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Antioxidant Actions of Dried Flower Extracts of *Hibiscus sabdariffa* L. On Sodium Arsenite - Induced Oxidative Stress in Rats

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Abstract: The antioxidant actions of 80% ethanolic extract of dried flowers of *Hibiscus sabdariffa* L. (HSE) on lipid peroxidation (LPO), reduced glutathione (GSH), glutathione-s-transferase (GST), catalase (CAT), superoxide dismutase (SOD) and vitamin C (VITC), were examined using a model of sodium arsenite (SA) - induced oxidative stress in rats. The oral administration of the extracts (200 and 300mg/kg body weight) significantly ($P < 0.05$) decreased by 37% SA - induced malondialdehyde (MDA) formation in liver, suggesting the role of the extract in protection against pro-oxidant induced membrane damage. Pretreatment with the extracts prior to the intra-peritoneal administration of 10mg/kg body weight of SA reduced significantly (by 86%) and induced non-significantly (by 37%) the level of GSH depletion and GST activity respectively in a dose - dependent manner. The extract also attenuated SA - induced reduction in the serum level of VITC as evidenced by a significant ($P < 0.05$) dose - dependent increase (by 60%) in serum VITC level. Pretreatment with the extracts showed a significant ($P < 0.05$) increase in liver and decrease in whole blood activities of SOD (by 369% and 85%) and CAT (by 829% and 58%) respectively, hence revealing the hepatoprotective and antioxidant effectiveness of the extracts. Furthermore, the extracts, evaluated (*in vitro*) by their capacity of quenching 1, 1-diphenyl - 2-picrylhydrazyl (DPPH) free radical, showed strong scavenging effects on DPPH free radical at concentration of 0.20mg/ml (1C50 = 0.20mg/ml). The extracts at low and high concentrations showed no inhibitory effect on nitric oxide radical. These findings are suggestive of the possible chemopreventive and antioxidative role played by dried flower extract of *Hibiscus sabdariffa* L.

Key words: Sodium arsenite, oxidative stress, *Hibiscus sabdariffa* L., antioxidant activity

Introduction

Reactive oxygen species (ROS) or free radicals are generated as byproducts or intermediates of aerobic metabolism and through reactions with drugs and environmental toxins. The elevated cellular levels of free radicals cause damage to nucleic acid, proteins, and membrane lipids and have associated with many aging-related problems including carcinogenesis and heart diseases (Halliwell *et al.*, 1992; Halliwell, 1996; Wang and Jiao, 2000). The balance between the production and scavenging of ROS can therefore determine the susceptibility of the body to oxidative damage. Although almost all organisms possess antioxidant defense and repair systems, which quench or minimize the production of oxygen-derived species, thus protecting them against oxidative damage, these protective systems are insufficient to entirely prevent the damage (Simic, 1988) caused by endogenous or exogenous oxidants (Sun, 1990). In view of this, much attention has been focused on the protective biochemical function of naturally occurring antioxidants in biological systems, and on the mechanisms of their actions. The phenolic compounds, which are widely distributed in plants, were considered to play an important role as dietary antioxidants for the prevention of oxidative damage in living systems (Block, 1992; Hertog and Feskens, 1993).

The study of numerous compounds that could be useful antioxidants has generated increasing interest in the field of food or medicine. The dried flowers of *Hibiscus sabdariffa* L. (Malvaceae) commonly called "zobo" by Nigerians have gained importance as local soft drink and medical herb in local regions. Studies revealed that the dried flowers of *Hibiscus sabdariffa* L., a Chinese herbal medicine, have been used effectively in folk medicine against hypertension, pyrexia, and liver disorders (Tseng *et al.*, 1997). The present study was aimed at evaluating the antioxidant effects of ethanolic extracts of dried flowers of *Hibiscus sabdariffa* L., on some biomarkers of oxidative stress in rats treated with sodium arsenite.

Materials and Methods

Plant material: The fresh flowers of *Hibiscus sabdariffa* L. were purchased at Bodija Market, Ibadan. They were dried at room temperature and Soxhlet extracted with 80% ethanol (three changes), lyophilized, weighed and preserved at 4°C and used as and when required (Rana *et al.*, 2000).

Experimental animals: Twenty female Wistar rats (160-200g) obtained from the Department of Physiology, University of Ibadan were used. They were maintained

and housed in cages in the departmental animal house and fed on commercial rat pellets obtained from Mokola, Ibadan.

Experimental design: Animals were randomized into five groups with four in each group and put on normal diet. The extract and sodium arsenite, dissolved in distilled water were administered orally and intraperitoneally (ip) respectively.

- Group A (control): Only normal diet. Group B: Animals were treated with only 10mg/kg body weight of SA.
- Group C: Animals were treated with 200mg/kg body weight of HSE for 7 days.
- Group D: Animals were treated for 6 days with 200mg/kg body weight of HSE and 10mg/kg body weight SA on the 7th day.
- Group E: Animals were treated for 6 days with 300mg/kg body weight of HSE and 10mg/kg body weight of SA on the 7th day.

Preparation of serum and microsomal fractions of liver homogenate: The animals were sacrificed 24 hours after the sodium arsenite administration by cervical dislocation. The blood was collected by heart puncture and serum was separated by centrifugation (3000rpm at 4°C for 10 mins). The serum was used for the determination of vitamin C. The whole blood was also collected for the determination of catalase and SOD activities. The liver was immediately removed, washed in ice cold 1.15%KCl solution, blotted, weighed and homogenized in 4 volumes of the homogenizing buffer (pH7.4) using a Potter-Elvehjem homogenizer. The resulting liver homogenate was centrifuged at 10,000g for 30mins, in a Beckman L5 - 50B ultracentrifuge with a 220.78VD2 rotor at 4°C. The solution (supernatant) was decanted and part of it used for GST assay. The other portion was further centrifuged at 105,000g for 1 hour with a type 35 fixed angle rotors in the same ultracentrifuge. Pellet microsomes were suspended in 0.25M sucrose solutions and this was stored in a frozen condition. These procedures were carried out at temperature between 0°C and 4°C so as to retain enzyme activity. The liver microsome was used to determine lipid peroxidation, reduced glutathione, SOD, catalase and free radical scavenging capacity.

Assessment of lipid peroxidation: Lipid peroxidation in microsomes prepared from liver was estimated spectrophotometrically by Thiobarbituric acid - reacting substances (TBARS) method as described by Varshney and Kale (1990). The reaction mixture contained 0.4ml of microsomal sample mixed with 1.6ml 0.15M Tris KCl buffer, 0.5ml of 30% TCA and 0.5ml of 52mM TBA. The mixture was placed in a water bath for 45min at 80°C,

cooled in ice and centrifuged at room temperature for 10min at 3,000rpm. The absorbance of the clear supernatant was measured against reference blank of distilled water at 532nm in a spectrophotometer.

Determination of catalase: Whole blood and liver catalase was estimated in a UV recording spectrophotometer at 240nm by monitoring the decomposition of H₂O₂ as described by Aebi (1984). The reaction mixture (1ml, vol.) contained 0.02ml of suitably diluted cytosol in phosphate buffer (50mM, pH7.0) and 0.1ml of 30mM H₂O₂ in phosphate buffer. The specific activity of catalase has been expressed as moles of H₂O₂ reduced per minute per mg protein.

Superoxide dismutase (SOD) assay: Whole blood and liver superoxide dismutase was assayed utilizing the technique of Fridovich (1989). 1ml of whole blood was diluted in 9ml of distilled water to make a one in ten dilution of whole blood. An aliquot of 2.0ml of the diluted blood was added to 2.5ml of 0.05M carbonate buffer (pH 10.2) to equilibrate in the spectrophotometer and the reaction started by the addition of 0.3ml freshly prepared 0.3mM adrenaline to the mixture which was quickly mixed by inversion. The reference cuvette contained 2.5ml buffer, 0.3ml of substrate (adrenaline) and 0.2ml of water. The increase in absorbance at 480nm was monitored every 30 seconds for 150sec. A single unit of enzyme is defined as the quantity of SOD required to produce 50% inhibition of autoxidation.

Hepatic reduced glutathione (GSH) determination: Hepatic GSH was estimated by a colorimetric method using Ellman's reagent as described by Sedlak and Lindsay (1968). Liver of rats was removed, blotted and homogenized in 4 volume of ice-cold homogenizing buffer (pH 7.4). An aliquot of the homogenate was deproteinized by addition of an equal volume of 4% sulfosalicylic acid and after centrifugation at 17,000g for 15min at 2°C. 0.5ml of the diluted supernatant was added to 4.5ml of Ellman's reagent. A blank was prepared with 0.5ml of the diluted precipitate solution (diluted twice with 0.1ml phosphate buffer and 4.5ml Ellman's reagent). GSH was proportional to the absorbance at 412nm. The GSH level was quantified using a standard curve prepared by plotting with different concentration of GSH.

Vitamin C assay: Vitamin C in serum was assayed according to the method of Wilson and Guilan (1969). 0.5ml of serum was added to 2ml of freshly prepared TCA (6g/100ml) in 13x100mm test tubes and mixed well on a vortex mixer. This mixture was centrifuged for 10min at 2500rpm. 1.2ml of the clear supernatant was pipetted into 12x100mm screw cap test tubes. The standards were prepared in duplicate. 1.2ml of TCA (6g/100ml)

were added to two test tubes to use as blank. 0.4ml of dinitrophenyl hydrazine-thiourea-copper sulphate (DTCS) reagent was added to all tubes, which were capped, mixed and incubated in a water bath at 37°C for 3 hours. The tubes were removed from water bath and chilled for 10min in ice bath, while mixing slowly. 2ml of cold 12M H₂SO₄ was mixed and the mixture checked to make sure it did not exceed room temperature. The spectrophotometer was adjusted with the blank to read zero absorbance at 520nm and then absorbance of standards and unknown read. The concentration of each working standard was plotted against absorbance. The vitamin C level was quantified using the standard curve.

Glutathione-s-transferase (GST) assay: The cytosolic GST activity was determined spectrophotometrically at 37°C according to the method of Habig *et al.* (1974). The total reaction mixture contained 2.79ml 0.1M phosphate buffer (pH 6.5), 0.15ml 20mM 1-chloro-2, 4-dinitrobenzene (CDNB), 0.03ml 0.1M GSH. The reaction mixture was preincubated at 37°C which after the reaction was started by the addition of 0.03ml diluted cytosol and the absorbance was read at 30 sec, 1min, 2min, 3min, interval at 340nm. The reaction mixture without the enzyme was used as blank. The specific activity of GST is expressed as μmol of GSH-CDNB conjugate formed/min/mg protein using an extinction coefficient of 9.6mM⁻¹CM⁻¹

Determination of nitric oxide radical inhibition activity: Nitric oxide, generated from sodium nitroprusside in aqueous solution at physiological pH, interacts with oxygen to produce nitrite ions, which were measured by Griess reaction. The method of Green *et al.* (1982) and Macocci *et al.* (1994) was used. The reaction mixture (3ml) containing sodium nitroprusside in phosphate buffered saline and the extract (from 10 to 1000 $\mu\text{g}/\text{ml}$) was incubated at 25°C for 150min. After incubation 0.5ml of Griess reagent was added. The absorbance of the chromophore formed was evaluated at 546nm.

Determination of free radical-scavenging capacity: This involves testing the free radical-scavenging capacity of the extract by bleaching (at 517nm) the staple 1,1-diphenyl-2-picrylhydrazine (DPPH) according to the method of Ursin *et al.* (1994). A reaction mixture containing 3ml of methanol, 30 μl of DPPH and the extract of *Hibiscus sabdariffa* (at a final concentration of 0.01, 0.05, 0.20, 0.30, 0.40 or 0.50 mg/ml) was left to stand at room temperature for 30min before being mixed with redistilled water (1ml) and toluene (3ml). The solution was then centrifuged, and the absorbance of the upper phase was read at 517nm against a blank without crude extract. The percentage of DPPH bleaching was calculated thus:

$$\% \text{ of DPPH bleaching} = \frac{[Ab_c - Ab_T / Ab_c] \times 100\%}{}$$

Where: Ab_c = Absorbance of control group
 Ab_T = Absorbance of the extract added group

Protein determination: Protein was determined by following the method of Lowry *et al.* (1951) using bovine serum albumin (BSA), at 660nm.

Statistical analysis: The results were reported as means \pm SD from four repeated determinations and evaluated with the analysis of student's t-test. Differences were considered to be statistically significant at $P < 0.05$.

Results and Discussion

An explosion of interest in studying the involvement of free radicals in carcinogenesis (Troll and Weisner, 1985), has led to the use of dietary antioxidant treatment to terminate free radical attacks, hence promoting general human health (Neff, 1997; Chung *et al.*, 1999; Baubles *et al.*, 2000). The antioxidants may act as free radical scavengers, reducing agents, chelating agents for transition metals, quenchers of singlet oxygen molecules, and activators of antioxidative defense enzyme systems to suppress radical damage in biological systems (Zielinski and Kozłowska, 2000).

It is evident from our results (Table 1) that pretreatment with HSE significantly ($P < 0.05$) decreased (by 37%) SA-induced hepatic lipid peroxidation when compared with the group that received only 10mg/kg of SA, hence exhibiting a dose dependent effect. Also increasing the conc. of HSE (from 200 to 300mg/kg) non-significantly delayed the lipid oxidation (by 2%) when compared with the control. The concentration of malondialdehyde (MDA), an index of lipid peroxidation was increased in rats treated with 10mg/kg of SA alone, but pretreatment with HSE decreased its formation.

The free radical chain reaction is widely accepted as a common mechanism of lipid peroxidation. Radical scavengers may directly react with and quench peroxide radicals to terminate the peroxidation chain reaction and improve the quality and stability of food products (Bran – Williams *et al.*, 1995; Chen and Ho, 1997). HSE may suppress lipid peroxidation through different chemical mechanisms, including free radical quenching, electron transfer, radical addition, or radical recombination (Liangli Yu *et al.*, 2002).

It is well established that reduced glutathione (GSH), the most important biomolecule protecting against chemically induced cytotoxicity, can participate in the elimination of reactive intermediates by conjugation and hydro peroxide reduction, or of free radicals by direct quenching. It plays a key role in the detoxification of its reactive toxic metabolites, and liver necrosis begins when GSH stores are markedly depleted (Davies *et al.*, 1974; Mitchell *et al.*, 1973). Our results (Table 1) demonstrated a significant ($P < 0.05$) decrease (by 43%)

Table 1: Effect of *Hibiscus sabdariffa* Extract (HSE) on Hepatic lipid peroxidation (LPO), Reduced Glutathione (GSH), Glutathione-s-transferase (GST) activity and protein concentration in rats treated with Sodium Arsenite (SA)

Group/ treatment	Mean ± SD			
	LPO(MDA mM/cm)	GSH (mg/ml)	GST (µmol/min/mg protein)	Protein (mg/ml)
A: Control (only normal feed)	102.60±9.051	5.80±0.46	0.11±0.07	38.11±19.28
B: 10mg/kg HSE only	160.30±23.13 (56%)	3.29±0.67 (43%)*	0.051±0.02 (116%)*	22.49±10.10
C:200mg/kg HSE only	86.53±37.18 (16%)* (46%)**	6.87±0.34 (18%)* (108%)**	0.13±0.07 (18%)* (61%)**	37.02±16.40
D:200mg/kg HSE and 10 mg/kg SA	111.5.40±33.92 (12%)* (28%)**	4.789±0.69 (17%)* (46%)**	0.053±0.03 (52%)* (3%)**	27.77±11.09
E: 300mg/kg HSE and 10 mg/kg SA	100.95±25.32 (29%)* (37%)**	6.13±0.79 (6%)* (86%)**	0.07±0.06 (36%)* (37%)**	25.16±6.36

and increase (by 18%) in the conc. of GSH in rats treated with only 10mg/kg of sodium arsenite and 200mg/kg of the extract respectively as compared to the control. The depletion of GSH promotes generation of ROS and oxidative stress with cascade of effects thereby affecting functional as well as structural integrity of cell and organelle membrane (De Leve *et al.*, 1996). However, pretreatment with HSE significantly reduced sodium arsenite - induced hepatic GSH depletion; the result which is probably due to the decreased bioactivation of sodium arsenite to reactive species by the extracts. The elevated level of GSH protects cellular proteins against oxidation through glutathione redox cycle and also directly detoxifies ROS and/or neutralize reactive intermediate species generated from exposure to xenobiotics including chemical carcinogens (Ketterer, 1998). Our *in vivo* study showed that SA reduced GSH levels in the rat liver and a high dose of HSE blocked the phenomenon effectively.

GSH is largely mediated through the activity of GST, and form adducts with the toxic metabolites of SA. Results in Table 1 showed that HSE also induced (by 37%) the activity of hepatic GST (though not significantly), by preventing the SA - induced reduction (by 116%) of this enzyme. GSTs catalyze the conjugation between electrophiles and nucleophiles GSH. Their primary function is to detoxify electrophiles capable of DNA binding, a typical reaction of phase II metabolism (Pickett *et al.*, 1995). They play a critical role in protecting tissues against product of oxidative stress and electrophiles. Persuasive evidences abound which support the induction of GST and protection against a wide spectrum of cytotoxic, mutagenic and carcinogenic chemicals (De Flora and Ramel, 1989; Ketterer, 1988; Reed, 1990). The protective effect of many naturally occurring chemopreventive agents against carcinogenesis have been ascribed to decreased bioavailability of potential DNA damaging entities and their destruction into excretable metabolites facilitated through induction of GST (Coles and Ketterer, 1990). In

this study the specific activity of the enzyme measured was the sum of all its isoforms. The results in our study of HSE against SA-induced hepatotoxicity may be related to increased levels of GSH content or increased GST activity and the possibility exists that HSE is involved in the direct quenching of the reactive metabolites of SA deactivation such as conjugation with glucuronide and sulphate.

The results in Table 2 revealed significant ($P < 0.05$) reduction (by 50%) and elevation by (10%) in levels of vitamin C in animals that received only the toxicant and the extract respectively as compared to the control. HSE pretreatment also prevented significantly SA-induced reduction of serum vitamin C levels, hence exhibiting a dose dependent effect. Vitamin C (ascorbic acid) is an outstanding powerful antioxidant that reacts rapidly with a variety of oxidants, including the rather poorly reactive superoxide anion radical (Nishikimi, 1975). Vitamin C within the body is maintained in the reduced form by shuttling the dehydroascobate across the erythrocyte membrane for reconversion to ascorbate (Orroinger and Roear, 1979), a mechanism that may not cope with oxidative stress. The decrease in the levels of vitamin C could be attributed to increased production of ROS, and the increased levels is possibly due to the ability of HSE to mitigate ROS produced by the compound.

The activities of physiological antioxidants such as catalase and superoxide dismutase were studied. The results (Table 2) revealed a dose - dependent change in the activities of whole blood and liver catalase, and whole blood and liver SOD in HSE treated groups. Catalase is a tetrameric hemoprotein that undergoes reduction at its active site in the presence of its substrate, H_2O_2 , and catalyses the dismutation of H_2O_2 to water and molecular oxygen. The result of our study showed an increase and a decrease in the activity of whole blood and liver catalase respectively due to administration of SA alone. This increase is thought to be due to an induction in response to increased production of H_2O_2 following increased oxidant stress

Table 2: Effect of Extract of *Hibiscus sabdariffa* (HSE) on Serum Vitamin C levels, Whole Blood and Hepatic Catalase (CAT) and Superoxide Dismutase (SOD) activities in rats treated with Sodium Arsenite

Group/ treatment	Mean ± SD				
	Vitamin C (mg/100ml)	Whole blood CAT (Kat f)	Liver CAT (Kat f)	Whole blood SOD Unit of activity	Liver SOD unit of activity
A: Control (only normal feed)	0.186±0.0087	0.89±0.09	2.63±0.07	0.58±0.23	1.56±0.46
B: 10mg/kg HSE only	0.081±0.022 (56%)*	1.32±0.29 (48%)*	0.28±0.02 (89%)	1.93±0.038 (232%)*	0.35±0.129 (75%)*
C:200mg/kg HSE only	0.206±0.012 (10%)* (56%)**	0.46±0.06 (48%)* (65%)**	2.99±0.13 (14%)* (968%)**	0.48±0.02 (17%)* (75%)**	1.97±0.15 (28%)* (462%)**
D:200mg/kg HSE and 10 mg/kg SA	0.126±0.030 (32%)* (56%)**	0.63±0.066 (29%)* (52%)**	2.36±0.061 (10%)* (742%)**	0.56±0.04 (3%)* (71%)**	1.42±0.46 (9%)* (31%)**
E:300mg/kg HSE and 10 mg/kg SA	0.130±0.030 (30%)* (60%)**	0.55±0.064 (38%)* (58%)**	2.60±0.005 (10%)* (829%)**	0.28±0.17 (52%)* (85%)**	1.64±0.31 (5%)* (369%)**

Table3: Effect of Extract of *Hibiscus sabdariffa* on Nitric Oxide radical and DPPH bleaching

Dose (mg/ml)	IC ₅₀	Dose (mg/ml)	% of DPPH Bleaching
10-1000	N.D	0.01	42.4±3.60
		0.05	44.8±5.70
		0.20	49.7±2.80
		0.30	57.7±7.10
		0.40	66.7±4.60
		0.50	75.6±8.10

* % change with respect to control group, A

** % Change with respect to toxicant treated group, B

Data are calculated from four experiments in each case

N.D = not detected

which injured the liver and caused a leakage of the enzyme into the blood, hence increasing and decreasing its activities in the blood and liver respectively. Moreso, the activities of whole blood catalase were shown to be decreased and liver catalase increased following HSE pretreatment which inhibited SA-induced liver injury in rats. The hepatoprotective capacity of the extract is evident in the significant ($P < 0.05$) increase in the activity of liver catalase when compared with the toxicant treated group alone. It has been proposed that glutathione peroxidase is responsible for the detoxification of H_2O_2 in low concentration whereas catalase comes into play when glutathione peroxidase pathway is saturated with substrate (Gaetani *et al.*, 1989).

Superoxide dismutase (SOD) has an antitoxic effect against the superoxide anion. The augmented activity of metalloenzyme, SOD accelerates dismutation of superoxide radicals to H_2O_2 , which is removed by catalase (Aebi, 1984). H_2O_2 , a reaction product of the SOD reaction, inactivates SOD, and in the presence of H_2O_2 , SOD acts as a pro-oxidant (Bast *et al.*, 1991; Yim *et al.*, 1990). It is observed from the results (Table 2) of this experiment that administration of SA alone increased and decreased respectively whole blood and liver SOD activities. This observation may not be unconnected with a response to an increased

production of ROS induced by the toxicant. The pharmacokinetics of SA may play a significant role in the difference observed on the activities of SOD in the blood and liver. The decrease in SOD activity observed in the liver might be attributed to the high distribution of the toxicant in the liver, a major target organ for most xenobiotics. The high concentration therefore in the liver and the resultant damage should be responsible for the decrease in SOD activity observed in this study. The affinity of xenobiotics for plasma protein determines the volume of distribution in this medium as well as the concentration of free (active) drug. The generation of H_2O_2 from metabolism of SA and perhaps elsewhere may be responsible for the induction of SOD activity in the blood coupled with relatively low distribution in plasma compared with the liver. The experiment also revealed a decrease and an increase respectively in the activity of whole blood and liver SOD following administration of the extract of *Hibiscus sabdariffa*. It is obvious from the result that HSE inhibits hepatotoxicity induced by 10mg/kg body weight of SA, evidently shown by a reduction and elevation in the whole blood and SOD activities. The elevated level of liver SOD suggests that HSE is hepatoprotective and contains antioxidant property.

Nitric oxide radical generation at physiological pH from sodium Nitroprusside was not found to be inhibited by the extract of *Hibiscus sabdariffa* as its $1C_{50}$ (mg/ml) was not detected, even at low and high concentrations of the extract (Table 3). This suggests that the extract may not scavenge nitric oxide radicals. The bleaching of DPPH by the extract of *Hibiscus sabdariffa* was measured in order to determine the free radical quenching capacity of the extract. DPPH was used to provide stable free radicals, which were scavenged by the extract in a dose-dependent manner (Table 3). Although a decrease in absorbance, in other words, loss of DPPH free radicals indicates the capacity of the extracts to capture free radicals; it is not a clear-cut definition of antioxidant effect (Tseng *et al.*, 1997). However, the present study revealed that the extract has the capacity to quench

about 50% DPPH free radicals at the concentration of 0.2mg/ml. This result might not be unconnected with the contribution of the phenolic constituents of the crude extract.

The present study demonstrates that the extracts of *Hibiscus sabdariffa* L. have dose-dependent protective effect against sodium arsenite-induced oxidative damage. It is not unlikely that this protective effect is probably mediated by its inhibitory effect on sodium arsenite bioactivation. More would have been comprehended if this study was extended to correlate the exact mechanisms and constituents of the extract on the levels of other parameters such as glutathione peroxidase, glutathione reductase, β -carotene, vitamin A, and phase I and II drug metabolizing enzymes in different tissues. Moreover, since the data reported in this study were generated for short-term treatment with HSE; it is recommended that long-term animal studies to evaluate the effects of these extracts on biomarkers of oxidative stress and biochemical mechanisms involving xenobiotic enzymes be carried out. This will increase our understanding of the role of *Hibiscus sabdariffa* L. in chemical carcinogenesis.

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