[10]. Fifty grams (50 g) powder of each plant material and the mixture were put in 500 ml 95% cold ethanol and was allowed to stand for 72 hours. The extracts thus obtained were filtered with muslin cloth and evaporated to dryness at a temperature of 40±2^{o}C. The resulting dried extracts of each plant material yielded 6.6 g, 6.5 g, 6.2 g, 5.9 g, and 6.1 g of roselle, moringa, ginger, 'ugwu' and mixture, respectively. These dry extracts were reconstituted in water and were the decoctions used for the experiment.

2.5 Acute Toxicity Test

The acute toxicity of the crude extracts of the plants was measured using the 'Classical LD_{50}' method described by Gabriel *et al.* (2008)[11]. Albino rats (36) of both sexes weighing between 183 and 205 g were used for the studies. The rats were randomly distributed into six groups of 6 rats each and were made to fast for 12 hours before commencing the study. The control group received only distilled water, while the test groups were orally administered doses of 200, 400, 500, 700, 1500, and 2000 mg kg^{-1} of the crude extracts. The general symptoms of toxicity were monitored and recorded for each group within 24 hours.

2.6 Dosage Administered to the Rats

The acute toxicity test showed the plant extracts were nontoxic to the rats even at a dose of 2000 mg kg^{-1}. However, a dose of 400 mg kg^{-1} was chosen because a previous study by Adedapo *et al*. (2009)[12] showed that moringa extracts work best on the biochemical and haematological parameters of rats at a dose of 400 mg kg^{-1}.

3. Methods

3.1 Study Design

The wild rats (*R. rattus*) were placed into seven groups, comprising 15 rats per group. Group one was tagged

control 1, and rats in this group were obtained from the cement dust-free zone (about 30 kilometers from the factory). Group two was tagged control 2 and, together with groups three through seven, consisted of rats collected from the cement factory. All the rats in the seven groups were kept in the cement dust-free zone. The ALT, AST, and ALP levels in the rats were evaluated using standard protocols, before commencing the experiment. Groups three through seven of the rats were subsequently treated with 400 mg kg^{-1} ethanolic extracts of roselle, moringa, ginger, 'ugwu' and mixture, respectively, while group one and two (control 1 and control 2) of the rats received only distilled water (10ml/day). Thereafter, the ALT, AST and ALP levels in the rats were again monitored for 180 days.

3.2 Biochemical Studies (Liver Enzymes)

The biochemical study of the rats was carried out at the Biochemistry Department, National Institute of Medical Research, Yaba, Lagos.

3.3 Determination of Alanine amino Transferase (ALT)

The ultraviolet method described by Bergmeyer and Bernt (1974)[13] was used to determine ALT activity using Randox test kits (RANDOX laboratories, Crumlin, Antrim, UK). The reagent for ALT assay composes of Phosphate buffer containing L-alanine and α-oxoglutarate. ALT activity was measured by monitoring the concentration of pyruvate hydrazone formed with 2, 4 – dintrophenyl hydrazine. The absorbance of the sample was read against the reagent blank.

Principle: α – oxoglutarate + L – alanine $\rightarrow$ L– glutamate + Pyruvate.

Enzyme activity is expressed as a Standard International Unit (U/I).

3.4 Determination of Aspartate Amino Transferase (AST)

The blood serum was extracted into plain bottles after centrifugation of the blood samples at 3, 500 rpm for 10 minutes. AST activity was determined by the Colorimetric method using Randox test kits as described by Bergmeyer and Bernt (1974)[13]. Reagent blank was prepared with 0.5 ml of phosphate buffer containing L-aspartate and α-oxoglutarate and 0.1 ml of distilled water. The reagent was stirred and incubated for 30 minutes at 37^0C. After incubation, 0.5 ml of 2, 4 – dinitrophenyl hydrazine was added and allowed to stand for 20 minutes at 20^0C. The serum (0.1 ml) was then added to 0.5 ml of phosphate buffer as described above, 0.5 ml of 2,4 – dinitrophenyl hydrazine was also added to reagent blank and sample, respectively. The absorbance of the sample was read against the reagent blank after 5 minutes at 546 nm.

Principle: α – oxoglutarate + L-aspartate $\rightarrow$ L – glutamate + oxalocetate.

Enzyme activity is expressed as a Standard International Unit (U/I).

3.5 Determination of Alkaline Phosphates activity (ALP)

The ALP activity was determined by the spectrophotometric method according to Bergmeyer and Bernt (1974)[13] using Randox test kits. The serum (0.02 ml) was added to 1.0 ml of reagent containing diethanol – amine buffer, pH 9.9, Magnesium Chloride ($Mg\ Cl_2$) and Substrate (p- nitrophenyl phosphate). The mixture produced was stirred and absorbance taken after 1, 2, and 3 minutes using a timer at 405 nm in a spectrophotometer. Change in absorbance taken after 2 and 3 minutes was used to determine the final absorbance of ALP.

Enzyme activity was expressed as a Standard International Unit (U/I).

3.6 Statistical Analysis

The Statistical Package for Social Sciences (SPSS) version 17 for windows was used for all analyses. Comparison of data between the test and control groups was calculated using Student's t-test. $p<0.05$ was considered statistically significant.

4. Results

4.1 Acute Toxicity Test

The results of the acute toxicity test showed the plant extracts were nontoxic to the rats even at a dose of 2000 mg kg^{-1}. The general observations showed no mortality occurred 24 hours after administering the plant extracts. However, the

rats that received roselle extract displayed a readiness to take more; they were licking the cannular used to administer the extract. The rats that received ginger, moringa, 'ugwu' and mixture extracts did not show any signs of illness.

4.2 Efficacy of the Plant extracts on the Liver Enzymes of the Wild rats

Tables 1- 3 show the normalizing efficacy of the plant extracts in the liver enzymes of the wild rats. The plant extracts significantly ($p<0.05$) normalized the liver enzymes of the test rats compared to the control 1 and control 2 rats that were fed with distilled water only. Table 1 shows both the control 1 and control 2 rats had 1.3 and 8.3 u l^{-1} ALT decrease, respectively, whereas the rats fed with roselle, moringa, ginger, 'ugwu', and mixture extracts had 16.1, 20.2, 22.6, 22.5 and 26.8 u l^{-1} ALT decrease, respectively. The AST decrease in the control 1 and control 2 rats are 0.9 and 9.7 u l^{-1}, respectively, whereas the rats administered with roselle, moringa, ginger, 'ugwu', and mixture extracts had AST decrease of 18.0, 20.9, 22.4, 18.8 and 23.8 u l^{-1}, respectively (Table 2). Table 3 shows the ALP decrease of the control 1 and control 2 rats are 0.7 and 23.7 u l^{-1}, respectively, while the ALP decrease of the rats fed with roselle, moringa, ginger, 'ugwu', and mixture extracts are 45.8, 56.8, 59.5, 55.2 and 60.9 u l^{-1}, respectively. Significant differences ($p<0.05$) were also noticed in the liver enzymes of the rats fed with the different extracts.

Figures 1-3 illustrate the percentage normalizing efficacy of the plant extracts in the liver enzymes (ALT, AST and ALP) of the wild rats treated with the plant extracts for 180 days. The test rats had significantly ($p<0.05$) higher percentage normalizing efficacy compared with the control. Also, at the end of the treatment, significant differences ($p<0.05$) were observed in the percentage normalizing efficacy of the test rats.

Table 1: The ALT level (u/l) of the exposed wild rats treated with different plant extracts for 180 days

Day	0	90	180	Min. Value	Max. Value	Amount Decrease	RD L Range
Control 1	$25.4^{a} \pm 2.3$	$25.2^{a} \pm 2.4$	$24.1^{a} \pm 2.1$	23.1	26.5	1.3 ± 1.1	10 – 40
Control 2	$46.4^{a} \pm 4.3$	$43.2^{a} \pm 4.4$	$38.1^{a} \pm 5.1$	36.1	48.5	8.3 ± 2.9	10 – 40
Roselle	$47.4^{a} \pm 4.4$	$40.3^{b} \pm 4.7$	$31.3^{a} \pm 4.0$	28.7	48.7	16.1 ± 3.1	10 – 40
Moringa	$46.5^{a} \pm 3.9$	$38.4^{b} \pm 4.4$	$26.3^{b} \pm 4.6$	24.4	47.9	20.2 ± 2.5	10 – 40
Ginger	$45.1^{a} \pm 3.3$	$33.1^{b} \pm 3.5$	$22.5^{b} \pm 4.0$	20.3	46.9	22.6 ± 2.5	10 – 40
'Ugwu'	$47.3^{a} \pm 5.1$	$37.9^{b} \pm 4.0$	$24.8^{a} \pm 4.4$	22.1	48.3	22.5 ± 2.4	10 – 40
Mixture	$46.5^{a} \pm 3.2$	$33.5^{b} \pm 3.5$	$19.7^{a} \pm 5.6$	17.9	47.8	26.8 ± 2.5	10 – 40

- Data are expressed as Mean ± SEM
- Mean values with different superscripts '[a]' and '[b]' along the same row are significantly different at $P<0.05$
- Control 2 = Exposed wild rats fed only with distilled water
- RDL = Randox Laboratory Services, UK

Table 2: The AST level (u/l) of the exposed wild rats treated with different plant extracts for 180 days

Day	0	90	180	Min. Value	Max. Value	Amount Decrease	RD L Range
Control 1	$21.4^{a} \pm 2.1$	$21.3^{a} \pm 2.2$	$20.5^{a} \pm 2.0$	18.6.	23.1	0.96 ± 0.43	10 – 34
Control 2	$55.2^{a} \pm 6.3$	$47.4^{a} \pm 5.2$	$45.5^{a} \pm 4.1$	43.3	57.0	9.7 ± 1.9	10 – 34
Roselle	$56.1^{a} \pm 5.0$	$46.5^{b} \pm 6.1$	$38.1^{a} \pm 3.4$	37.8	57.6	18.0 ± 2.9	10 – 34
Moringa	$57.3^{a} \pm 6.4$	$43.2^{b} \pm 4.7$	$36.4^{a} \pm 4.6$	34.9	58.1	20.9 ± 3.0	10 – 34
Ginger	$56.5^{a} \pm 4.2$	$41.1^{b} \pm 4.1$	$34.1^{a} \pm 5.8$	33.2	57.8	22.4 ± 2.4	10 – 34
'Ugwu'	$55.8^{a} \pm 4.3$	$43.1^{b} \pm 6.0$	$37.0^{a} \pm 5.0$	35.5	56.9	18.8 ± 3.1	10 – 34
Mixture	$56.3^{a} \pm 4.0$	$38.5^{b} \pm 5.2$	$32.3^{a} \pm 5.3$	30.1	58.3	23.8 ± 3.4	10 – 34

- Data are expressed as Mean ± Sem
- Mean values with different superscripts 'a' and 'b' along the same row are significantly different at; $P<0.05$.
- Control 2 = Exposed wild rats fed only with distilled water
- RDL = Randox Laboratory Services, UK.

Table 3: The ALP level (u/l) of the exposed wild rats treated with different plant extracts for 180 days

Day	0	90	180	Min. Value	Max. Value	Amount decrease	RDL Range
Control 1	$100.6^{a} \pm 10.13$	$100.3^{a} \pm 9.00$	$99.9^{a} \pm 8.81$	97.5	103.8	0.7 ± 0.2	44 – 147
Control 2	$200.7^{a} \pm 14.23$	$189.3^{a} \pm 13.25$	$177.0^{a} \pm 15.71$	164.3	202.6	23.7 ± 2.1	44 – 147
Roselle	$201.6^{a} \pm 15.07$	$185.5^{a} \pm 15.5$	$155.8^{b} \pm 15.36$	152.8	203.9	45.8 ± 2.5	44 – 147
Moringa	$202.6^{a} \pm 15.86$	$155.9^{b} \pm 14.16$	$145.8^{a} \pm 18.00$	140.5	204.8	56.8 ± 3.8	44 – 147
Ginger	$202.5^{a} \pm 10.00$	$151.7^{b} \pm 11.16$	$143.0^{a} \pm 14.21$	137.9	203.6	59.5 ± 3.7	44 – 147
'Ugwu'	$199.8^{a} \pm 12.45$	$150.3^{b} \pm 18.42$	$144.6^{a} \pm 13.21$	138.1	202.5	55.2 ± 4.6	44 – 147
Mixture	$201.5^{a} \pm 13.43$	$148.4^{b} \pm 14.11$	$140.6^{a} \pm 14.01$	135.4	204.2	60.9 ± 4.4	44 – 147

- Data are expressed as Mean ± SEM
- Mean values with different superscripts 'a' and 'b' along the same row are significantly different at $P < 0.05$.
- Control 2 = Exposed wild rats fed only with distilled water.
- RDL = Randox Laboratory Services, UK.

Figure 1: Percentage ALT decrease (u/l) of the wild rats after treatment with the plant extracts for 180 days.

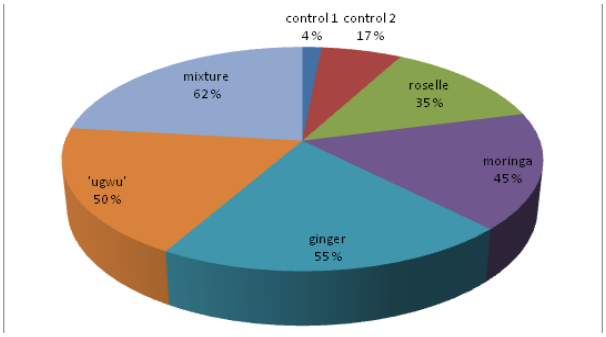


Figure 2: Percentage AST decrease (u/l) of the wild rats after treatment with the plant extracts for 180 days.

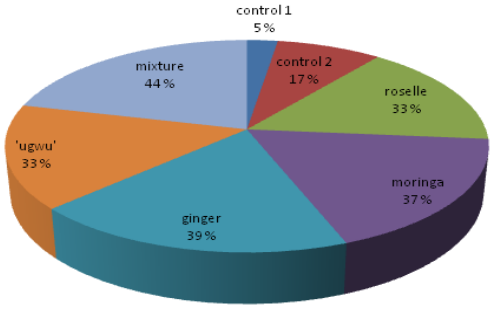
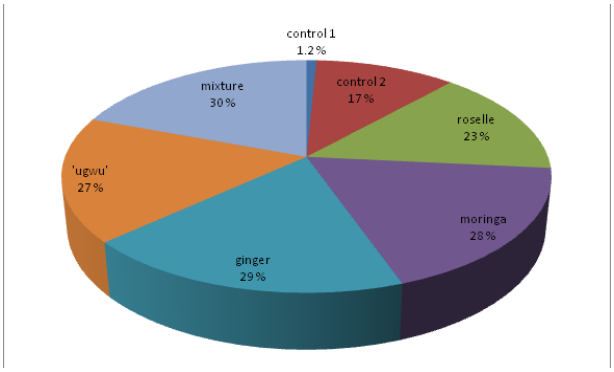


Figure 3: Percentage ALP decrease (u/l) of the wild rats after treatment with the plant extracts for 180 days.



5. Discussion

Exposure to environmental toxins even at low concentrations can elevate the levels of liver enzymes in animal, including humans. The abnormal high values of Alanine amino transferase (ALT), Aspartate amino transferase (AST) and Alkaline phosphates (ALP) observed in the wild rats collected from the vicinity of the cement plant could be the effects of liver damage caused by toxic elements in the cement dust. Bilen (2010)[14] reported that dust and gas emission from cement production facilities contain toxic elements, which pose a significant threat to the environment and humans. Yahaya *et al*. (2011)[4] also found high concentrations of aluminium, silicon, lead and chromium in the lungs of cement dust-exposed rats that showed multiorgan damage. The decreases observed in these enzymes after administering the plant extracts to the rats could be the results of the normalizing and therapeutic properties of the phytochemicals and phytonutrients present in the plants extracts. Harper (2011)[15] reported that phytochemicals and phytonutrients can be found in high amounts in fruits, vegetables and spices. Phytochemicals and phytonutrients present in roselle include riboflavin, niacin, flavonoids, hibiscetine, sadderetine, gossypetine, calcium, iron[16,17]. Moringa contains carotenoids, niazimimicin, pterogospermin, flavonoids, vitamins, calcium, zinc and magnesium[18,19]. Ginger has gingerol, eugenol, polyphenol, tannins and flavonoids[20]. 'Ugwu' contains iron and vitamins, and its extracts are used in the management of liver problems[21]. All these phytochemicals and phytonutrients reportedly present in the selected food plants could have worked together and produced a synergy for the well being of the rats.

6. Conclusion

The findings of this study have shown that the selected food plants could be used to normalize or reverse abnormal values of liver enzymes of rats exposed to cement dust. People living in environments polluted with cement dust should be advised to include these food plants in their diets for their overall well being.

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