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Free radical scavenging and antigenotoxic activities of natural phenolic compounds in dried flowers of *Hibiscus sabdariffa L*

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The antioxidant and free radical scavenging effects of two fractions of the ethanolic extract (HSCF, chloroform soluble fraction and HSEA, ethyl acetate soluble fraction) obtained from the dried flowers of *Hibiscus sabdariffa* L were investigated. The total antioxidant activity of the extracts was estimated to be 4.6 and 8.6 mM of vitamin C for HSCF and HSEA, respectively. Both HSCF and HSEA scavenged hydrogen peroxide (H_2O_2) (79 – 94%) at the dose of 500 μg. Similarly, the extracts showed inhibitory (70 – 80%) effects on superoxide anions radicals ($O_2^{-\bullet}$) at a dose of 1000 μg. The concentrations required for a 50% scavenging of hydroxyl radical (OH) (IC_{50}) were 380 and 200 μg for HSCF and HSEA, respectively. HSEA and HSCF were better scavengers of $O_2^{-\bullet}$, $^{\bullet}OH$ and H_2O_2 as compared to BHA, quercetin and α-tocopherol. At a concentration of 25 μg/mL HSCF and HSEA exhibited 32 and 38% inhibition on CCl_4-NADPH-induced lipid peroxidation, respectively, while both extracts exhibited 80 and 89% inhibitory effects at 100 μg/mL. Pretreatment with *H. sabdariffa* extracts orally with 100 mg/kg and 250 mg/kg simultaneously with intraperitoneal injection $FeCl_2$-ascorbic acid-ADP mixture reduced ($p < 0.01$) the formation of malondialdehyde content. Treatment of rats with HSCF, HSEA and vitamin C (standard antioxidant) significantly inhibited the induction of micronucleated polychromatic erythrocytes by sodium arsenite (2.5 mg/kg) ($p < 0.001$) after 24 h by 60, 70 and 50%, respectively. The results indicate that extracts of *H. sabdariffa* showed strong antimutagenic activity and free radical scavenging effects on active oxygen species.

Keywords: *Hibiscus sabdariffa* / Micronucleus / Phenolics / Radical scavenger

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1 Introduction

Free radicals are thought to be involved in the development of a number of pathological effects and diseases such as diabetes and atherosclerosis as well as aging [1]. The radicals implicated in these processes are mainly oxygen-centered radicals such as superoxide, hydroxyl, peroxyl and nitric oxide [2]. There is sufficient evidence that exposure to reactive oxygen species (ROS) leads to deleterious changes of cell function by a number of alterations such as lipid peroxidation, enzyme inactivation and oxidative DNA damage [2].

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Abbreviations: HSCF, chloroform soluble fraction; **HSEA**, ethyl acetate soluble fraction; **MNPCE**, micronucleated polychromatic erythrocytes; **MDA**, malondialdehyde; **ADP**, adenine dinucleotide phosphate; **NADP**$^+$, nicotinamide adenine dinucleotide phosphate

Fatty acids are susceptible to attack such as hydroxyl radicals, which stimulates lipid peroxidation. Abstraction of hydrogen is easier with unsaturated fatty acids than with saturated thus making the former more susceptible to ROS attack [1]. The toxicological significance of this process has been linked to spontaneous mutagenesis and carcinogenesis [3].

Arsenic, a known human carcinogen and teratogen, is ubiquitously present in the environment, where it occurs as compounds of arsenite (As^{3+}) and (As^{5+}) [4, 5]. Arsenic contamination of drinking water is a serious environmental problem worldwide and the risk of developing arsenic-induced human disease from environmental exposure is particularly high in many developing countries [5]. A recent study of arsenic contamination of ground water in West Bengal, India, has been reported [6].

Although the mechanism by which arsenic induces toxicity is not completely understood, free radicals have been implicated [7, 8]. In addition, ROS, particularly hydroxyl radi-

cals, have also been shown to play pivotal causal role in the genotoxicity of arsenical compounds in mammalian cells [9, 10].

The administration of antioxidative compounds or natural products to treat or prevent environmentally induced toxicity is attractive. Thus, the characterization of natural plant substances as antioxidant prophylactic is necessary [11, 12]. A number of herbs and diets rich in fruits and vegetables have been found to be protective against life-threatening diseases and this effect has been attributed partly to the antioxidants contained therein, in particular phenolic compounds [13]. Polypehnols are common constituents of the human diet, present in most foods and plant materials. They are known to contribute to the prevention of various diseases, including cancer, neurodegenerative diseases, osteoporosis and cardiovascular diseases [14]. Additional evidence for a protective role of phenolic compounds against chronic diseases have emanated from epidemiological studies as well as clinical trials and experiments in animal models [15]. The health effects of polyphenols, however, depend on the amount consumed and on their bioavailability [16].

Hibiscus sabdariffa L (Malvaceae) exists as shrubs often with fibrous stems believed to be native to Africa [17] and it is lemon tasting. It has gained wide acceptance in folk medicine in treating many diseases. Thus, it has been shown that tea infusion of the plant lowered both the systolic and diastolic blood pressures in patients presenting with essential hypertention [18]. Its anti-atherosclerotic activity in rabbits fed cholesterol has also been reported [19]. It is used in making 'Sobo', a local popular drink in Nigeria. The compounds isolated from the flowers of *H. sabdariffa* include anthocyanins [17] and protocatechuic acid a simple phenolic compound [20]. However, the biological properties of this plant and its effect on drug-induced mutagenesis have not been fully elucidated.

In the present study, we have focused on the ability of the polyphenolic compounds in *H. sabdariffa* dried flower to scavenge ROS using established *in vitro* and *in vivo* assays. Since oxidative stress has been implicated in arsenic toxicity, we investigated additionally the potential preventive effects of flavonoid compounds of *H. sabdariffa* on the sodium arsenite-induced micronucleus induction in the rat bone marrow.

2 Materials and methods

2.1 Chemicals

The 2,2-azino-bis-(3-ethyl benz-thiazoline-6-sulfonic acid); (ABTS), nitroblue tetrazolium, thiobarbituric acid, glucose 6-phosphate, glucose 6-phosphate dehydrogenase, ascorbic acid, adenine dinucleotide phosphate (ADP), nicotinamide adenine dinucleotide phosphate ($NADP^+$), butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), quercetin and α-tocopherol were obtained from Sigma Chemical (St. Louis, MO, USA). All other reagents were of analytical grade and were obtained from British Drug Houses (Doorset, UK).

2.2 Sample preparation

Hibiscus sabdariffa dried flowers were purchased from a local market, Bodija, in Ibadan, Nigeria and stored for 1 week at room temperature. A voucher sample was authenticated at the Department of Botany University of Ibadan, Ibadan, Nigeria. One kilogram of the dried flowers was subjected to Soxlet extraction with ethanol. The average yield of the extract was 42%. The ethanol extract was extracted with chloroform to obtain a soluble fraction designated (HSCF) and the insoluble fraction was re-extracted with ethyl acetate and the ethyl acetate fraction was named (HSEA). The fractions were concentrated in a rotary evaporator at 40°C. The yields of HSCF and HSEA were 480 and 520 mg/g, respectively. The extracts were stored in a moisture-free container at 4°C. Protocatechuic acid was used for the standardization of the batches in this experiment. Both HSCF and HSEA were dissolved in DMSO. HSCF has been shown to contain β-sitosteroid and flavonoids using a combination of column and TLC methods [21], while HSEA was shown to contain protocatechuic acid using silical gel column chromatography, analysis of spectral data and comparison with an authentic sample using TLC [20].

2.3 Determination of total phenolic content of HSCF and HSEA

The phenolic content of HSCF or HSEA was determined as described by Yen *et al*. [22]. The extract (0.1 g) was dissolved in 5 mL of 0.3% HCl in methanol/water (60 : 40, v/v). The resulting solution (100 μL) was added to 2 mL of 2% Na_2CO_3. After 2 min, 50% Folin-Ciocalteau reagent (100 μL) was added to the mixture, which was then left for 30 min. Absorbance was measured at 750 nm spectrophotometrically using protocatechuic acid as standard and concentrations were expressed as protocatechuic acid equivalents.

2.4 Determination of total antioxidant activity

Total antioxidant activity of the HSCF and HSEA fractions was determined using the horseradish peroxidase catalyzed oxidation of 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfo-

nic acid) (ABTS) [23]. The reaction mixture contained 2 mM ABTS, 0.1 mM H_2O_2 in 50 mM glycine-HCl buffer, pH 4.5 and 5 mg of the extract (HSCF or HSEA) in a total volume of 2 mL. The reaction was started by the addition of 0.25 nM peroxidase. After 10 min, absorbance was measured at 414 nm spectrophotometrically. Ascorbic acid was used as standard and total antioxidant activity was measured as mM of vitamin (Vit) C equivalent.

2.5 Hydroxyl radical scavenging assay

Hydroxyl radical scavenging activity was determined by assessing the ability of deoxyribose to compete with test compounds for hydroxyl radicals. Hydroxyl radicals were generated by a Fenton-type reaction system (Fe^{3+} + EDTA/H_2O_2/ascorbic acid) as described by Aruoma and Halliwell [24]. The reaction mixture contained deoxyribose (2.8 mM), $FeCl_3$ (0.1 mM), EDTA (0.1 mM), H_2O_2 (1 mM), ascorbate (0.1 mM), KH_2PO_4-KOH buffer (20 mM, pH 7.4) and HSCF (0–1000 μg) or HSEA (0–1000 μg) in a final volume of 1 mL. The mixture was incubated for 1 h at 37°C followed by the addition of 1.0 mL TBA (0.7% in 0.05 M KOH) and 1.0 mL of 2.5 mL of 2.5% TCA. The mixture was heated at 100°C for 8 min, cooled and the absorbance was measured at 532 nm.

2.6 Superoxide anion radical production

Superoxide was generated non-enzymatically in a mixture composed of HSCF (10–1000 μg) or HSEA (10 1000 μg) 10 μM phenazine methosulfate, 78 μM NADH, 25 μM NBT and 0.1 M phosphate buffer (pH 7.4). The mixture was incubated for 5 min at room temperature and the color was measured at 560 nm against blank samples [25].

2.7 Scavenging of hydrogen peroxide

The scavenging of extracts from *H. sabdariffa* on hydrogen peroxide was determined as described by Yen and Chen [26]. Hydrogen peroxide concentration was determined in a 4 mM solution prepared in PBS, pH 7.4 using molar absorptivity of 81 M^{-1} cm^{-1}. HSCF (0–0.5 μg) and HSEA (0–0.5 μg) dissolved in 4 mL distilled water was added to hydrogen peroxide solution (0.6 mL). Absorbance of hydrogen peroxide was determined at 230 nm 10 min later against a blank solution containing the extracts without hydrogen peroxide.

2.8 *In vivo* lipid peroxidation

Thirty-five male rats, Wistar strain (145–165 g), were randomly divided into 7 groups. Rats in group 1 served as control and group-2 rats were administered daily a mixture of $FeCl_2$ (30 mg/kg body weight), ascorbic acid (170 mg) and ADP (5 mg) intraperitoneally for 3 days [27]. Rats in the groups 3, 4, 5 and 6 received orally 100 mg/kg HSCF, 250 mg/kg HSCF, 100 mg/kg HSCEA and 250 mg/kg HSCEA daily, respectively, in addition to $FeCl_2$/As/ADP mixture as in group 2 for 3 days. Animals in group 7 received 25 mg/kg α-tocopherol per day 1 h before administration of the $FeCl_2$/AS/ADP mixture. Rats were sacrificed 24 h after the last treatment by cervical dislocation. Microsomes were prepared from the excised liver samples by centrifuging the homogenate at $10\,000 \times g$ for 20 min and the supernatant was centrifuged at $105\,000 \times g$ for 1 h to obtain the microsomes. Lipid peroxidation was estimated by the TBA test as described previously [11]. Malondialdehyde (MDA) was quantitated by using $\Sigma = 1.56 \times 10^5$ M^{-1} cm^{-1} [28].

2.9 CCl_4-NADPH-stimulated lipid peroxidation

In vitro microsomal lipid peroxidation was evaluated in the CCl_4-NADPH-stimulated system. [29]. Reaction mixture contained microsomal protein (1.5 mg/mL), 0.2 mM $NADP^+$, 4 mM glucose 6-phosphate, 0.6 units glucose 6-phosphate dehydrogenase (NADPH generating system) in 0.1 M Tris HCL, 1.15% KCl (pH 7.4) and HSCS (0–100 μg) or HSEA (0–100 μg). Lipid peroxidation was induced by the addition of CCl_4 (0.02 M final concentration). The reaction mixture was incubated for 15 min and TBA test was performed as described above.

2.10 Genotoxicity studies

Initial time-course and dose-response studies were carried out to establish the required dose for micronucleus induction and sampling time. Rats were treated with a single oral administration of sodium arsenite at doses of 1.5, 2.5 and 3.5 mg/kg body weight and were sacrificed at 0, 24, 48 and 72 h and micronuclei assay was conducted. Control rats received oral saline alone.

In order to evaluate the antigenotoxic potential of HSCS and HSEA and ascorbic acid on sodium arsenite-induced genotoxicity, 35 male rats were divided into seven groups of 5 rats per group. Rats in group 1 served as control and group-2 rats were administered single dose of sodium arsenite (2.5 mg/kg) by gavage. Rats in groups 3, 4, 5 and 6 received 100 mg/kg HSCF, 250 mg/kg HSCF, 100 mg/kg HSCEA and 250 mg/kg HSCEA daily, respectively and

sodium arsenite (2.5 mg/kg) was orally administered on the 7th day. Animals in group 7 received 200 mg/kg ascorbic acid per day and sodium arsenite (2.5 mg/kg) was administered on the 7th day. Rats were sacrificed 24 h after the last treatment by cervical dislocation.

2.11 Bone marrow preparation and micronuclei assay

Bone marrow smears were prepared and micronucleus test carried out according to Asanami and Shimono [30]. Rats were sacrificed at 24 h after sodium arsenite treatment. Briefly, the femurs of each rat was removed and stripped clean of muscle. A syringe was then introduced into the marrow canal at the epiphyseal end; the marrow was flushed out through the hole at the iliac end with fetal bovine serum. The bone marrow was placed on a slide and mixed to obtain a homogenous mixture and spread as a smear. After drying of the slides, they were fixed in absolute ethanol for 5 min and air dried to remove the solvent. The slides were stained in 5% Giemsa for 30 min and rinsed in phosphate buffer (pH 7.4) for 30 s and distilled water for 2 min and air dried. The slides were coded screened to avoid bias and scored using a compound microscope with the aid of tally counter for the presence of micronucleated polychromatic erythrocytes.

2.12 Statistics

All variables were tested for normal distribution using the Kolmogorov-Smirnov test ($p > 0.05$) and for homogeneity of variance among groups using the Levene's test ($p > 0.05$). The groups were compared using one-way ANOVA. If significant differences were found ($p < 0.05$), the treatment groups were compared with the control group using Dunnett's test. All the statistics were carried out in SAS (The SAS System for windows, v8; SAS Institute Inc., Cary, NC).

3 Results

The results of the total antioxidant activity and total phenolic compounds in HSCF and HSEA extracts of *H. sabdariffa* are presented in Table 1. The HSEA fraction contained a significantly higher concentration of phenolic compounds than HSCF.

Figure 1 shows the scavenging activity of *H. sabdariffa* extracts on hydrogen peroxide. Both HSCF and HSEA were capable of scavenging hydrogen peroxide in a concentration

Table 1. Total phenolic content and total antioxidant activity of *H. sabdariffa* extracts[a)]

Sample	Total antioxidant activity TAA	Total phenolic content mg/g extracts
HSCF	4.6 ± 0.2	98.5 ± 2.6
HSEA	8.6 ± 0.4*	201.4 ± 2.1**

a) Values are means ± SD of three replicate analyses. Total antioxidant activity (TAA) was measured as mM equivalent of Vit C.

* Significantly different from HSCF ($p < 0.05$); ** significantly different from HSCF ($p < 001$).

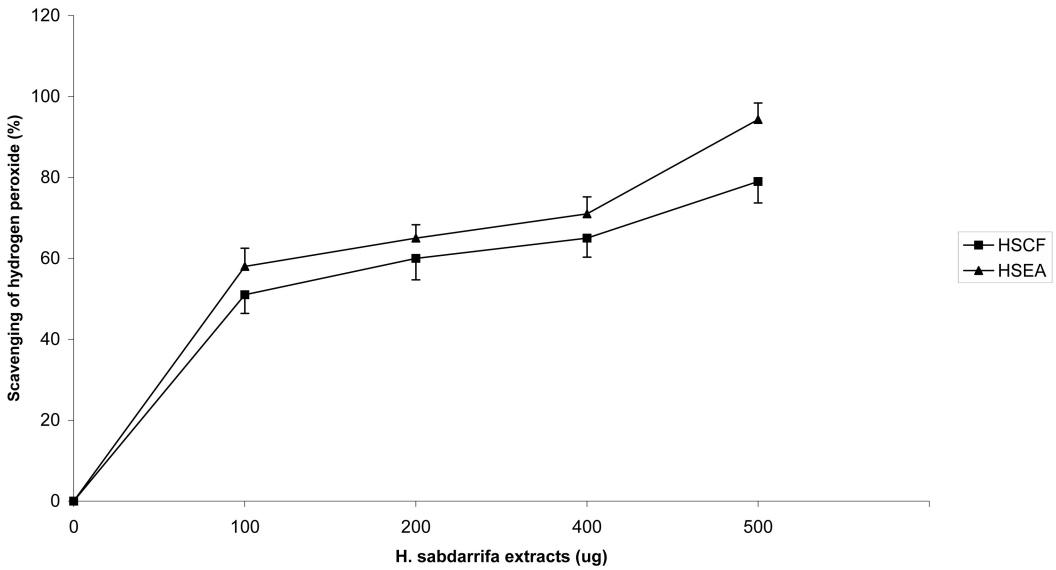

Figure 1. Scavenging effects of *H. sabdariffa* extracts (HSCF, chloroform soluble fraction; HSEA, ethyl acetate soluble fraction) on hydrogen peroxide. Values are mean ± SD of three determinations.

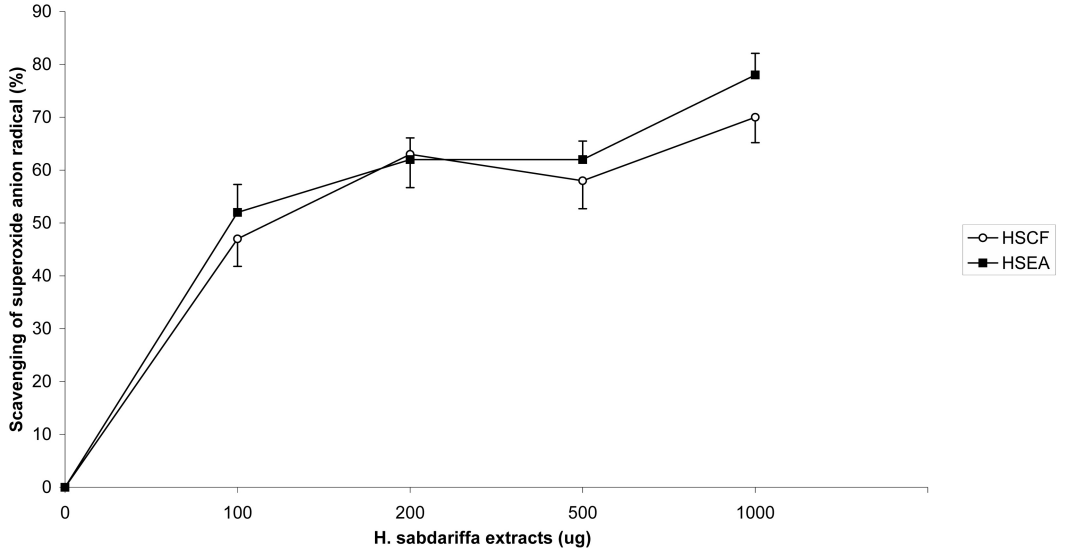

Figure 2. Scavenging effects of *H. sabdariffa* extracts (HSCF, chloroform soluble fraction; HSEA, ethyl acetate soluble fraction) on superoxide anion radical. Values are mean ± SD of three determinations.

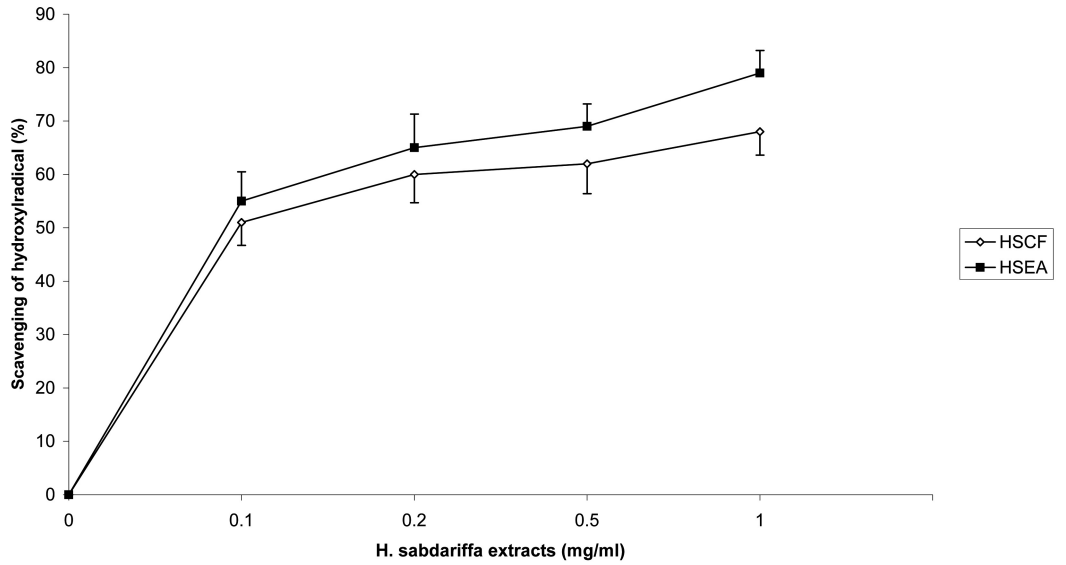

Figure 3. Scavenging of hydroxyl radicals by *H. sabdariffa* extracts (HSCF, chloroform soluble fraction; HSEA, ethyl acetate soluble fraction). Values are mean ± SD of three determinations.

dependent manner. They exhibited scavenging effect of 79 – 94% on H_2O_2 at the dose of 500 µg.

The ability of the extracts to scavenge superoxide anions is shown in Fig. 2. The scavenging effect of both HSEA increased with increasing concentration of the extracts. The extracts showed 70 – 80% inhibitory effects on superoxide anions at a dose of 1000 µg. HSEA was a better scavenger than HSCF in this assay system.

Figure 3 shows the effects of HSCF and HSEA on oxidation of deoxyribose. Both extracts inhibited the deoxyribose degradation in a concentration-dependent manner. The concentrations required for a 50% decrease (IC_{50}) were 380 and 200 µg for HSCF and HSEA, respectively (Table 2).

The inhibitory concentrations at 50% (IC_{50}) of the extracts of *H. sabdariffa* and some antioxidants on superoxide anion radicals, hydroxyl radicals and hydrogen peroxide are presented in Table 2. HSEA and HSCF were better radical scavengers compared to BHA, quercetin and α-tocopherol.

Table 3 shows the effect of HSCF and HSEA on lipid peroxidation in rat liver stimulated by administration of $FeCl_2$-ascorbic acid-ADP mixture. Following intraperitoneal injection of the oxidant mixture, there was a significant ($p <$

Table 2. Effect of *H. sabdariffa* extracts on rat liver lipid peroxidation induced by intraperitoneal administration of $FeCl_2$-ascorbic acid-ADP mixture[a)]

Treatment	Dosage mg/kg/day	MDA nmol/mg protein
Control	–	410.5 ± 23.1
$FeCl_2$-As-ADP	–	1117.6 ± 35.5*
$FeCl_2$-As-ADP + HSCF	100	880.4 ± 33.1**
$FeCl_2$-As-ADP + HSCF	250	680.6 ± 36.1**
$FeCl_2$-As-ADP + HSEA	100	874.1 ± 5.1**
$FeCl_2$-As-ADP + HSEA	250	654.3 ± 34.1**
$FeCl_2$-As-ADP + α-tocopherol	100	600.2 ± 23.1**

a) Values are means ± SD for five rats in each group. $FeCl_2$-As-ADP = $FeCl_2$-ascorbic acid-ADP mixture.
* Significantly different from control ($P < 0.001$); **significantly different from control ($p < 0.01$).

Table 3. Scavenging activities of *H. sabdariffa* extracts and standard antioxidants on superoxide anion radicals, hydrogen peroxide and hydroxyl radicals[a)]

Antioxidants	IC_{50} on		
	Superoxide anion radical µg/mL	Hydroxyl radical µg/mL	Hydrogen peroxide µg/mL
HSCF	130 ± 3.6	100 ± 4.1	110 ± 6.5
HSEA	98 ± 4.3	90 ± 3.1	91 ± 5.3
BHA	650 ± 5.1	710 ± 4.8	440 ± 4.2
Quercetin	480 ± 4.4	250 ± 4.1	345 ± 3.2
Vit E	375 ± 3.7	520 ± 4.6	245 ± 5.1

a) The IC_{50} were calculated from the concentration-dependent percentage inhibition plots. Values are the means ± SD from three separate determinations. BHA = butylated hydroxyl anisole.

0.001) increase in MDA content compared to control. However, pretreatment with H *sabdariffa* extracts at 100 mg/kg and 250 mg/kg simultaneously with the oxidant mixture reduced ($p < 0.01$) the MDA content.

The inhibitory effect of HSCF and HSEA on CCl_4-NADPH-stimulated lipid peroxidation is shown in Fig. 4. Both HSCF and HSEA inhibited CCl_4-NADPH-induced lipid peroxidation dose dependently. At a concentration of 25 µg/mL, HSCF and HSEA elicited 32 and 38% inhibition on CCl_4-NADPH-induced lipid peroxidation, respectively, while both extracts exhibited 80 and 89% inhibitory effects at 100 µg/mL.

The time-response curves of MNPCE induction by sodium arsenite at the doses 1.5, 2.5 and 3.5 mg/kg body weight tested are shown in Fig. 5. At a dose of 1.5 mg/kg body weight, the MNPCE induction was not pronounced, whereas at doses of 2.5 and 3.5 mg/kg body weight significant induction of MNPCE ($p < 0.01$) was observed. Induction of MNPCE occurred at all the sampling times; thus, the sampling time of 24 h and dose of 2.5 mg/kg were considered appropriate.

The effects of HSCF, HSEA and vitamins C on sodium arsenite induced formation of MNPCE are shown in Fig. 6. HSCF, HSEA and vitamin C treatment did not induce MNPCE. Treatment of rats with both HSCF and HSEA significantly inhibited the induction of MNPCE by sodium arsenite ($p < 0.001$) after 24 h by 60 and 70%, respectively. Similarly, the standard antioxidant vitamin C significantly inhibited sodium arsenite-induced MNPCE formation by 50%.

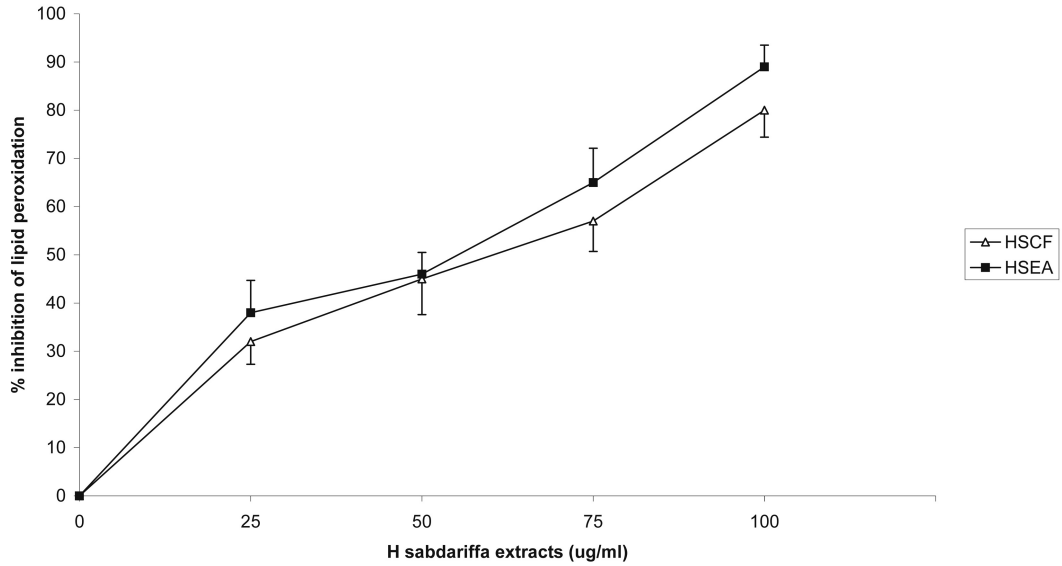

Figure 4. Antioxidant activities of *H. sabdariffa* extracts (HSCF, chloroform soluble fraction; HSEA, ethyl acetate soluble fraction) in the rat liver microsome peroxidation induced by CCl_4-NADPH system. Values are mean ± SD of three determinations.

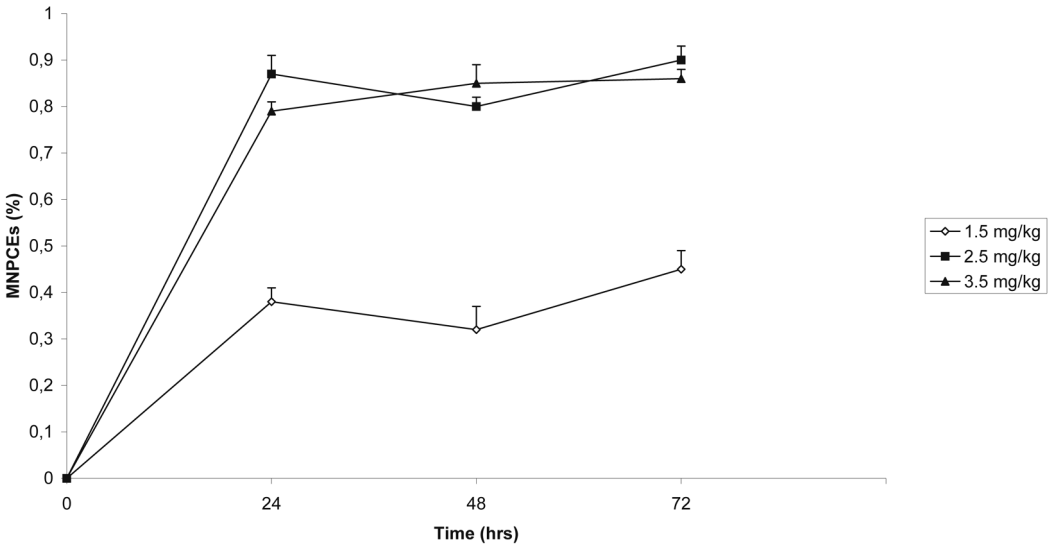

Figure 5. Time-response curves of the frequency of occurrence of micronucleated polychromatic erythrocytes (MNPCE) induced by administration of sodium arsenite at doses of 1.5 mg/kg, 2.5 mg/kg and 3.5 mg/kg. Values are means ± SD for five rats.

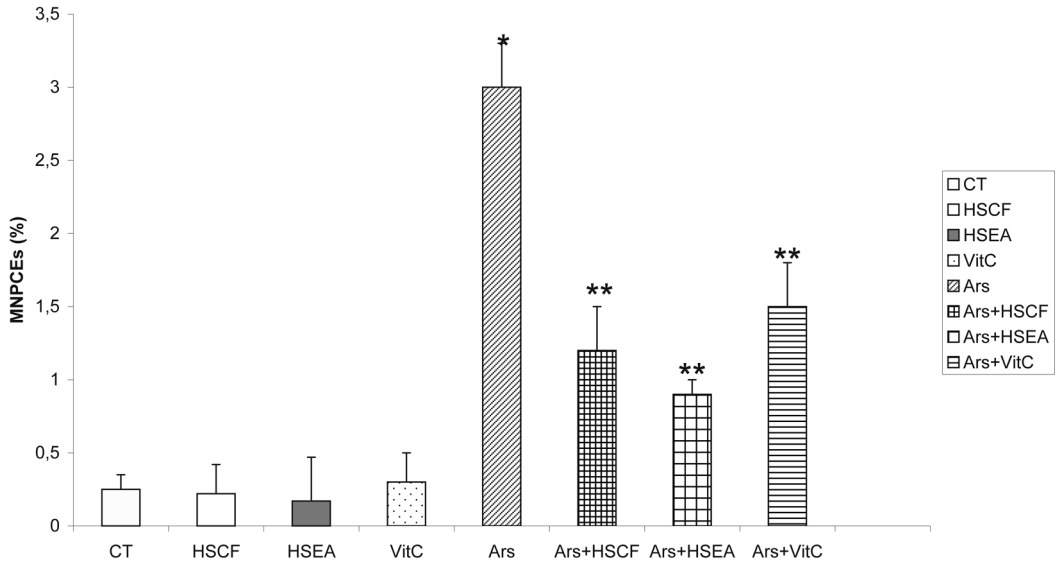

Figure 6. Effects of pretreatment of *H. sabdariffa* extracts (HSCF, chloroform soluble fraction; HSEA, ethyl acetate soluble fraction) and Vit C on the frequency of occurrence of micronucleated polychromatic erythrocytes (MNPCE) in rats treated with sodium arsenite (2.5 mg/kg). Values are mean ± SD for five rats in each group. *$p < 0.001$ significantly different from control; **$p < 0.01$ significantly different from sodium arsenite group.

4 Discussion

The search for natural chemical compounds with potential antimutagenic and anticarcinogenic properties is of great importance because of adverse consequences of increased rate of mutations and cancer risks in humans [31]. Among the naturally occurring antimutagens, the antioxidants ubiquitously present in herbs, fruits and vegetables are known to protect against oxidative damage. The beneficial effects of plant flavonoids have been attributed to their antioxidant properties including their ability to scavenge superoxide anions, hydroxyl and nitric oxide radicals and to inhibit lipid peroxidation in a variety of experimental systems [32].

The results of the present investigation indicate that HSCF and HSEA containing the phenolic compounds of the ethanol extract of dried flowers of *H. sabdariffa* L can scavenge oxygen free radicals and nitric oxide radicals. The results

further revealed that the phenolic compounds could prevent sodium arsenite-induced *in vivo* rat bone marrow micronucleus induction.

Earlier investigations on *H. sabdariffa* showed that the two fractions examined in the present study, HSCF and HSEA contained several polyphenolic compounds and dihydrobenzoic acids such as protocatechuic acids [20]. Thus, Hibiscus protocatechuic acid has been shown to protect against oxidative damage in rat primary hepatocytes.

In the present study, a relationship was found between the total phenolic content, total antioxidant activity and free radical scavenging ability of the extracts. The results are in agreement with those reported earlier [22]. Since several phenolic compounds express antioxidant activity, it can be inferred that the antioxidant activity of HSCF and HSEA is attributed to these phenolic compounds.

The apparent scavenging effects of the extracts on H_2O_2 may be attributed to their phenolics, which could donate electrons to H_2O_2 thus neutralizing it to water [1].

Superoxide radicals and hydroxyl radicals, two oxygen-derived free radical species and nitric oxide, reactive nitrogen intermediates, were used to study the free radical-scavenging capacity of *H. sabdariffa* extracts. In the present study, the phenolic compounds from *H. sabdariffa* scavenged superoxide anion radical. Phenolics and their derivatives have been reported to be excellent free radical scavengers [22]. Furthermore it has been demonstrated that dihydroxy benzoic acids as protocatechuic acids were capable of quenching superoxide anion radical by hydrogen donation. Therefore, the scavenging effect of superoxide anion radicals by the extracts of *H. sabdariffa* may depend on hydrogen donation by their different flavonoid compounds, which lead to secondary radicals that are resonance, stabilized like many phenolic antioxidants [33].

The hydroxyl radical is an extremely reactive free radical formed in biological systems and reacts rapidly with almost every type of molecule found in living cells such as sugars, proteins, lipids and DNA [34]. Namiki [35] indicated that the hydroxyl radical is the major active oxygen species causing lipid oxidation by abstracting hydrogen atoms from membrane lipids. In mechanistic terms, extracts of *H. sabdariffa* may compete with deoxyribose for hydroxyl radicals thereby preventing its oxidation. Zhao and Jung [36] alluded to the possibility of hydroxyl radical scavengers preventing against deoxyribose oxidation through competition with hydroxyl radicals. Therefore, the ability of extracts from H. *sabdariffa* to scavenge hydroxyl radical by preventing the oxidation of deoxyribose in the present study may relate directly to prevention of propagation of the process of lipid peroxidation *in vivo.*

Our data indicate that extract from *H. sabdariffa* inhibited NADPH-dependent CCl_4-induced peroxidation of liver microsomal lipids. CCl_4 is activated by the NADPH-cytochrome P450 system of the liver endoplasmic reticulum with formation of trichloromethyl radical ($CCl_3^{\bullet}$) and in aerobic conditions of the trichloromethylperoxylradicals (CCl_3O_2) [37].

Flavonoids have been shown to act by interfering with the metabolism of CCl_4 either by scavenging CCl_4-derived radicals or by impairment of the microsomal enzyme system necessary for CCl_4 activation [37]. Therefore, the apparent inhibition of CCl_4-induced peroxidation by extracts of *H. sabdariffa* may be due to either scavenging of radicals produced by CCl_4 or inhibition of enzymes involved in CCl_4 activation.

Furthermore, extracts from *H. sabdariffa* effectively inhibited $FeCl_2$-ascorbic acid-ADP-induced lipid peroxidation *in vivo*. Since the presence of free ferrous iron itself is known to induce the formation of superoxide anion and thus initiate the chain of reactions such as peroxidation of lipids [38], the ability of the extracts to decrease microsomal MDA concentration may possibly be due to a reduction in low molecular weight iron by means of chelation, thus diminishing the amount of iron available for catalytic role in the generation of oxygen free radicals.

Our data demonstrate that sodium arsenite induced formation of polychromatic erythrocyte formation in rats. Several assays performed *in vivo* and *in vitro* on mammalian cells have shown that exposure to arsenicals induces chromosome damage as indicated by aberrations and formation of micronuclei [10, 39]. In the present study, administration of extracts from *H. sabdariffa* together with sodium arsenite prevented the formation of micronucleus by this mutagen. Previous studies indicate that co-treatment of mammalian cells with the oxygen radical scavenger dimethyl sulfoxide significantly reduces the mutagenicity of arsenite and provide convincing evidence that reactive oxygen species, particularly hydroxyl radicals, play an important causal role in the genotoxicity of arsenical compounds in mammalian cells [5, 9]. The inhibitory effects of *H. sabdariffa* extracts further underscore that active oxygen species are involved in its mutagenicity. Our results suggest that the antioxidant activities of these extracts are related to their antimutagenic properties. It has been suggested that compounds, which possess antioxidant activity, can inhibit mutation and cancer because they can scavenge free radicals or induce antioxidant enzymes [40]. Katiyar *et al*. [41] also demonstrated that oral feeding of green tea extracts in drinking water to mice showed significant antioxidant activity and this was indicated to play an important role with regard to their antimutagenicity. Furthermore, antioxidants such as cysteine,

vitamin C and GSH were shown to prevent micronucleus formation induced by potassium bromate [42].

Taken together, our results indicate that extracts of *H. sabdariffa* showed strong antimutagenic activity, antioxidant activity and scavenging effects on active oxygen and free radicals. The protective effects of the extracts against arsenite-induced genotoxicity imply that they eliminate active oxygen species generated by the compound. In addition, the antioxidant properties of the extracts seem to explain their anti genotoxic effects.

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