

EFFECTS OF CO-ADMINISTRATION OF AQUEOUS EXTRACTS OF TELFERIA OCCIDENTALIS AND AGERATUM CONIZOIDES ON HAEMATOLOGICAL INDICES, UREA AND CREATININE IN ALBINO WISTAR RATS

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ABSTRACT: *The effect of the combination of aqueous extracts of the leaves of Telferia occidentalis and Ageratum conizoides compared with a standard hematinic, Astyfer, on some haematological indices, urea and creatinine in albino Wistar rats was studied. Thirty (30) males and females albino Wistar rats of average weight 140g were randomly selected into 5 groups of 6 animals per group. The parameters investigated included Red blood cells, (RBC), White blood cells (WBC), Haemoglobin (HGB), Haematocrit (HCT), Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH), Mean corpuscular haemoglobin concentration (MCHC), Platelets count (PLT), urea and creatinine. The results revealed a significant ($P<0.01$) increase in urea level in the group administered with combination of A. conyzoides and T. occidentalis as well as a significant ($P<0.05$) increase in the group that A. conyzoides was administered when compared to control. Also groups treated with T. Occidentalis, A. conizoides and combination of T. occidentalis and A. conizoides significantly ($P<0.01$) increased the RBC, HGB, HCT, while a significant ($P>0.01$) decrease in PLT in the groups treated with T. occidentalis and A. conizoides was observed when compared to control. When compared to standard hematinic, astyfer, groups treated with T. occidentalis showed a significant ($P<0.01$) increase in HGB and MCH at ($P<0.05$), while those treated with A. conizoides, and combination of T. occidentalis and A. conyzoides showed a significant ($P>0.01$) decrease in PLT. The results suggest that T. Occidentalis significantly increases haematological parameters when compared with standard hematinics. A. conyzoides also increases haematological parameters though not significantly when compared with standard hematinics while it raises urea levels in the blood which could suggest the presence of toxic phytochemicals. From the result, we can deduce that T. occidentalis is a better blood boosting vegetable than A. conyzoides.*

KEYWORDS: Telferia Occidentalis, Ageratum Conizoides, Haematology, Urea, Creatinine

INTRODUCTION

Telferia occidentalis which belongs to the family Cucurbitaceae is one of the leafy vegetables widely consumed in Nigeria for its nutritional and therapeutic benefits. It is widely cultivated in the south eastern, south western and south southern parts of Nigeria and utilised in the preparation of soups and medicines. Dietary intake of *Telferia occidentalis* could reduce garlic induced haemolysis in rats (Obboh, 2005). The aqueous extracts of *Telferia occidentalis* has been found to reduce blood glucose levels and also have anti diabetic effects in glucose induced hyperglycemic and streptozotocin induced diabetic mice (Aderibigbe *et al*, 1999). Also aqueous extracts of *Telferia occidentalis* has been reported to assist in the purging of the gastro intestinal tract as revealed by the purgative effects of the aqueous extracts of *Telferia occidentalis* leaf on isolated Guinea pig ileum (Dina *et al*, 2001). Based on its use as hematinic, its effect on haematological indices has been scientifically investigated and reported. According to Alada, 2000, the diet preparation of the air-dried leaves of the plant significantly

increased the red blood cell count, white blood cell count, packed cell volume and the haemoglobin concentration in rats. The use of the aqueous extract of the leaves of *Telferia occidentalis* for the enhancement of the haematological parameters has been in use for a very long time.

Ageratum conyzoides (Goat weed) is a member of the family of *Asteraceae* and is widely utilised in traditional medicine for the treatment and control of various ailments including epilepsy and convulsions. The plant often regarded as a weed has been known since ancient times for its curative properties and has been utilised for the treatment of various ailments, such as burns and wounds, for antimicrobial properties, for many infectious conditions and bacterial infections, arthrosis, headache and dyspnea, pneumonia, analgesic, anti-inflammatory, antiasthmatic, antispasmodic and homeostatic effects, stomach ailments, gynaecological diseases, leprosy and other skin diseases (Anjoo *et al*, 2008). *Ageratum conyzoides* has been used in various parts of Africa for the treatment of various diseases, as a purgative, and for wound dressing. Abbiw in 1990 reported that, the oil obtained from the plant has a powerful nauseating odour found to be poisonous to rabbits due to the presence of HCN and coumarin. The aqueous extracts of the leaves have been reported to prevent coagulation of the whole blood while causing precipitation of some blood materials and a decrease in the overall bleeding time (Akah, 1988). The leaves of the plant are reported to have anti-inflammatory properties with no apparent hepatotoxicity (Moura *et al.*, 2005).

MATERIALS AND METHODS

Materials

Telferia occidentalis and *Ageratum conyzoides*

The fresh leaves of *Telferia occidentalis* (Pumpkin) and *Ageratum conyzoides* (Goat weed) were obtained from the Itam market, in Uyo Akwa Ibom State.

Astyfer

The standard hematinic, Astyfer which was utilized in the course of this research was obtained from Pharmablaze Pharmacy, a pharmaceutical outlet located along Oron road in the Uyo Metropolis, Akwa Ibom State.

Experimental Animals

Thirty (30) male and female albino Wistar rats weighing between (145-170)g were used for this experiment. The animals were obtained from the animal house, Faculty of Basic Medical Sciences, University of Uyo, Uyo, Nigeria. The animals were acclimatised for two months and maintained on water and animal feed ad libitum.

Reagents

Reagent kits manufactured by Randox, Teco Diagnostics and Oxis Research were used for the biochemical analysis.

METHODS

Experimental design and Treatment of Animals

A total of thirty (30) albino Wistar rats (male and female) were weighed and randomly selected into five (5) groups of six (6) animals per group and put into rat cages. After the acclimatization period of two months, the rats were subjected to different treatments of the test sample. Group V animals served as control and were placed on normal saline as placebo while groups I, II, III and IV were treated with a combination of *Ageratum conyzoides* and *Telferia occidentalis*, *Telferia occidentalis*, *Ageratum conyzoides* and a standard hematinic, astyfer respectively. Group I animals were treated with 200mg/kg body weight of *Telferia occidentalis* and 400mg/kg body weight of *Ageratum conyzoides* while group II animals were treated with 200mg/kg body weight of *Telferia occidentalis*. Group III animals were treated with 400mg/kg body weight of *Ageratum conyzoides* while Group IV animals were treated with the standard haematinic, astyfer. The administration was carried for 15 days after which they were sacrificed.

Sample Preparation and Administration

The freshly collected samples were air dried and pulverised to obtain a fine homogenate. The homogenate obtained was immersed in water for 30 minutes and filtered to obtain the filtrate which was further subjected to extraction using a hot water bath which was maintained at 50°C to obtain the crude extract. The crude extract which was in a semi solid form was used for the purpose of the administration. The crude extracts of *T. occidentalis* and *A. conyzoides* was dissolved in the equivalent amount of solution to prepare a 200mg/kg and 400mg/kg solution which was administered to the rats according to their body weight.

Animal Sacrifice and Preparation of Plasma and Sera for Analysis

At the end of the administration (i.e after 21 days), the animals were fasted overnight (12 hours) and euthanized by dropping each of them in a transparent jar saturated with chloroform vapour. Incision was made on the abdomen and the blood sample was collected through cardiac puncture into sterile plain tubes for sera preparation and anticoagulant (E.D.T.A.) bottles for plasma preparation which were used for whole blood analysis. Serum samples were obtained from clotted blood into sterile plain tubes after centrifugation at 2000rpm for 15mins using a bench-top centrifuge. The serum was stored in the refrigerator for analysis.

Assay of Biochemical Parameters

Determination of serum creatinine concentration was carried out according to the method of Narayanan and Appleton, (1980). Determination of serum urea concentration was carried out according to the method of Tobacco *et al*, (1979).

Haematological Studies

Haematological analysis were conducted using Sysmex automated Haematology Analyser Model KX-21N. The parameters analysed included Red blood cells, (RBC), White blood cells (WBC), Haemoglobin (HGB), Haematocrit (HCT), Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH), Mean corpuscular haemoglobin concentration (MCHC) and Platelets count (PLT).

Statistical Analysis

The statistical analysis was carried out using the Student T Test while tables were done on Microsoft word and Microsoft excel. The data was expressed as mean standard deviation and the value of $P < 0.05$ and $P < 0.01$ was considered significant.

RESULT AND DISCUSSION

Results

Table 1: Effect of *Telferia occidentalis* and *Ageratum conyzoides* on the Haematological Parameters.

GROUPS	RBC	WBC	HGB	HCT
I 200mg/kg & 400mg/kg	$7.62 \pm 0.58^*$	17.15 ± 4.40	$13.4 \pm 0.75^{**}$	$50.48 \pm 3.37^{**}$
II 200mg/kg	$7.39 \pm 0.28^*$	$13.16 \pm 3.49^*$	$13.18 \pm 0.28^{**}$	$47.8 \pm 1.89^*$
III 400mg/kg	$7.02 \pm 0.36^*$	$13.40 \pm 3.61^*$	$12.08 \pm 0.76^*$	$45.95 \pm 2.93^*$
IV 10ml/70kg	$7.31 \pm 0.19^*$	$12.87 \pm 4.17^*$	$12.57 \pm 0.36^*$	$47.07 \pm 1.12^*$
V (Control)	6.61 ± 0.24	17.16 ± 3.13	11.32 ± 0.33	42.68 ± 1.67

Mean \pm standard deviation, $n=6$, $^*=P < 0.05$, 0.01 when compared with control.

$^{**}=P < 0.05$, 0.01 when compared with standard hematinic, astyfer.

Table 1 contd.

GROUPS	MCV	MCH	MCHC	PLT
I 200mg/kg & 400mg/kg	66.80 ± 4.89	17.65 ± 0.83	26.58 ± 1.02	879.33 ± 81.30
II 200mg/kg	64.68 ± 2.64	$17.82 \pm 0.40^*$	$27.58 \pm 0.80^{**}$	$793.4 \pm 112.7^{**}$
III 400mg/kg	65.23 ± 2.56	17.20 ± 0.68	$26.30 \pm 0.30^*$	809.0 ± 89.73
IV 10ml/70kg	64.45 ± 2.28	17.20 ± 0.67	26.68 ± 0.31	946.0 ± 78.87
V (Control)	64.48 ± 1.93	17.14 ± 0.60	26.36 ± 1.04	977.6 ± 52.92

Mean \pm standard deviation, $n=6$, $^*=P < 0.05$, 0.01 when compared with control.

$^{**}=P < 0.05$, 0.01 when compared with standard hematinic, astyfer.

Comparison with Control

The results of the haematological studies are shown in Table 2 above. The few haematological parameters that were studied include; RBC, WBC, HGB, HGT, MCV, MCH, MCHC and PLT.

Results obtained from the data analysis showed a significant ($P < 0.01$) increase in RBC in groups treated with a combination of *A. conyzoides* and *T. occidentalis*, *T. occidentalis* and Astyfer (i.e. groups I, II and IV) while a significant ($P < 0.05$) increase in the RBC was obtained in group treated with *A. conyzoides* (group III) when compared with control. There was also a significant ($P < 0.01$) increase in the HGB and HCT levels of groups treated with a combination of *A. conyzoides* and *T. occidentalis* (group I), *T. occidentalis* (group II) and Astyfer (group 3) while a significant ($P < 0.05$) increase in was observed in the group treated with *A. conyzoides* when compared with the control group. Group I (66.80 ± 4.89) and group 3 (64.48 ± 1.93) showed an increase in their MCV levels when compared with the control, though not significant. *T. occidentalis* significantly ($P < 0.05$) increased the MCH and MCHC levels in the group in which it was administered only (group II) when compared with control,

and also significantly ($P > 0.01$) decreased the PLT levels in the blood of animals treated with its aqueous extract.

Comparison with Standard Haematinic, Astyfer

Astyfer, a standard haematinic was also utilized in this experimental study to ascertain the effect of the aqueous extracts of the administered samples on the haematological parameters. Group I animals, treated with a combination of *T. occidentalis* and *A. conyzoides* showed a significant ($P < 0.05$) increase in HGB and HCT when compared with the standard haematinic astyfer while group II animals showed a significant ($P < 0.01$) increase in HGB and a significant ($P > 0.05$) decrease in PLT. Group II animals treated with *T. occidentalis*, showed a significant ($P < 0.05$) increase in MCHC.

Table 2: Effect of *Telferia occidentalis* and *Ageratum conyzoides* on Urea and Creatinine.

GROUPS	CREATININE	UREA
I 200mg/kg & 400mg/kg	93.01 \pm 19.47	7.80 \pm 0.85**
II 200mg/kg	90.82 \pm 33.03	6.34 \pm 1.06
III 400mg/kg	108.88 \pm 23.95	6.88 \pm 0.25*
IV 10ml/70kg	122.35 \pm 54.91	6.73 \pm 0.58*
V (Control)	108.08 \pm 28.11	6.06 \pm 0.64

Mean \pm standard deviation, $n=6$, *= $P < 0.05$, **= $P < 0.01$ when compared with control.

Results obtained from the assay of urea and creatinine, are listed on Table 2 above.

Results obtained from the careful analysis of data, obtained from the assay of creatinine reveal that group treated with *A. conyzoides* (108.88 \pm 23.95) and group treated with astyfer (122.35 \pm 54.91) had an increase in their creatinine level when compared with control (108.07 \pm 28.11) though the increase was not significant. Groups treated with combination of *T. occidentalis* and *A. conyzoides*, (93.01 \pm 19.47), and *T. occidentalis* only (90.82 \pm 33.03) had a decrease in their creatinine levels, though not significant when compared with control (108.07 \pm 28.11). The marked decrease in the creatinine levels of groups I and II could be due to the anti-oxidant and free radical scavenging properties of *T. occidentalis* as reported by Oboh, 2005.

Results for the assay of urea showed that there was a significant ($P < 0.01$) increase in the urea level of group treated with a combination of *A. conyzoides* and *T. occidentalis* (7.80 \pm 0.85) while a significant ($P < 0.05$) increase was obtained in groups treated with *A. conyzoides* (6.88 \pm 0.25) alone and astyfer (6.73 \pm 0.58) when compared with control (6.06 \pm 0.64).

DISCUSSION

In this present study, *A. conyzoides* was found to increase the urea concentration significantly. Urea and creatinine levels in the albino Wistar rats are presented on Table 2. The significant increase in urea in the group treated with a co-administration of *A. conyzoides* and *T. occidentalis* and the group treated with *A. conyzoides* could suggest the presence of toxic phytochemicals within *A. conyzoides* whose effect is modulated in the presence of *T. occidentalis*. The blood urea nitrogen (BUN) test was utilized in the determination of urea. It measures the amount of nitrogen in the blood that comes from the waste product urea which is

a by-product of protein metabolism, produced in the liver and excreted through urine. Elevated urea levels could be a positive marker of reduced kidney function and could also result in conditions like; nausea, fatigue, insomnia, reduced sense of taste and smell. Though elevated urea levels in the human system may signify reduced kidney function and mild kidney damage, it is not essentially utilized in the evaluation of kidney function because a lot of other factors may contribute to the elevation of urea levels in the blood.

Creatinine is usually utilized in the assessment of the kidney function. It is a breakdown product that is produced in proportion to active muscle mass and removed from the body by filtration in the kidney. Elevated levels of creatinine in the blood may be used to access the functionality of the kidneys. When blood creatinine increases, it may indicate reduced blood flow to the kidneys with a resulting decrease in the filtration and clearance of creatinine from the system. It may also indicate functional damage to the kidneys especially at the site of glomerular filtration.

Though *A. conyzoides* may possess some anti-microbial effects, it has also been observed to possess toxic effects hence resulting in elevated urea levels and modulated enzyme levels in human systems (Nair et al., 1977). It can be inferred from this study that *A. conyzoides* and hence the co-administration of *A. conyzoides* and *T. occidentalis* may pose toxic consequences to human systems and should be consumed with caution due to its toxic potentials. The aqueous extract of *T. occidentalis* has no toxic effects in human systems and hence should be consumed readily for the provision of essential phytochemicals needed for the normal functioning of human systems.

The assay of haematological parameters reveals that *T. occidentalis* significantly increases haematological parameters in the blood. The present study has shown that the aqueous extract of *T. occidentalis* caused significant increases in packed cell volume, haemoglobin concentration, red blood cell count, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration. This study is consistent with the observations made when rats were fed with the diet preparation of the air-dried leaves of *T. occidentalis* for four weeks (Alada, 2000).

The increases in the haematological indices observed following treatment with *T. occidentalis* extract might not be unconnected with the chemical composition of the leaves of *T. occidentalis*. The chemical composition had been shown to include proteins, fat, vitamin A, thiamine, riboflavin, nicotinamide, vitamin C and minerals such as zinc, iron, calcium and magnesium. The amino acid profile of *T. occidentalis* had also been shown to be very rich and includes alanine, aspartate, glycine, glutamine, histidine, lysine, methionine, tryptophan, cystine, leucine, arginine, serine, threonine, phenylalanine, valine, tyrosine and isoleucine (Tindal, 1968; Fasuyi, 2006).

Telferia occidentalis has been proven to be rich in phenols which possess high antioxidant and blood boosting activities. Some evidence has shown that flavonoids present in *T. occidentalis* could protect membrane leaves from oxidation (Amic et al., 2003). The additive and synergistic effects of phytochemicals in fruits and vegetables are responsible for this potent blood boosting capacity. *Telferia occidentalis* is a rich source of phytochemicals that possess a protective potential against diseases such as anaemia and diabetes hence its use in folk medicine in Nigeria could be as a result of its ability to increase haematological parameters. According to Oboh, 2004, the high phenol contents in the aqueous extracts could have contributed to the treatment and management of haemolytic anaemia.

The co-administration of *T. occidentalis* and *A. conyzoides* also increased the red blood count, haemoglobin and packed cell volume concentrations. This result suggests that the concomitant administration had a reduced effect on the haematological parameters when compared with the singular administration of *T. occidentalis*. This may be due to the presence of some toxic phytochemicals within *A. conyzoides* which reduce the proven blood boosting effects of *T. occidentalis*. Though the co-administration of the aqueous extracts of the two leaves did not increase as much haematological parameters as the administration of *T. occidentalis* alone, it is pertinent to note that the toxic effect of *A. conyzoides* was down regulated when it was administered in the combined form. This could be due to the presence of antioxidant phenolic and flavonoid compounds present in *T. occidentalis* that mop off the effect of free radicals and toxic intermediates produced by *A. conyzoides*. Another mechanism to explain the down-regulating property of the concomitant administration could be the formation of ligand-ligand complexes and protein-protein complexes within the two aqueous extracts that serve to down regulate the effects of some toxic intermediates that were present within the individual extracts.

SUMMARY AND CONCLUSION

Summary

The results of haematological indices indicate that the aqueous extract of *T. occidentalis* increases blood parameters considerably even when compared with a standard hematinic and hence should be consumed readily for the boosting of haematological indices during times of blood loss and anaemic conditions. The aqueous extracts of the combination of *T. occidentalis* and *A. conyzoides* also significantly increase haematological parameters like the HGB and HCT. This effect may be linked to the already proven blood boosting capacity of *T. occidentalis* and the collaborative activity of some phytochemicals in *T. occidentalis* and *A. conyzoides*. This work centres on the effect of the concomitant administration of the aqueous extracts of *T. occidentalis* and *A. conyzoides* and has gone to prove that with respect to the haematological indices the concomitant administration reduces the toxic effects of *A. conyzoides* through the modulating ability of *T. occidentalis* but increases Urea levels significantly which could be a marker for kidney malfunction. Hence the concomitant administration though has a positive effect on the haematological parameters, may have a mild to medium nephrotoxic effect.

A comprehensive assessment and consideration of the effect of co-administration of the aqueous extracts of *Telferia occidentalis* and *Ageratum conyzoides* was understudied in this research work. The study was divided into two parts; haematological studies and urea & creatinine assay. The experimental or alternate hypothesis of the first part of the study was that the co-administration of the aqueous extracts of *T. occidentalis* and *A. conyzoides* significantly increases the haematological indices in albino Wistar rats while the null hypothesis was that there was no significant increase in the haematological indices of the albino Wistar rats when treated with the concomitant administration. The experimental or alternate hypothesis of the second part of the study was that the co-administration of the aqueous extracts of *T. occidentalis* and *A. conyzoides* does not significantly increase the urea and creatinine levels in albino Wistar rats while the null hypothesis was that the concomitant administration significantly increased the urea and creatinine levels in albino Wistar rats treated with the concomitant administration. From the results obtained at the end of the study, the experimental hypothesis is accepted for the first part of the study while a null hypothesis is accepted for the second part of the study.

CONCLUSION

The co-administration of the aqueous extracts of *T. occidentalis* and *A. conyzoides* have been proven to increase some blood parameters and hence should be recommended as a better blood boosting source than most hematinics. Nonetheless, though not properly ascertained, it may pose some mild nephrotoxic effects.

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