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Ameliorative Potentials of Yoyo Bitters and Aqueous Leaf Extract of *Moringa oleifera* in Arsenite Induced Inflammatory Dysfunctions in Male Wistar Rats

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Authors' contributions

Author OEB designed the research work, was involved in the collection of data and data analysis, as well as the interpretation of data and drafted the manuscript. Author AOK was involved in the collection of data and the interpretation of the result. Author OBT provided some reagents, conducted the arsenic quantifications in organs and data analysis. Author AMA was involved in the design of the research work and read the final manuscript.

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ABSTRACT

Background: The use of alternative therapy to combat heavy metal intoxications has emerged recently, due to scientific evidences indicating superior efficacy in the use of botanicals that possess high antioxidative capabilities with or without dimercaprol or succimer in treating heavy metal intoxication.
Methods: To investigate the amelioration potentials of yoyo bitters and *Moringa oleifera* aqueous leaf extract in arsenite induced health perturbations, 100 ppm of arsenite, *M. oleifera* and yoyo bitters at respective doses of 250 mg/kg and 0.308 ml/kg body weight were administered to rats. Parameters such as reduced glutathione, glutathione peroxidase, catalase, protein carbonyl,

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malondialdehyde, protein thiol, nitric oxide, s-glutathionylated protein, prealbumin, cortisol, carbon clearance, zinc sulphate turbidity, neutrophil adhesion, interleukin-6, C-reactive protein, tumor necrosis factor-alpha and leukocyte analyses were determined.

Results: Administrations of arsenite and herbal supplements reduced the body weights in the rats ($p<0.05$), altered the oxidant-antioxidant activities by enhanced oxidative activities ($p<0.05$), and triggered chronic inflammatory responses that were ameliorated by the administration of yoyo bitters (0.308 ml/kg body weight) and *Moringa oleifera* (250 mg/kg body weight) especially when combined ($p<0.05$). Paradoxically, leukocyte counts were increased ($p<0.05$) in the herbal supplement treated rats compared to the untreated arsenite administered rats, but the phagocytic capabilities were all reduced ($p<0.05$). The herbal supplement did not prevent the assimilation of arsenic in the organs, but rather reduced the assimilations.

Conclusion: Our study indicated that yoyo bitters and *Moringa oleifera* singly or combined can ameliorate some health perturbations precipitated through arsenite intoxication, but the avoidance of the repeated exposures to arsenic contaminated environment and food substances is recommended.

Keywords: Ameliorate; arsenite intoxication; health perturbations; Moringa oleifera; yoyo bitters.

1. INTRODUCTION

Arsenic is an element with immense concerns in both environmental and human health standpoints. It is semi-metallic compound that is found in the soil, groundwater, surface water, air, food substances, etc. As it is ubiquitous in nature and its abundance ranks 20th in the earth's crust, 14th in seawater and 12th in the human body, which gave it a key front in various epidemiological and clinical studies. Exposures of man to arsenic is mostly from the food ingested, such as seafood, mushrooms, poultry products, fresh water foods, drinking water, etc., although exposures through the air is well documented [1]. Arsenic could exist in various oxidative states, such as the metalloid arsenide (0), arsenites (+3) and arsenates (+5), but arsenites are the most renowned of the toxic manifestations, due the high absorption and reactivity they possess [2,3]. [4] reported the severe insidious health impact of the consumption of water contaminated with arsenic that affected over 43 million people in Bangladesh and India due to a single act of groundwater contamination. Several epidemiological studies have revealed that chronic exposures to arsenic has been linked to a myriad of human diseases, such as atherosclerosis, diabetes, cardiovascular diseases, various cancers, kidney failure, death etc.

The treatment of arsenic toxicity with the chelation therapy using dimercaprol or succimer (2, 3-dimercaptosuccinic acid, DMSA), is often faced with its prolonged usage adverse effects [5]. Recently, the uses of botanicals, such as garlic extract have been reported to be highly effective in the treatment of heavy metal intoxication with less undesirable adverse effects [5,6]. In this vein, the few botanicals scientifically proven to ameliorate heavy metal intoxications were reported to achieve so due to the high antioxidative principles inherent in them.

Herbal supplements have remarkable uses, such as antimalarials, antiviral, antibacterial, cardio-protective, anti-aging, immune boosters, detoxifiers, etc., in modern medicine [7]. They can be in the form of granulated powder or suspended in aqueous or alcoholic solution singly or combined. They are commonly used as a prophylactic medicine in most developed and developing countries due their acclaimed antioxidative properties, health benefit and non toxicity. Herbal bitters are renowned for the recent publicities in varieties and usage in many countries of the world. They are a blend of various parts of plants with alleged health restoration capabilities in folkloric medicine and are often characterized with the bitter taste [8]. Herbal bitters are tinctures suspended in either water or 30-42%$^{v}/_{v}$ alcohol. They are characterized by the alleged improved digestion, detoxifiers, blood cleanser, vermicidal, anti-pile, antibacterial, immunomodulators and so on [7,9].

Moringa oleifera (*M. oleifera*) is a fast growing drought-resistant tree of the family of *Moringacaea*, native to sub-Hamalayan tracts of northern India and now distributed in many tropic and subtropics of the world [10,11]. Almost all the parts of this plant: root, bark, gum, leaf, fruit (pods), flowers, seed and seed oil have been used for various ailments in indigenous

medicines, including the treatment of inflammation and infectious diseases along with cardiovascular, gastrointestinal, hematological and hepato-renal disorders [12]. The leaf has been reported as a good source of natural antioxidant due to the presence of various types of antioxidant compounds such as ascorbic acid, flavonoids, phenolics and carotenoids [11]. Consequentially, herbal supplements have found a role as a component of the diet in most homes in many developing and developed countries due to the acclaimed health restoration benefits and wide pharmacological uses. Thus, the use of these herbal supplements might be imperative in combating arsenic intoxications in individuals or communities prone to arsenite intoxication. Our study, therefore, investigated the health restoration potentials of yoyo bitters and aqueous leaf extract of *M. oleifera* in sub-chronic arsenite induced inflammatory disorders in male Wistar rats.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Herbal supplements

Fresh leaf of *M. oleifera* was collected before sunrise in November, 2014 from the natural habitat around Ogbomoso, Oyo State. The plant was authenticated in the Department of Crop Science, Ladoke Akintola University of Technology, Ogbomoso, Oyo State.

Yoyo Bitters was a product of Abllat Company Nigeria Limited, 12 Ajayi Close, Ikotun-Ijegun Road, Ikeja, Lagos State. Nigeria.

2.1.2 Experimental animals

A total of eighty four (84) male Wistar rats with a weight range between 171 and 192 g, age between 4-5 months were obtained locally from the animal care facility, Ladoke Akintola University of Technology, Osogbo, Nigeria. The eighty four Wistar male rats were kept in wooden cages with metal netting and allowed access to standard feed and tap water *ad libitum*. They were allowed to acclimatize for 14 days before the commencement of the study.

2.1.3 Sodium arsenite

Sodium arsenite was a product of 1/20 B, Narayan Plaza, 26-A, Chandivali Road, Andheri (E), Mumbai-400072, Maharashsta, India.

2.1.4 Reagent Kits

Assay kits for the quantitative determinations of glutathione peroxidase, gamma glutamyl transferase, pre-albumin and catalase were products Fortress Diagnostic Laboratory, unit 2c, Antrim Technology Park, AntrimBT41, United Kingdom. The ELISA kits for interleukin 2, interleukin 6, tumour necrosis factor-α and C-reactive protein were products of RayBio Technology, inc. USA, while s-glutathionylated protein was a product of Cell Biolabs, inc. USA.

2.1.5 Carbon ink suspension

The carbon ink suspension was a product of Pelica AG, Germany.

2.1.6 Drugs and chemicals

All other chemicals and reagents were of analytical grade, either product of the British Drug House (BDH) Poole England, or Sigma Aldrich, Wisconsin U.S.A.

2.2 Methods

2.2.1 Preparation of aqueous leaf extract

The fresh leaf of *M. oleifera* was rinsed thoroughly in distilled water and dried in the shade for 12 days. The dried leaf was pulverized to a fine powder, using a domestic electric grinder and suspended in distilled water at room temperature. The filtrates were pulled together and lyophilised using a freeze dryer. The lyophilised extract was stored airtight and kept away from light in a dessicator containg preheated silica.

2.2.2 Experimental procedures

The animals were randomly allotted into six groups of fourteen (14) rats each and handled as indicated below:

Group A received distilled water
Group B received sodium arsenite
Group C received sodium arsenite and 250 mg/kg body weight of *M. oleifera*
Group D received sodium arsenite and 0.308 ml/kg body weight of yoyo bitters
Group E received sodium arsenite and dimercaprol
Group F received sodium arsenite, 250 and 0.308 mg/kg body weight of *M. Oleifera* and yoyo bitters

The respective dose of the administered sodium arsenite was as described by [13], yoyo bitters by [7] with slight modifications and *M. oleifera* by [11]. Sodium arsenite was introduced into the drinking water of the rats at the dose of 100 ppm and the rats were allowed free access to the water. The rats were administered concurrently arsenite, herbal supplements and dimercaprol for 42 days, after which a set of the rats in each group were sacrificed, while administration of the herbal supplements and dimercaprol continued further for 28 days. Yoyo bitters was administered once daily to the respective rats for five days in every seven days, observing a two days break in administration. Administrations of the herbal supplements were performed at 17 hours ± 30 minutes GMT and the body weights were monitored. Four rats in each group were sacrificed after 42 and 70 days of the administration, while three rats in each group were also used for the carbon clearance rates at stated days.

*This research was conducted in accordance with the National Institutes of Health's Guide for the Care and Use of Laboratory Animals [14].

2.2.3 Biological assays

The concentrations of the biological parameters were determined by standard methods, such as; reduced glutathione, protein thiol [15], glutathione peroxidase [16], catalase [17], protein carbonyl [18], malondialdehyde [19], nitric oxide [20], prealbumin [21], cortisol [22], carbon clearance test [23], zinc sulphate turbidity test [24], neutrophil adhesion test [25], arsenic [26], interleukin-6, C-reactive protein, tumor necrosis factor-alpha (Sandwich ELISA as contained on the instruction manuals in RAYbiotech) and S-glutathionylated protein (Sandwich ELISA as contained on the instruction manual in Cell Biolabs).

2.3 Statistical Analysis

The statistical model of this research study was a Completely Randomised Design (CRD) and the results were expressed as mean ± standard error of mean (S.E.M.) of at least three determinations. The results were subjected to ANOVA at $p<0.05$ and the Tukey test was utilized to identify the variation(s) within treatment groups, also at $p<0.05$.

3. RESULTS

3.1 Body Weight

The administration of arsenite presented reductions ($p<0.05$) in the body weights of the rats that spanned through the experiment, although the administration of dimercaprol, *M. oleifera* and yoyo bitters indicated consistent weight increases ($p<0.05$) in the arsenite administered rats (Fig. 1).

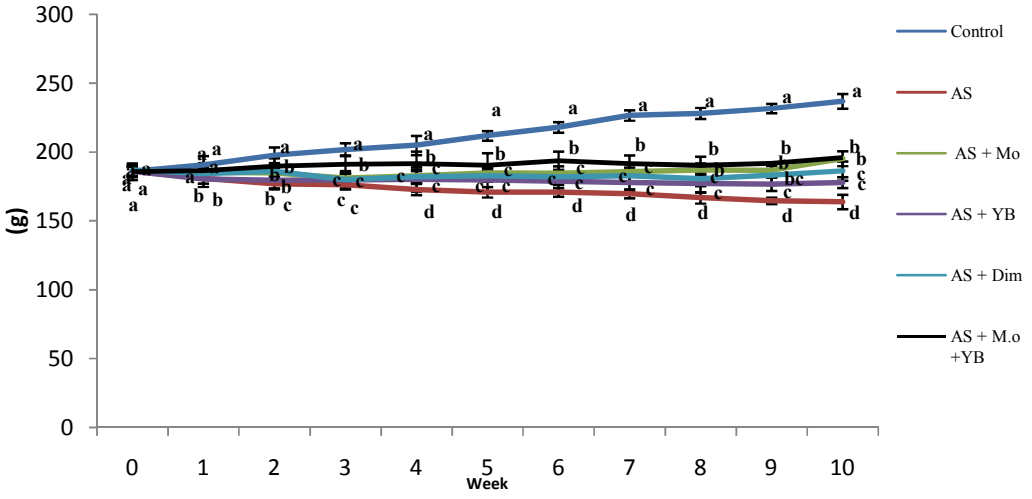

Fig. 1. Patterns in the body weight of rats administered with arsenite, herbal supplements and dimercaprol

(Values are means ± SEM; n=5-7. (Mean values bearing different alphabets are significantly different ($p<0.05$). AS= arsenite, M.o = M. oleifera, YB = yoyo bitters and Dim = dimercaprol.

3.2 Immunomodulatory Parameters

3.2.1 Oxidative stress indices

In Table 1, various trends that indicated significant decreases ($p<0.05$) were obtained in the concentrations of reduced glutathione, glutathione peroxidase, protein thiol and catalase in all the arsenite administered rats, but with marked increases ($p<0.05$) in the order: *M. oleifera* and yoyo bitters co-administered rats> yoyo bitters> *M. oleifera* within the arsenite administered rats. In a manner contrary to the foregoing, the concentrations of s-glutathionylated protein, protein carbonyl, malondialdehyde and nitric oxide were increased ($p<0.05$) following the administration of arsenite in the rats, and were reduced ($p<0.05$) with the administration of the herbal supplements (Table 1).

3.2.2 Inflammatory indices

The serum concentrations of prealbumin, cortisol, c-reactive protein, interleukin-6 and tumor necrosis factor-α all indicated increases ($p<0.05$) in the arsenite administered rats that were reduced ($p<0.05$) in the herbal supplemented rats with the *M. oleifera* and yoyo bitters co-administered rats presenting the best recovery ($p<0.05$) in the arsenite administered rats, as depicted in Figs. 2-6 respectively.

3.2.3 Leukocyte parameters

In Table 2, arsenite administration gave marked decreases ($p<0.05$) in the counts of the total leukocytes and monocytes, while the lymphocyte counts did not give a specific pattern in the rats ($p<0.05$). Although, the administration of the *M. oleifera* and yoyo bitters indicated alterations ($p<0.05$) contrary to those obtained in the arsenite only administered rats, but the *M. oleifera* and yoyo bitters co-administered rats presented alterations ($p<0.05$) closest to the control. Marked neutropenia ($p<0.05$) were recorded only in the non-treated arsenite administered rats (Table 2). The result of the total immunoglobulins indicated an initial marked increase ($p<0.05$) in concentration that was later reduced ($p<0.05$) in the non-treated arsenite administered rats, while consistent decreases ($p<0.05$) were obtained in the herbal supplement treated rats (Fig. 7). Leukocytes functions recorded decreases ($p<0.05$) in the phagocytic capabilities in all the arsenite administered rats (Figs. 8 and 9).

3.4 Arsenic in Tissues

The administration of arsenite recorded marked bio-assimilation ($p<0.05$) of arsenics in the various tissues, such as the liver, intestine, kidney, heart and brain in the male rats, in which

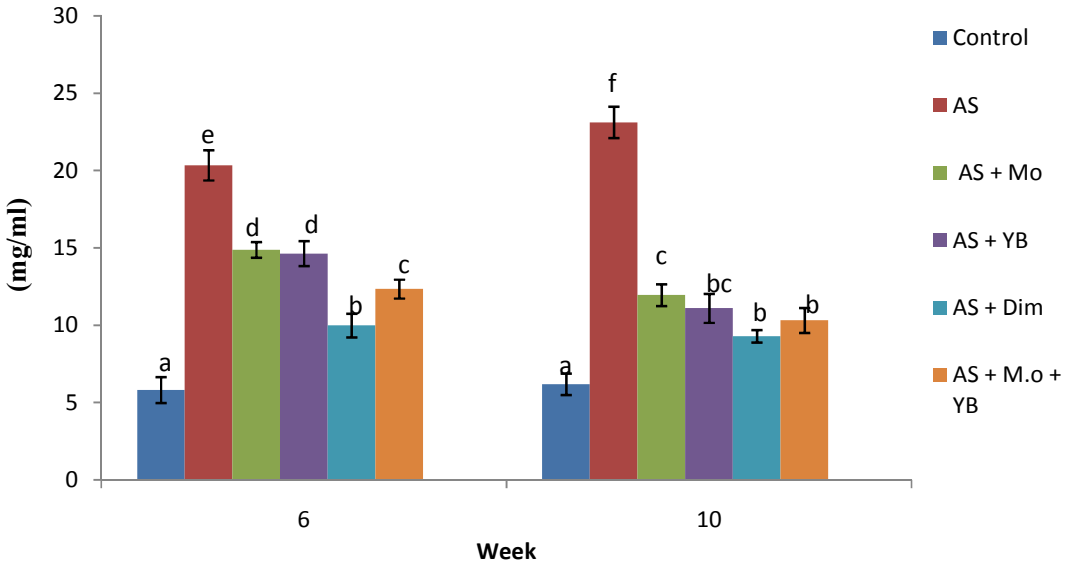

Fig. 2. Conentrations of prealbumin in treated arsenite fed rats

(Values are means ± SEM; n=3-4. (Mean values bearing different alphabets are significantly different ($p<0.05$). AS= arsenite, M.o = M. oleifera, YB = yoyo bitters and Dim =dimercaprol.

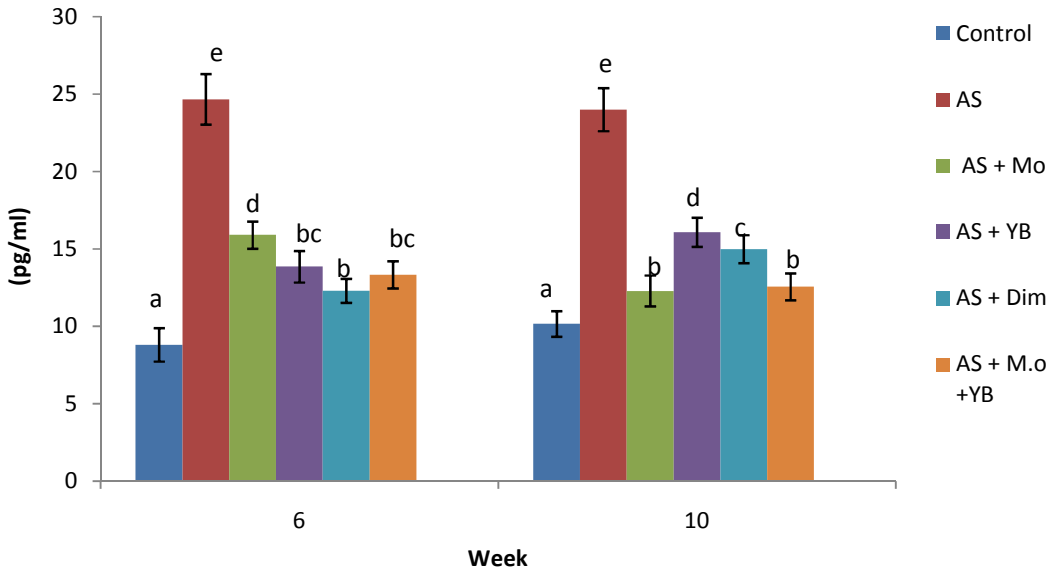

Fig. 3. Serum concentration of cortisol in treated male fed arsenite

Values are means ± SEM; n=3-4 (Mean values bearing different alphabets are significantly different (p<0.05). AS= arsenite, M.o = M. oleifera, YB = yoyo bitters and Dim = dimercaprol.

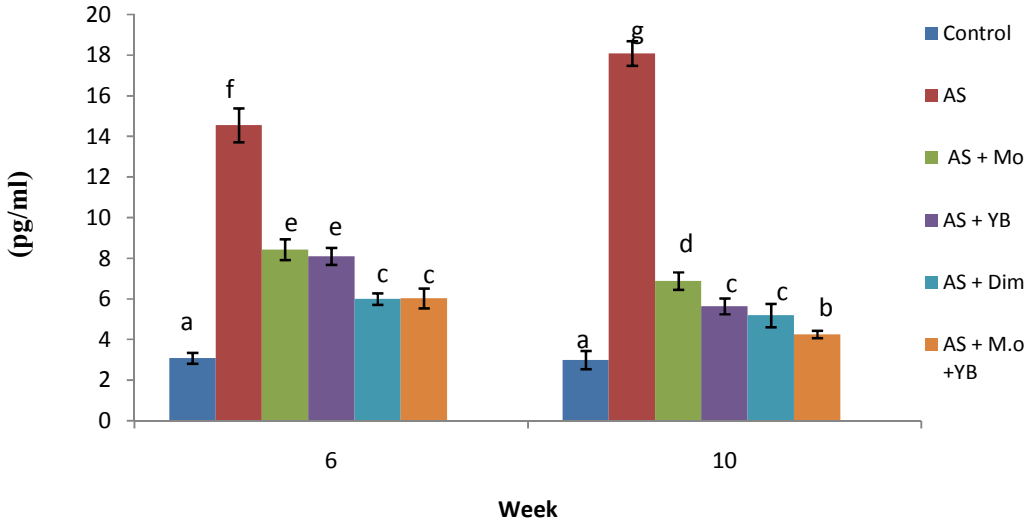

Fig. 4. C-reactive protein concentration in arsenite fed rats treated with herbal supplements and dimercapol.

(Values are means ± SEM; n=3-4. (Mean values bearing different alphabets are significantly different (p<0.05). AS= arsenite, M.O = M. oleifera, YB = yoyo bitters and Dim = dimercaprol.

the administration of the herbal bitters gave markedly reductions (p<0.05) in the bio-assimilation (Table 3).

4. DISCUSSION

In nature, virtually all the environmental components, including the abiotic and biotic factors have been consistently threatened by the excessive contamination of heavy metals that are released continuously from various human activities. Heavy metals are generally characterized as inorganic elements that have specific gravity five times than water's specific gravity. They are listed into the d-orbital elements of the modern periodic table, arsenic, cadmium,

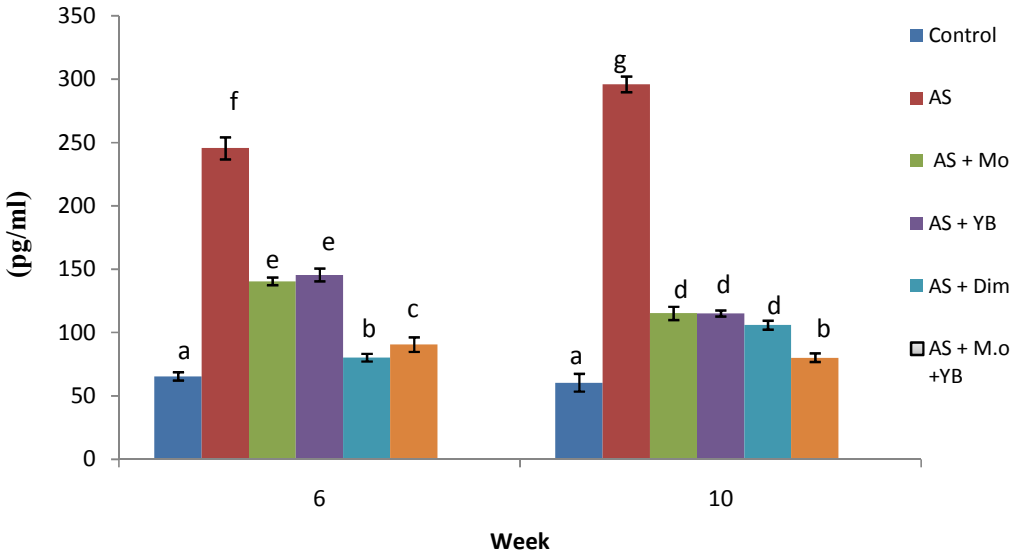

Fig. 5. Plasma concentration interleukin-6 in treated male rats fed with arsenite

Values are means ± SEM; n=3-4. (Mean values bearing different alphabets are significantly different (p<0.05). AS= arsenite, M.o = M. oleifera, YB =yoyo bitters and Dim = dimercaprol.

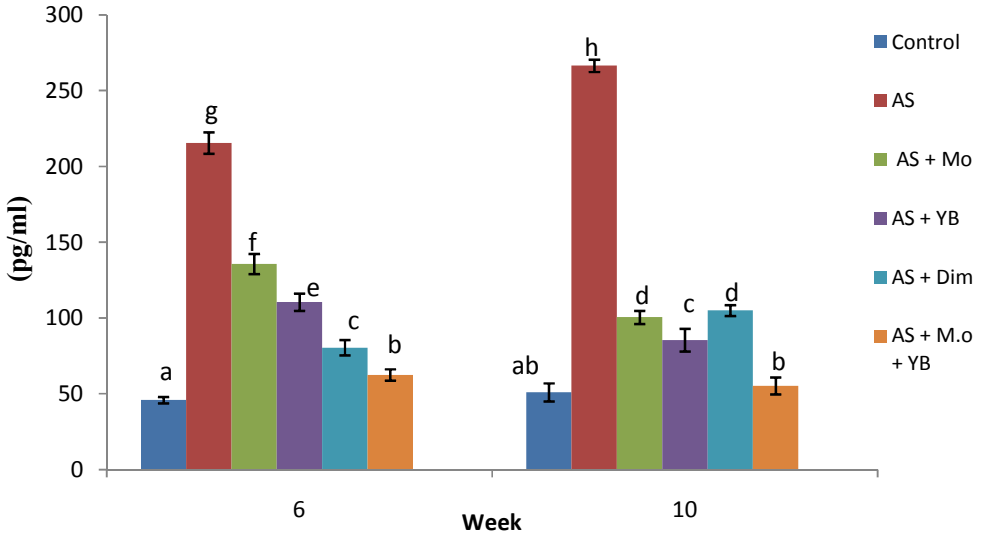

Fig. 6. Tumour necrosis factor-alpha concentration in arsenite fed rats treated with herbal supplements and dimercaprol

(Values are means ± SEM; n=3-4. (Mean values bearing different alphabets are significantly different (p<0.05). AS= arsenite, M.o = M. oleifera, YB = yoyo bitters and Dim = dimercaprol.

mercury and lead, and have been given prime importance due to their pathophysiological significance, as their bioaccumulation in living systems might cause severe damage to the vital organs, such as the immune system, nervous system and reproductive systems, gastrointestinal tract, mucous tissues etc [5]. The patterns reported in the body weight of rats administered arsenite indicated a probable disruption in food assimilation and utilization in the animals (Fig. 1). Arsenite had been reported to alter adversely carbohydrates, lipid, proteins and energy metabolism [13,27,28]. However, the trends in the body weights in rats singly and co-administered *M. oleifera* and yoyo bitters might connote an amelioration in the disruption in food assimilation and utilization, in which the herbal co-administered rats displayed the most recovery processes.

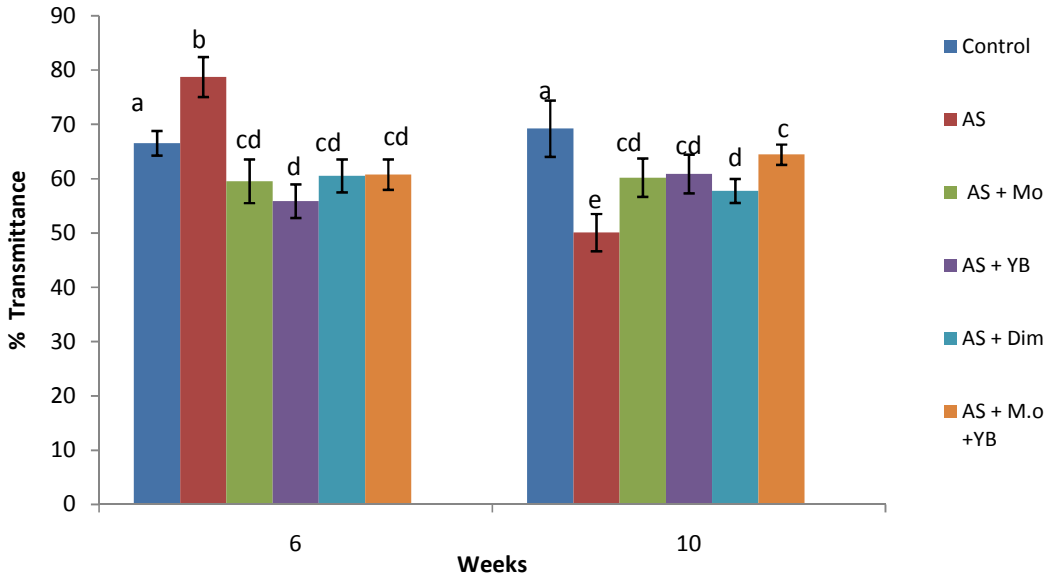

Fig. 7. Total immunoglobulin concentration in treated arsenite fed rats.

(Values are means ± SEM; n=3-4

(Mean values bearing different alphabets are significantly different (p<0.05). AS= arsenite, M.o = M. oleifera, YB = yoyo bitters and Dim = dimercaprol.

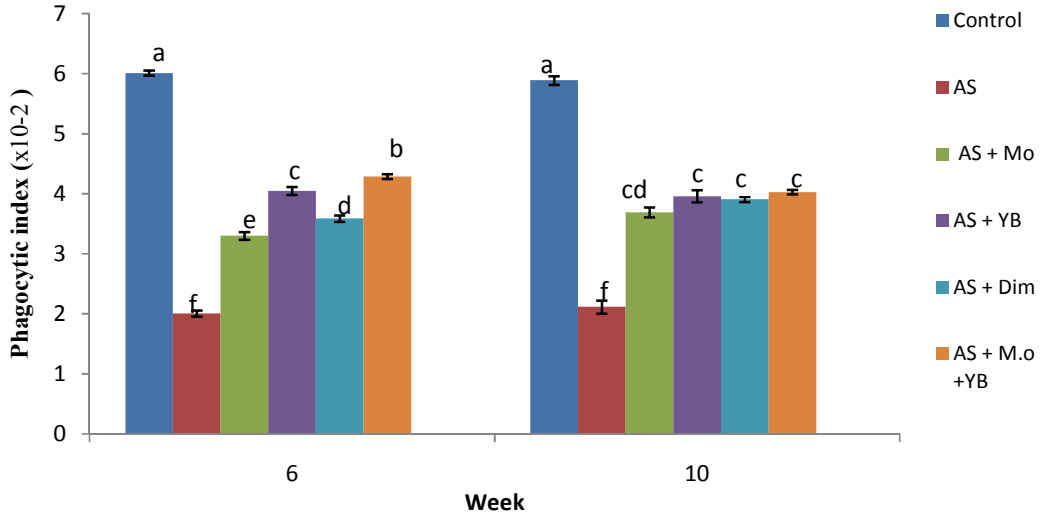

Fig. 8. Carbon clearance rate of in arsenite fed male rats treated with herbal supplements and dimercaprol.

(Values are means ± SEM; n=3. (Mean values bearing different alphabets are significantly different (p<0.05). AS= arsenite, M.o = M. oleifera, YB = yoyo bitters and Dim = dimercaprol

Naturally, all forms of life maintain a reducing environment within the cellular contents of the cell. This reducing environment in the cell is preserved by enzymes that maintain the reduced state through the constant input of metabolic energy. The results presented in all the oxidative stress indices following the administration of arsenite in the rats (Table 2) supported our previous report [13]. Heavy metals, such as arsenite can cause disturbances in the normal redox state of a cell by the indirect production of peroxides and free radicals that concomitantly induce oxidative stress [29]. Although, the exact mechanism of the pathogenicity of arsenic is not known scientifically, but various studies have implicated the exposure to arsenic or their

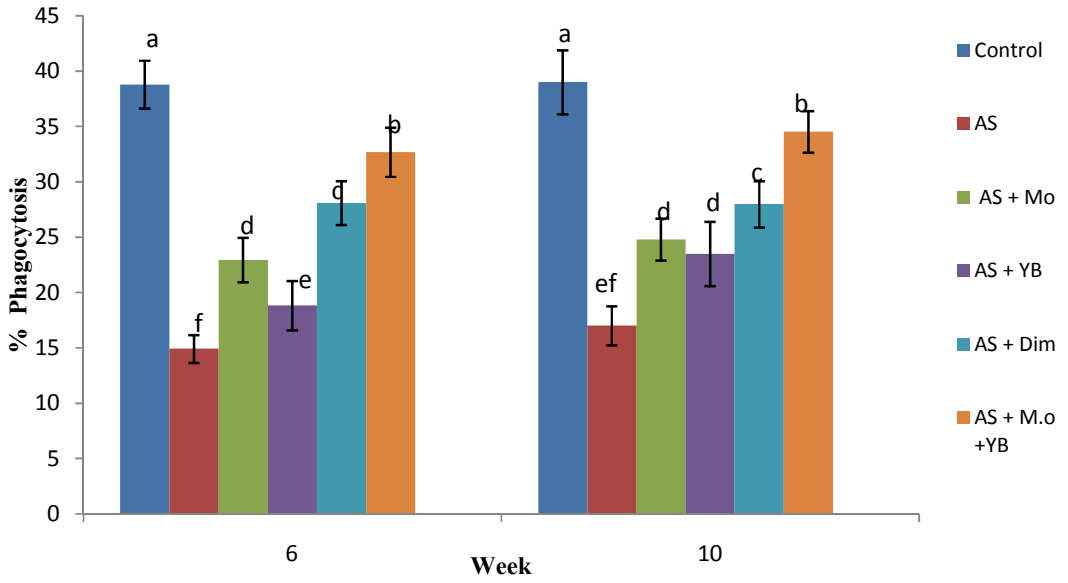

Fig. 9. Adhension capabilities of neutrophils in treated arsenite fed male rats. Values are means ± SEM; n=3.

(Mean values bearing different alphabets are significantly different (p<0.05). AS= arsenite, M.O = M. oleifera, YB = yoyo bitters and Dim = dimercaprol.

excess accumulation in the body tissues on the induction or production of free radicals, particularly, the reactive oxygen species (ROS) and reactive nitrogen species (RNS) which finally culminate into production of oxidative stress [13,27,28,30].

The administration of the herbal supplements, however, seemed to reverse the arsenite induced oxidative stress. This can only be possible through the activities of the active principles in the herbal supplements, which could either quench the free radical generated by arsenite in the cells, e.g. flavonoids, polyphenols etc. and, or chelate the arsenite ingested, making it unavailable for absorption, e.g. oxalate etc. It is plausible that the two processes were responsible for the amelioration of the arsenite induced oxidative stress. This is because the concentration of blood nitric oxide concentration supported the reduction in the generated free radicals, while the patterns obtained in the concentration of total thiol, reduced glutathione, glutathione peroxidase, s-glutathionylated protein and catalase, a sulphur containing enzyme all supported the inhibition of arsenite cross reactions with sulphur bridges. Various researches have elucidated that arsenites induce oxidative stress, bind sulfhydryl groups, disrupts the enzymatic process and cellular process and cell membranes, induce DNA damage and inflammatory responses, anemia etc. [13,27,28,29]. In addition, our results supported the report of [31] on the protective properties of some botanicals, such as *Annona muricata* leaf extracts against sodium arsenite-induced toxicity.

Inflammatory indices or markers are good indicators of tissue damage, assaults, invasion, compromise and, or health. The patterns presented in the plasma levels of cortisol, prealbumin, interleukin 6, C-reactive protein and tumor necrosis factor-α in the rats administered the herbal supplements were all indicators of the recovery from the arsenite induced tissue damage (Figs. 2-6). Inflammatory responses are consequential upon the acute and, or chronic induction of oxidative stress and tissue damage [7]. Paradoxically, the results obtained in the oxidative stress indices underline the trends in the inflammatory markers. It is noteworthy that the indicated inhibitions in the levels of the inflammatory indices were more desirable in the long term than in short terms and the co-administration of the herbal supplements elucidated the best anti-inflammatory properties. The increases in the levels of the inflammatory markers indicated a response towards healing due to cellular damage or a call up for enhanced immune activities to contain any tissue invasion, whose continuous stimulation might lead to various immunodegenerative or immunodeficiency disorders, loss of organ functions and death.

Table 1. The oxidative stress indices in male rats administered with arsenite, *M. oleifera* and yoyo bitters

			Groups					
			A	**B**	**C**	**D**	**E**	**F**
Malondialdehyde (nmole/mg of protein)	Liver	(x)	1.39 ± 0.40^{a}	8.27± 0.31^{e}	4.81± 0.58^{d}	4.74± 0.67^{d}	3.58 ± 0.39^{c}	4.39± 0.55^{d}
		(y)	1.11 ± 0.27^{a}	8.48± 0.51^{e}	3.26± 0.37^{b}	4.19±0.41^{d}	3.43± 0.62^{bc}	2.85± 0.48^{b}
	Heart	(x)	0.88 ± 0.11^{a}	4.85± 0.37^{d}	2.79± 0.28^{c}	2.87±0.35^{c}	1.93±0.22^{b}	2.31±0.42^{bc}
		(y)	0.97 ± 0.08^{a}	5.03± 0.45^{d}	2.68± 0.22^{c}	2.69±0.52^{c}	2.41± 0.54^{bc}	1.70± 0.21^{b}
	Intestine	(x)	1.21 ± 0.18^{a}	7.98±0.77^{e}	4.06± 0.56^{d}	3.88±0.37^{d}	2.07±0.49^{bc}	3.58±0.51^{d}
		(y)	1.38 ± 0.21^{a}	6.87±0.55^{e}	3.79±0.41^{d}	3.61±0.54^{d}	2.22±0.17^{b}	2.72±0.35^{c}
	Blood	(x)	1.03 ± 0.20^{a}	3.79±0.34^{d}	2.55± 0.43^{bc}	2.87±0.21^{c}	2.15±0.19^{b}	2.21±0.41^{b}
		(y)	1.12 ± 0.09^{a}	3.61±0.53^{d}	2.65± 0.51^{bc}	2.91±0.27^{c}	2.33± 0.26^{bc}	2.57±0.32^{bc}
Liver	Glutathione peroxidase (µmol/ml)	(x)	21.60± 1.23^{a}	7.08 ± 0.92^{e}	13.66 ± 1.25^{c}	14.84 ± 1.30^{c}	16.82± 1.61^{b}	15.76 ± 2.00^{bc}
		(y)	23.05± 1.78^{a}	6.64 ± 1.12^{e}	15.48 ±0.86^{bc}	14.22±1.06^{c}	15.90±1.78^{bc}	18.80± 1.34^{b}
	Reduced Glutathione (mg/ml)	(x)	40.12± 2.68^{a}	21.76± 2.28^{d}	28.94±3.62^{c}	28.48± 2.32^{c}	33.80 ±2.20^{b}	31.74±3.61^{bc}
		(y)	41.66± 3.04^{a}	19.81±3.34^{d}	32.58±3.26^{bc}	30.24±1.90^{c}	30.36±3.12^{bc}	34.06± 3.08^{b}
	Protein thiol (µg/mg protein)	(x)	12.66± 0.32^{a}	5.21 ± 0.09^{f}	7.72 ± 0.44^{e}	7.91 ± 0.26^{e}	8.16 ± 0.51^{de}	9.68 ± 0.43^{c}
		(y)	13.21± 0.67^{a}	4.98 ± 0.36^{f}	9.84 ± 0.24^{c}	10.63 ± 0.47^{bc}	8.62 ± 0.38^{d}	11.27 ± 0.21^{b}
	Protein carbonyl (nmol/mg protein)	(x)	3.40 ± 0.44^{a}	9.06 ± 0.82^{d}	5.32 ± 0.51^{c}	6.00 ± 0.29^{c}	4.33 ± 0.17^{b}	5.65 ± 0.45^{c}
		(y)	3.06 ± 0.28^{a}	8.44 ± 0.40^{d}	5.30 ± 0.63^{c}	5.25 ± 0.34^{c}	4.16 ± 0.26^{b}	4.01 ± 0.19^{b}
Catalase (U/ml)	Liver	(x)	72.52 ± 4.18^{a}	48.64 ± 2.96^{e}	53.88 ± 3.21^{d}	56.15 ± 2.42^{cd}	57.36± 5.22^{c}	58.28 ± 2.74^{c}
		(y)	71.20 ± 5.00^{a}	44.38 ± 1.47^{e}	58.92 ± 3.38^{c}	59.90 ± 3.83^{c}	58.02 ± 4.40^{c}	64.66 ± 4.20^{b}
	Intestine	(x)	56.00 ± 2.78^{a}	32.70 ± 2.24^{d}	41.36 ± 2.58^{c}	39.78 ± 1.80^{c}	49.20 ± 3.02^{b}	48.04 ± 4.22^{b}
		(y)	57.10 ± 3.42^{a}	29.62 ± 3.36^{d}	42.04 ± 3.22^{c}	42.64 ± 2.34^{c}	45.68 ± 3.32^{bc}	52.20 ± 2.88^{b}

		Groups					
		A	B	C	D	E	F
Plasma	S-glutathionylated protein (µg/ml)						
	(x)	0.92 ± 0.02^{a}	6.11 ± 0.82^{f}	4.16 ± 0.48^{de}	4.24 ± 0.76^{e}	3.06 ± 0.30^{bc}	3.34 ± 0.26^{c}
	(y)	0.97± 0.14^{a}	5.69 ± 0.63^{f}	3.47± 0.36^{c}	3.86 ± 0.18^{d}	3.18 ± 0.41^{c}	2.70 ± 0.43^{b}
	Nitric oxide (µmol/mg protein)						
	(x)	115.05± 7.20^{a}	188.46 ±8.17^{e}	145.63 ±7.49^{c}	142.27±8.01^{bc}	131.34 ±6.87^{b}	138.83±8.20^{bc}
	(y)	108.93± 8.35^{a}	161.67± 6.83^{d}	123.55±6.76^{ab}	128.49 ±8.43^{b}	130.76 ±7.82^{b}	121.80±7.07^{ab}

Values are means ± SEM; n=3-4. (Mean values bearing different alphabets are significantly different (p<0.05). A= distilled water, B= arsenite, C= arsenite + M. oleifera, D= arsenite + yoyo bitters, E= arsenite, + dimercaprol and F= arsenite + M. oleifera + yoyo bitters. X= 6 weeks and y= 10 weeks.

Table 2. Leukocyte counts in rats administered arsenite, *M. oleifera* and yoyo bitters

Parameter		Groups					
		A	B	C	D	E	F
Total Leukocyte (10^{9}/L)	(x)	6.85 ± 0.30^{a}	8.31 ± 0.07^{b}	5.50 ± 0.43^{cd}	5.11 ± 0.27^{d}	5.45± 0.52^{cd}	5.42 ± 0.40^{d}
	(y)	7.05 ± 0.54^{a}	4.20 ± 0.39^{e}	5.82 ± 0.50^{c}	5.80 ± 0.41^{c}	5.68 ± 0.26^{c}	5.95 ± 0.20^{c}
Monocytes (%)							
	(x)	6.11 ± 0.08^{a}	8.85 ± 0.11^{b}	5.79 ± 0.22^{c}	5.15 ± 0.10^{d}	5.31 ± 0.21^{cd}	5.26 ± 0.20^{d}
	(y)	6.06 ± 0.17^{a}	3.41 ± 0.09^{e}	6.01 ± 0.12^{a}	5.71 ± 0.34^{c}	5.46 ± 0.39^{cd}	5.91 ± 0.26^{ac}
Lymphocyte (%)							
	(x)	63.85 ± 1.91^{a}	77.80 ± 2.95^{b}	55.70 ± 2.50^{cd}	57.75 ± 3.10^{cd}	55.50 ± 3.10^{cd}	58.00 ± 2.51^{c}
	(y)	63.45 ± 3.05^{a}	79.60 ± 4.56^{b}	58.90 ± 3.05^{c}	56.65 ± 2.11^{cd}	54.75 ± 1.11^{d}	59.90 ± 2.76^{ac}
Neutrophil (%)							
	(x)	29.15 ± 1.00^{a}	15.40 ± 0.76^{b}	30.81 ± 1.66^{a}	29.46 ± 2.16^{a}	32.80 ± 2.41^{a}	30.30 ± 1.91^{a}
	(y)	31.30 ± 2.21^{a}	13.80 ± 0.82^{b}	31.99 ± 1.70^{a}	32.70 ± 2.21^{a}	30.66 ± 2.80^{a}	30.06 ± 2.45^{a}

Values are means ± SEM; n=3-4. (Mean values bearing different alphabets are significantly different (p<0.05). A= distilled water, B= arsenite, C= arsenite + M. oleifera, D= arsenite + yoyo bitters, E= arsenite, + dimercaprol and F= arsenite + M. oleifera + yoyo bitters. X= 6 weeks and y= 10 weeks.

Table 3. Concentration of arsenic in male rats treated with *M. oleifera* and yoyo bitters

Arsenic (µg/g dry weight)		Groups					
		A	B	C	D	E	F
Liver	(x)	1.06 ± 0.51^{a}	65.22 ± 2.30^{g}	21.43 ± 2.22^{e}	13.27 ± 2.00^{d}	8.81 ± 1.60^{c}	10.49 ± 2.00^{cd}
	(y)	1.25 ± 0.22^{a}	57.55 ± 1.00^{f}	10.74 ± 2.50^{cd}	8.85 ± 1.81^{c}	7.33 ± 1.02^{bc}	6.68 ± 1.55^{b}
Intestine	(x)	1.03 ± 0.29^{a}	90.78 ± 1.87^{g}	23.27 ± 1.35^{e}	24.39 ± 2.20^{e}	6.98 ± 1.00^{c}	14.32 ± 2.30^{d}
	(y)	0.71 ± 0.32^{a}	79.62± 1.21^{f}	15.54 ± 2.04^{d}	15.73 ± 1.28^{d}	3.58 ± 2.26^{b}	5.78 ± 1.47^{c}
Heart	(x)	0.03 ± 0.01^{a}	8.57 ± 0.12^{f}	2.06 ± 0.20^{e}	1.81 ± 0.31^{e}	0.42 ± 0.23^{b}	1.25 ± 0.33^{cd}
	(y)	0.05 ± 0.01^{a}	8.42 ± 0.11^{f}	1.58 ± 0.33^{d}	1.22 ± 0.40^{cd}	0.31 ± 0.08^{b}	0.84 ± 0.18^{c}
Brain	(x)	0.00 ± 0.00^{a}	3.16 ± 0.14^{e}	1.22 ± 0.17^{d}	1.08± 0.02^{d}	0.76 ± 0.08^{c}	1.00 ± 0.03^{d}
	(y)	0.00 ± 0.00^{a}	3.37 ± 0.30^{e}	0.70 ± 0.06^{bc}	1.00 ± 0.02^{d}	0.57 ± 0.10^{b}	0.68 ± 0.11^{bc}
Kidney	(x)	0.22 ± 0.09^{a}	12.40 ± 0.61^{g}	5.06 ± 0.59^{f}	4.13 ± 0.25^{e}	1.58 ± 0.16^{c}	2.40 ± 0.52^{d}
	(y)	0.30 ± 0.05^{a}	10.17 ± 0.88^{g}	2.19 ± 0.44^{d}	2.02 ± 0.61^{d}	0.90 ± 0.20^{b}	1.29 ± 0.29^{bc}

Values are means ± SEM; n=3-4. (Mean values bearing different alphabets are significantly different ($p<0.05$). A= distilled water, B= arsenite, C= arsenite + M. oleifera, D= arsenite + yoyo bitters, E= arsenite, + dimercaprol and F= arsenite + M. oleifera + yoyo bitters. X= 6 weeks and y= 10 weeks.

Administrations of *M. oleifera* and yoyo bitters modulated the activities and secretion of the leukocytes in the arsenite fed rats (Figs. 7-9 and Table 2). This is not surprising, as the results of the oxidative stress and inflammatory indices all indicated a recovery from the challenge induced by arsenite in the rats. Although, the trends obtained in the leukocytes counts in the herbal supplemented rats were very much comparable to one another, but the co-herbal supplement administered rats indicated the consistent marked recovery. The inconsistent hyper and hypo secretions recorded in the leukocytes in untreated arsenite administered rats indicated an upset in the immune systems (Table 2). The upset might be sure manifestations of the immune disorders due to tissue degradation, functional losses, etc., which are precipitated by the chronic stimulation of inflammatory responses. [32,33] reported a close relationship between the chronic stimulation and release of inflammatory markers and tissue degeneration. The immune hypersecretion could connote the initial massive response to arsenite reactions in the tissues that went uncontrolled and precipitated massive innate cell penetrations in tissue spaces (extravasation) manifested in the hypo-innate immune secretions later recorded in our study (Table 2). Decreases that were reported in the phagocytic properties of the innate immune cell, neutrophils in the arsenite administered rats indicated an inhibition in the phagocytic activities. The losses in phagocytic capabilities in the herbal supplemented rats are an interesting piece because the levels of neutrophils were not altered in the rats (Figs. 7-9 and Table 2). Arsenite might have interfered with the secretion of neutrophils and perhaps altered or bind one or more important antigen recognition site or protein on the neutrophils. Although, our study did not include the mapping of antigen recognition proteins in neutrophils, but the result of the % neutrophil and the phagocytic capabilities in the untreated arsenite administered rats underlined our finding.

The increases recorded in the assimilations of arsenic in the various organs in the all the arsenite administered rats were due to the ingested arsenite and the administrations of the herbal supplement could not eliminate, but rather inhibited the assimilation (Table 3). However, the administrations of the herbal supplements indicated remarkable decreases in the assimilated arsenic that supported the results obtained in the oxidative stress indices, which was probably due to the chelating activities phytochemicals that prevented the absorption and assimilation of arsenite into tissues. This is logical as the few botanicals reported to combat heavy metal intoxications were alleged to possess remarkable chelating properties [6,34].

5. CONCLUSION

The findings obtained from the current study, depicted that yoyo bitters and *M. oleifera* aqueous extract prevented the absorption of arsenite and restored some health perturbations induced by sub-chronic arsenite intoxication. However, the amelioration of the arsenite induced health perturbations was best with by the co-administrations of yoyo bitters and *M. oleifera*. Therefore, yoyo bitters and *M. oleifera* are recommended in treating arsenic intoxication, but the avoidance of arsenite intoxication in contaminated environments and food substances is preferred.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Jones FT. A broad view of arsenic. Poult Sci. 2007;86:2-14.
2. Bishayi B. Sodium arsenite induced alteration in functional murine peritoneal macrophages. Ind J Pharmacol. 2000;32: 192-7.
3. Huges MF, Bech BD, Chen Y, Lewis AS, Thomas DJ. Arsenic exposure and toxicology: A historical perspective. Toxicol Sci. 2011;123:305-32.
4. Chowdhury U, Biswas B, Chowdhury T., Samanta G, Mandal BK. Groundwater arsenic contamination in Bangladesh and West Bengal, India, Environ. Health Prespect. 2000;108:393-7.
5. Gupta VK, Singh S, Agrawal A, Siddiqi NJ, Sharma B. Phytochemicals Mediated

Remediation of Neurotoxicity Induced by Heavy Metals, Biochemistry Research International. 2015; Article ID 534769: 9 pages.

6. Das B, Mandal S, Chaudhuri K. Role of arginine, a component of aqueous garlic extract, in remediation of sodium arsenite induced toxicity in A375 cells. Toxicology Research. 2014;3(3):191-6.
7. Oyewo EB, Adetutu A, Adebisi JA. Immunomodulatory activities of yoyo bitters: Recommended dose precipitated inflammatory responses in male wistar rats. Pak J of Biol Sci. 2013a; (Online) ISSN 1028-8880/ doi: 10.3923/pjbs.2013
8. Bussmann RW, Glen A, Meyer K, Kuhlman A, Townsmith A. Herbal mixtures in traditional medicine in Northern Peru. Journal of Ethnobiology and Ethnomedicine. 2010;6-10.
9. Mythilypriya R, Shanthi P, Sachdanandam P. Oral and sub-acute toxicity studies with Kalpaamrutha, a modified indigenous preparation in rats. J of Health and Sci. 2007;53(4):322-4.
10. Fuglie LJ. The Miracle Tree: *M. oleifera*: Natural Nutrition for the Tropics. Church World Service, Dakar. 68 pp.; revised in 2001 and published as The Miracle Tree: The Multiple Attributes of Moringa. 1999;172.
11. Oyewo EB, Adetutu A, Adesokan AA, Akanji MA. Repeated oral administration of aqueous leaf extract of *Moringa oleifera* modulated immunoactivities in Wistar Rats. J of Natural Sci Res. 2013b;3(6):100-8.
12. Siddhuraju P, Becker K, Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (*Moringa oleifera Lam.*) leaves. Journal of Agricultural and Food Chemistry. 2003;51:2144-2155
13. Afolabi OK, Oyewo EB, Adekunle AS, Adedosu OT, Adedeji AL. Assessment of lipid peroxidation markers and proinflammatory cytokines in arsenite-exposed rats. Asian Journal of Natural & Applied Sciences. 2013;2(2):8-18.
14. National Institutes of Health. Guide for the Care and Use of Laboratory Animals. U.S. Department of Health and Human Services, Public Health Service, (NIH) Bethesda, MD., USA. 1985;83.
15. Sedlak J, Lindsay RH. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem. 1968;24-25(1):192-205.
16. Thomas JP, Geiger PG, Maiorino M, Ursini F, Girotti AW. Enzymatic reduction of phospholipid and cholesterol hydroperoxides in artifi- cial bilayers and lipoproteins. Biochim Biophys Acta. 1990;1045:252-60.
17. Sinha AK. Colorimetric assay of catalase. Anal Biochem. 1972;47:389-94.
18. Levine RL, Williams JA, Stadtman ER. Carbonyl assays for determination of oxidatively modified proteins. Method Enzymol. 1994;233:346-63.
19. Varshney R, Kale RK. Effect of calmodulin antagonists on radiation induced lipid peroxidation in microsomes. Int J Radiat Biol. 1990;58:733-43.
20. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR. Analysis of nitrate, nitrite, and [15 N] nitrate in biological fluids. Anal Biochem. 1982;126:131-8.
21. Guder WG, Narayanan S, Wiisser H, Zawta B. List of analytes preanalytical variable. Broschure in: samples: from the patient to the laboratory. Darmstadt: GIT-Verlag; 1996.
22. Clark I. A colometric reaction for the estimation of cortisone, hydrocortisone, aldosterone and related steroids. Nature. 1955;75:123-4.
23. Gokhale AB, Damre AS, Saraf MN. Investigations into immunomodulatory activity of *Argyreia speciosa*. J Ethnopharmacol. 2003;84:109-14.
24. Pfeiffer NE, Mcguire TC, Bendel RB. Quantitation of bovine immunoglobulins: comparison of single radial immunodiffusion, zinc sulfate turbidity, serum electrophoresis, and refractometer methods. Am J Vet Res. 1977;8:693-8.
25. Fulzele SV, Satturwar PM, Joshi SB, Dorle AK. Study of the immunomodulatory activity of *Haridradi ghrita* in rats. Indian J Pharmacol. 2003;35:51-4.
26. Delgado C, Navarro M, Lopez H, Lopez MC. Determination of total arsenic levels by hydride generation atomic absorption spectrometry in foods from south-east Spain: estimation of daily dietary intake. J. Food Additives and Contaminants. 2003;10(20):923-32.
27. Flora S, Mittal M, Mehta A. Heavy metal induced oxidative stress and its possible reversal by chelation therapy. Indian J Med Res. 2008a;128(4):501-23.

28. Stice SA. Speciation, metabolism, toxicity and protein-binding of different arsenic species in human cells," FIU Electronic Theses and Dissertations 1203; 2014.
29. Valko M, Morris H, Cronin MTD. Metals, toxicity and oxidative stress. Current Medicinal Chemistry. 2005;12(10):1161-208.
30. Sharma B, Singh S, Siddiqi NJ. Biomedical implications of heavy metals induced imbalances in redox systems BioMed Research International. 2014. Article ID 640754, 26 pages.
31. George C, Kumar DR, Suresh PK, Kumar RA. *In vitro* protective potentials of *Annona muricata* leaf extracts against sodium arsenite-induced toxicity. Current Drug Discovery Technologies. 2015;12(1):59-63.
32. Oyewo EB, Akanji MA, Iniaghe MO, Fakunle PB. Toxicological implications of aqueous leaf extract of *Andrographis paniculata* in Wistar Rat. Nature and Science. 2012;10(2):91-108.
33. Oyewo EB, Fakunle PB. Safety concerns of the repeated administration of Yoyo bitters in male Wistar rats. International Journal for Pharmacy and Pharmaceutical Sciences. 2013c;5(3):323.
34. Lawal AO, Ellis EM. The chemopreventive effects of aged garlic extract against cadmium-induced toxicity. Environmental Toxicology and Pharmacology. 2011;32(2): 266-74.

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