Toxicological Evaluation and Antioxidant Properties of Moringa Oleifera Leaf, Hibiscus Sabdariffa Calyx and Their Blends

ODUSOLA, Kikelomo B.*[1], ADEMILUYI, O. Adedayo[*2], NWOKOCHA, Kate E.[3].,OBOH, Ganiyu.[2]and AKINDAHUNSI, A.A.[2], Awe, A.O[1]

[1]Federal college of Animal Health and Production Technology, Ibadan[2]Functional Food and Nutraceutical Unit, Department of Biochemistry, Federal University of Technology, Akure[3]Department of Biochemistry, University of Ibadan, Ibadan

This study assessed the acute and sub-acute toxicity profiles of *Moringa oleifera (*MO), *Hibiscus sabdariffa* (HS) and also the antioxidant ability of their blends in different ratio(1:1, 1:2 and 1:3). For the acute toxicity study, the rats were administered single dose of 5,000mg/kg body weight of HSand 3,000mg/kg body weight of MO in separate groups and were observed for 24 hours. Also, forsub-acute experiment, the rats were administered 250mg/kgBW,750mg/kgBW and 1,500mg/kgBW of *Moringa oleifera* (MO) and *Hibiscus sabdariffa* (HS) respectively but separately for 28days. At termination, the blood sera were subjected to biochemical analysis. The antioxidant capacity of the blends was also analysed. The results showed that LD_{50} of HS was above 5,000mg/kg BW while that of MO was more than 3,000mg/kg BW. ALT and creatinine levels were significantly reduced($p<0.05$)compared to control by *Moringa oleifera* but AST levels and ALP activity were not altered by *HS* and MO. *Moringa oleifera*leaf and *Hibiscus sabdariffa* calyx had high flavonoid and phenolic contents but M. oleifera had higher flavonoid and phenolic contents.The blends in all ratio had significantly higher($p<0.05$) DPPH radical scavenging and Fe^{2+} chelation ability than either 100% *HS*or *MO.*Infusion of *Moringa oleifera and Hibiscus sabdariffa* may serve as a good source of antioxidants and do not have toxic/deleterious effect at the doses studied except at the highest dose. Furthermore, there is synergistic effect in all the ratio of the blends

Keywords: blends, biochemical, infusion, synergistic, toxicity

I. INTRODUCTION

In the last few decades, there is increase in awareness and intake of food and beverages that originates from natural products. *Moringa oleifera* and *Hibiscus sabdariffa* are popular for several products that have been reported to have ameliorative and health promoting effects derived from them. They are widely used as tea, food and medicine (Da-Costa-Rocha et al., 2014). The calyces of *H. sabdariffa* are taken as a common local drink popularly known as "Zobo" in Nigeria and has been reported to be antiseptic, aphrodisiac, astringent, digestive, sedative, anti-stress, purgative and antioxidant (Akindahunsi and olaleye , 2003; Joshi and Parle, 2006). Leaves and many other parts of Moringa oleifera has been touted to have antipyretic, anti-inflammatory, antispasmodic, diuretic, antihypertensive, cholesterol lowering, antioxidant, anti-diabetic, hepatoprotective and antimicrobial activities (Anwar *et al.*, 2007; Sodamade*et al.*, 2013)Blending vegetable/fruit juices is one of the ways to improve its nutritional benefits and antioxidant activity depending on the type and quality of vegetables used (Hussein et al., 2017)

'Zobo' drink has become popular in many parts of Nigeria and other parts of Africa may be due to its acclaimmed medicinal value and that it is cheaper than many carbonated drinks. On the other hand Moringa leaves are eaten raw, used in soups in large quantities without consideration for possible toxicological effects. Since two or more vegetable juices could be blended to improve its nutritional

quality, this study aims at assessing the antioxidants value and toxicological effects of the blends of these two vegetables.

II. MATERALS AND METHODS

Plants collection and production of infusion

Calyces of *H. sabdariffa* and *M. oleifera* leaves were sourced from a local farm in Akure, Nigeria. Thereafter, they were hand-picked, washed under running tap and air-dried. The samples were authenticated at Forest Research Institute of Nigeria (FRIN), Ibadan where a voucher sample was deposited. Five hundred (500) grams each of the samples was extracted in 2L of boiling water for 10 min and thereafter allowed to cool to room temperature. The mixture was filtered and the filtrate later stored at 4 ^{o}C until use.

Evaluation of Antioxidant Potential

Determination of Total Phenol Content

The total phenol content was determined according to the method of Singleton et al. (1999). The infusions(25µl each) oxidized with 2.5ml 10% FolinCiocalteau's reagent and then neutralized with 2.0ml of 7.5% sodium carbonate. The reaction mixture was incubated for 40minute at 45oC and the absorbance was measured

Determination of Total Flavonoid Content

The total flavonoid content was determined using a slightly modified method of Meda et al. (2005)

Determination of DPPH (1, 1-diphenyl–2 picrylhydrazyl) free radical scavenging ability

The free radical scavenging ability of the infusions against DPPH (1, 1-diphenyl–2 picrylhydrazyl) free radical was evaluated according to the method of Gyamfi et al. (1999).

Determination of Iron (Fe2+) Chelation ability

The Fe2+ chelating ability of the infusionss was determined using a modified method of Minotti and Aust (1987) with a slight modification by Puntel et al., (2005).

Animals and Experimental treatment

Male albino rats weighing 180 – 200g were purchased and maintained at 25^{o} C on a 12 hour light/dark cycle with free access to food and water ad libitum. Prior to the commencement of the study, the animals were acclimatized under these conditions for two weeks.

Acute Toxicity Test

Male Wistar albino rats(n=6) were placed in cages and administered 5,000mg/kg body weight Hibiscus sabdariffa, and 3,000mg/kg body weight Moringa oleifera to another group. Physical and behavioral changes of rats were monitored for 24 hours

Sub- Acute Test

Six groups of mature albino rats were administered varying doses of 250, 750, and 1,500 mg/kg body weight of HS and MO respectively via oral gavage for 28days, with the seventh group serving as control. At the end of the treatment period, the rats were fasted for 24 hours and sacrificed by cervical dislocation. Blood samples were collected from the rats by cardiac puncture into non-heparinized plastic tubes, allowed to clot; serum separated from the clot and centrifuged into clean tubes for biochemical investigations. Liver and kidney were isolated, immediately placed on ice, weighed and homogenized in sodium phosphate buffer (pH 6.9). The homogenate obtained were centrifuged at 5,000 x g and the supernatant was used for biochemical tests such as AST, ALT, ALP, urea and uric acid.

III. Results and Discussion

Table1.Total phenol and Flavonoid contents of *Moringa oleifera* and *Hibiscus sabdariffa* infusions

	Total phenol (mgGAE/g)	Total flavonoid (mgQUE/g)
Moringa oleifera	39.18±0.13^{b}	27.02±0.09^{b}
Hibiscus sabdariffa	36.02±0.42^{a}	22.76±0.55^{a}

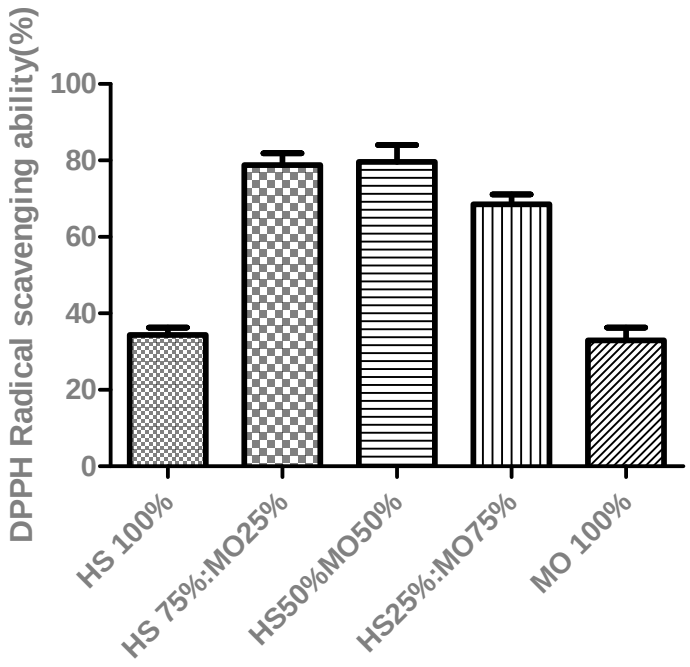

Figure 1: DPPH radical scavenging ability (%) of *Hibiscus sabdariffa*, *Moringa oleifera* and their blends

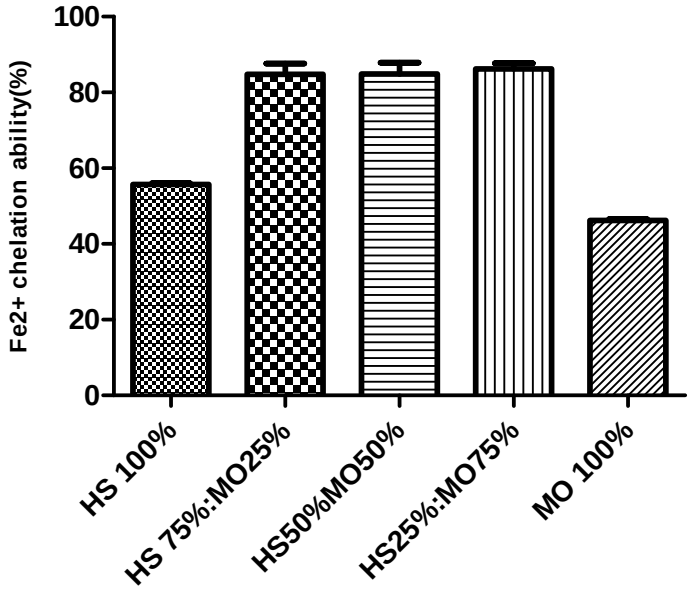

Figure 2. Fe^{2+}chelation ability of *Hibiscus sabdariffa, Moringa oleifera* and their blends

Table 2: Effect of *Moringa oleifera* and *Hibiscus sabdariffa* infusion on kidney function

Parameters	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII
Urea	6.64±0.39	5.79±0.23a	7.29±0.31a	8.23±0.42b	6.38±0.19a	6.54±0.21a	7.33±1.23a
Uric acid	0.31±0.01	0.35±0.02a	0.46±0.04b	0.47±0.04b	0.22±0.05a	0.29±0.02a	0.38±0.06a
Creatinine	15.24±0.31	8.45±1.32b	9.25±1.25b	12.51±0.89a	12,21±2.19a	21.59±1.32b	33.49±2.05b
MDA	14.81±2.98	13.36±1.72a	16.74±2.65a	20.38±0.58a	16.87±3.02a	19.63±2.19a	26.63±4.07b

Values are mean±SD of triplicate reading, a= not significantly different (P< 0.05) across the rows compared to control(group I), b= significantly different(P< 0.05) across the rowsin fusion compared to control

Key.

I = Control (Male rats), II = 250mg/kg BW *MO*, III = 750mg/kg BW *MO*

IV =1,500mg/kg BW *MO*, V =250mg/kg BW *HS*, VI =750mg/kg BW HS

VII =1,500mg/kg BW HS

Table 3: Effect of *Moringa oleifera* and *Hibiscus sabdariffa* infusion on liver function

Parameters	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII
AST	47.66±5.00	59.5±4.33a	65.5±2.64a	62.16±5.34a	36.00±4.44	48±3.46a	85.83±4.31b
ALT	46.00±5.19	15.33±2.88b	18.00±5.19b	22.33±4.61b	52.00±0.00	55.33±2.88a	53.66±2.88a
ALP	221.62±4.38a	229.03±6.00a	214.53±4.15a	228.80±8.57a	282.38±9.0a	253.64±3.89a	259.26±3.65a
MDA	11.00±0.77	7.75±0.0.33a	9.43±1.4a	13.13±0.89a	18.71±2.21b	21.09±2.71b	22.94±1.93b

Values are mean±SD of triplicate reading, a= not significantly different (P< 0.05) across the rows compared to control (group I), b= significantly different (P< 0.05) across the rows compared to control

Key.

I = Control (Male rats), II = 250mg/kg BW *MO*, III = 750mg/kg BW *MO*

IV =1,500mg/kg BW *MO*, V =250mg/kg BW *HS*, VI =750mg/kg BW HS

VII =1,500mg/kg BW HS

Antioxidant Potential of the Blends of Infusion of Hibiscus sabdariffa And *Moringa oleifera*

Table 1 revealed the total phenolic and total flavonoid contents of *Moringa oleifera* and *Hibiscus sabdariffa* infusions. MO leaf and HS calyx had high flavonoid and phenolic contents but *MO* had higher flavonoid and phenolic contents. *Hibiscus sabdariffa* had higher ABTS* scavenging ability than MO. Figure 1 shows the DPPH radical scavenging ability of the blends of the infusion compared to 100% HS and MO. Blending had a synergistic effect on the ability of these vegetables to scavenge DPPH radials. The same trend was observed in the ability of the blends to chelate Fe^{2+}as depicted by Figure 2.

Toxicological Evaluation of *Moringa oleifera* and *Hibiscus sabdariffa* Infusion on Kidney and Liver

Table 2 shows the kidney function test and Malondialdehyde (MDA) levels. Urea levels were not significantly different (P<0.05) from the control for both infusions except at the highest dose for MO. On the other hand, uric acid levels increased in a dose-dependent manner for MO but HS did not significantly change uric acid levels. Creatinine levels were significantly reduced by 250 and 750mg/kg body weight dosage of MO but on the contrary HS did not alter creatinine level at all doses. Both MO and HS infusion did not elicit an increase in the MDA produced in the kidney of Wistar rats The liver function tests, AST, ALT and ALP (Table 3) revealed that the infusion of Moringa oleifera significantly (P<0.05) lowered levels of Alanine amino transferase compared to control but infusion of Hibiscus sabdariffa did not alter the levels of ALT in all the animals compared to control. AST levels was not altered by the infusions at all doses except at the highest dose of HS. There was no alteration in ALP activity of animals across the groups at all doses. Contrary to what was observed in the kidney, infusion of MO reduced MDA levels at 250 amd 750 mg/kg body weight while HS infusion did not affect levels of MDA produced.

IV. DISCUSSION

Blending vegetable/fruit juices is one of the ways to improve its nutritional benefits and antioxidant activity depending on the type and quality of vegetables used (Hussein et al., 2017). In this study, the values obtained for total phenol and total flavonoid content (Table 1) were higher than what was earlier reported by Fakurazi *et al.,* (2012) ,Mohamed *et al.,* (2015) and Oboh*et al*., (2015) but lower than the total phenolic reported for almond (Oyeleye *et al*., 2017). There is a strong correlation between polyphenolic content of fruit/vegetable juices and their antioxidant capacity (Mohamed*et al*., 2015). Phenolics act by scavenging free radicals or chelating process(Kessler *et al*., 2003). The blends of Moringa oleifera and Hibiscus sabdariffa showed a significantly higher (P<0.05) DPPH radical scavenging ability than either 100% MO or HS(FIG 1). This is not surprising because several studies (Wang et al., 2000; Akindahunsi and Olaleye, 2003;Farombi and Fakoya, 2005; Olaleye and Rocha, 2007) have reported that both plants have strong antioxidant abilities but this study confirms a synergistic effect of blending on the DPPH radical scavenging capacity of these plants. The same trend was observed for Fe^{2+}chelation of MO and HS(Fig 2)

The kidney function markers (Table 2) revealed that infusion of HS did not significantly increase the levels of both urea and uric acid up to the highest dose. This is in contrast with the report of Fakeye et al., 2009 but agrees with the report of Emelike and Dapper (2013) that showed no significant difference of urea at high doses of administration of HS. This confirms the nephroprotective ability of HS infusion against assault on the kidney especially at low doses. However, there was a significant increase of urea at the highest dose(1,500mg/kg body weight) for MO infusion and also for uric acid at both the second and highest doses. Infusion of MO produced a significant decrease (P<0.05) in creatinine levels up to 750mg/kg while that of HS only had a similar effect at the lowest dose of 250mg/kg. MDA levels were not altered by both infusions except at the highest dose for HS. This possibly mean that these infusions inhibit lipid peroxidation in the kidney of rats

Activities of liver function markers (ALT, AST and ALP) have been used to determine and monitor onset and progression of liver injury/damage. Infusion of MO and HS did not significanly increase the activities of ALP and AST except for HS at the 1,500mg/kg dosage. This is in contrast with results of Oyagbemi *et al.,* (2013) that methanolicextract of MO when administered to rats at 200 and 400mg/kg led to a significant increase in ALT activity and eventual liver damage. Hepatoprotective effect of *Hibiscus sabdariffa* calyx extract has been attributed to its strong antioxidant activity which reduces cellular damage by reducing oxidative stress (Lee *et al*., 2012). Blending with MO laves which has also been reported to have antioxidant ability led to synergistic effect on their antioxidant ability.

V. Conclusion

This study revealed that blending of infusion of *Moringa oleifera*leaf *and Hibiscus sabdariffa*calyx increased the antioxidant potentials of the two vegetables which makes the blend a good source of antioxidants and each vegetable did not have deleterious effect on the kidney and liver up to

750mg/kg body weight in male Wistar rats.

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