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Effect of *Amaranthus hybridus* on the immunity of laboratory white albino rats

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Abstract This study determined the role of *A. hybrids* in boosting the immunity of female albino rats in the laboratory. They were randomly assigned to four groups, A to D and followed for 16 weeks during which groups A to C had their immunity suppressed and thereafter, group A given raw vegetable while group B cooked. The rats were bled four times and levels of three immune indicators determined (CD3%, CD^+4 and CD^+8 counts). Results indicated that the positive control (group C) was worse off than the groups who were given vegetables after suppressing immunity (groups A and B) in terms of CD^+4/CD^+8 ratio. There was, however no, significant difference ($p>0.05$) within the groups who were given raw or cooked vegetable. The negative control group (D) was the best performing group as per this indicator.

Key words: Cyclosporine A, female albino, immunity

Introduction

Amaranth is an indigenous African leafy vegetable consumed world over, especially in Africa. It is diverse with different varieties and species (Abukutsa-Onyango, 2007) and has a high density of vitamins, minerals and other bioactive ingredients. The crop is highly associated with immune boosting and other health benefits (Kimiywe *et al*., 2007) due to it's high composition of micronutrients. This study investigated the effect of effect of feeding on amaranthus on the immunity of white albino rats.

Materials and methods

Materials

Animals. The thirty female white albino weaner rats were acquired from the animal Unit of the College of Biological and Physical Sciences, University of Nairobi and were acclimatized for one month before deworming. They were housed in spacious and high cages allowing free movement as described by Lawlor M (Guttman, 1990). There were six cages of 5 rats distributed as follows; 2 cages each for groups A and B and 1 cage each for groups C and D. The Animal house was kept clean, well ventilated with temperature/humidity maintained at 19-21^{0}C/61-66g/m^3. Lighting was at 12hr light/12hr dark and the rats fed on rat pellets and tap water ad libitum. The rats were kept clean by changing beddings (wood shavings) every third day or earlier as was necessary. The health of the rats was monitored from time to time throughout the experimental period. The weights of the rats were taken every third day throughout the experiment period to ensure dosage of drug and vegetable was appropriate.

Feeding. The rats were fed to homogenous and fed cleaned *Amaranthus hybridus* harvested everyday early in the morning from a plot in the College of Agriculture and Veterinary Sciences, University of Nairobi. One portion was fed as raw while the other was boiled in its water, stirring frequently for 5 minutes. Both the raw and cooked samples were pound separately using mortar and pestle and passed through a sieve to make juice for the rats fed by oral tubation.

Blood. The rats were bled four times by retro- orbital bleeds. Each time 0.5ml of blood per rat was drawn and used for T-cell determination collected in EDTA (1ml tubes and capillary tubes).

Methods. After initial drawing of blood (T1) to determine baseline data of CD3% in blood, T-killer cells CD^+4 and CD^+8 counts, the rats were randomly allocated to the 4 experimental groups A,B,C,D, and followed for 16 weeks. During the period, the immunity of the albino rats in groups A, B and C was suppressed for 22 days using cyclosporine A at a rate of 10mg/kg body weight, after which blood was drawn from all the rats (T2). Thereafter, the immunity of the rats was immediately suppressed for a further 45 days at 30mg/kg body weight. Then blood was again drawn from all the rats (T3). Afterwards rats in groups A and B were given raw and cooked *Amaranthus hybridus* respectively and orally in juice form to supplement their normal diet, of rat pellets every day for 24 days at a rate of 1-2% body weight which translated into 20% of the daily feed. The volume of vegetable juice used in this efficacy

trial was the maximum amount that the rats could comfortably take. Rats in Group C (positive control) were not given any vegetable after immunity was suppressed while, those in group D were taken as the negative control (no treatment). At the end of the 24 days all the rats were bled for final checking of levels of the three parameters (T4).

Analytical procedure

CD3, CD^+4, CD^+8 determinations. 0.5ml (500µl) blood was used to determine the T-cell counts using the following procedure; 80µl of blood was put in a falcon tube of 1ml volume. The white blood cells were then stained using antibodies, 2µl each of anti-body for CD3, CD^+4 and CD^+8. These antibodies were added following each other and mixing after each. The sample was incubated for 20minutes. 1000µl of lysing buffer was added and mixed in thoroughly to destroy any red blood cells and also to clean the blood sample. The sample was then incubated for a further 10 minutes then span at 1500 rpm for 5 minutes. Any unbound antibodies were then washed off using Phosphate buffered saline (PBS) washing buffer for 2-3 times until the sample was 'clean'. Then 500µl of washing buffer was added to suspend the T-cells for reading in the Facs callibar, Flow Cytometry

Statistical analysis. Statistical analysis was performed using SAS Software. One-way analysis of variance (ANOVA) was followed by t-test. Results are expressed as means±SD of triplicate samples. Differences were considered significant at 95% confidence interval ($p<0.05$).

Results

The CD^+4 and CD^+8 immune indicators had varied trends across the groups (Table 1) over time. When the vegetable was introduced at T3 the changes of the ratio CD^+4/CD^+8, between T3 and T4 was negative for groups A, B and C and positive change for group D (negative control).

There was significant difference ($p<0.05$) for CD+4/CD+8 ratio between groups A/C at T1, and A/C, B/D at T3; and T4 for groups A/B and A/D.

Discussion

These findings corroborate with those of the present efficacy trial which indicates that *A. hybridus* boosts the immunity of white albino rats as seen in the changes of CD^+4/CD^+8 ratios between T3 and T4 after the introduction of *Amaranthus hybridus*. The positive control group is worse than the other 3 experimental groups because they needed the vegetable to boost immunity after its suppression.

The ill health of laboratory animals may be restored with supplementation of an anti-oxidant rich diet (Gupta & Prakash, 2009). Dietary supplementation with garlic, ginger and cabbage in male Wister rats reversed anemia caused by the toxic effect of cadmium (Eteng *et al*., 2012) and orchidectomized rats had improved anti-oxidant activity with orange pulp (Deyhim *et al*., 2007) and reduced osteoporosis due to improved femoral density with citrus juice (Deyhim *et al*., 2006). Pomegranate seed oil improved the immunity of mice (Yamasaki *et al*., 2006) while introduction of an extract from a plant traditionally used for medicinal purposes, *Alstonia boonei,* had beneficial effect in rats by lowering the lipid profile (Gabriel *et al*., 2008).

Vegetables are very rich in micronutrients and have been known to bring about various positive changes in laboratory rats in both hematological parameters and immune boosting. Supplementing the diet of rats with an African leafy vegetable. Therefore has remarkable increase in weight, hemoglobin and white blood cells, and reduction

Table 1. CD+4, CD+8 Counts and CD+4/CD+8 Ratio.

Treatment	Time	CD+4	CD+8	CD+4/CD+8
Raw (A)	T1	23.37±7.22(10)	17.62±7.87(10)	1.36
	T2	32.26±7.16(10)	20.76±3.83(10)	1.54
	T3	18.68±3.13(8)	9.03±3.75(8)	2.14
	T4	20.77±5.82(8)	19.71±8.35(8)	1.09
Cooked (B)	T1	23.03±6.29(10)	16.95±8.32(10)	1.39
	T2	31.65±6.66(10)	16.65±6.82(10)	1.92
	T3	14.90±3.26(10)	6.93±3.98(10)	2.34
	T4	23.58±4.63(9)	15.63±5.53(9)	1.53
Post.control (C)	T1	29.74±5.23(5)	14.38±2.84(5)	2.03
	T2	24.58±6.69(5)	13.21±8.08(5)	1.98
	T3	11.65±3.60(4)	3.55±2.94(4)	3.60
	T4	28.18±2.22(4)	18.35±9.07(4)	1.58
Negcontrol (D)	T1	31.59±3.95(5)	22.27±4.16(5)	1.42
	T2	26.35±4.64(5)	15.88±8.86(5)	1.73
	T3	24.80±5.73(5)	18.30±6.75(5)	1.38
	T4	35.57±4.30(4)	17.17±2.93(4)	2.07

The results are expressed as mean±SD(n)

in serum protein and lipid peroxidation (Iweala *et al*., 2009). Diabetic laboratory rats showed increased total red blood cells and white blood cells (Saliu *et al*., 2012) which is an indication of immune stimulation.

A healthy functioning immune system requires a variety of vitamins and minerals including anti-oxidants which are abundant in *A. hybridus* given to the white albino rats in this study. In conclusion therefore *A. hybridus* boosted the immunity of white albino laboratory rats and there was no significant difference ($p<0.05$) within and between the groups receiving raw and cooked.

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