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COMPARATIVE EFFECTS OF SOME MEDICINAL PLANTS ON SODIUM ARSENITE-INDUCED CLASTOGENICITY

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ABSTRACTS

Aims and Objectives: The effectiveness of crude methanolic extracts of *Bridelia ferruginea, Tridax procumbens, Ocimum gratissimum* and *Lawsonia inermis* against clastogenicity induced by sodium arsenite (SA) was evaluated using an *in-vivo* rat bone marrow cell micronuclei test and the DPPH radical scavenging assay. Methods: 50 mg/kg body weight of the extracts were given by gavaging to the rats in five groups for seven consecutive days as a dietary supplement followed by a single dose of SA (2.5 mg/kg per body weight) which was administered intraperitoneally. Results: Preliminary screening of the clastogenicity showed that aqueous extracts of these plants have no significant clastogenic activity in rat. Pre-treatment of rats for seven days with extracts orally before exposure to SA resulted in a significant reduction of the degree of formation of micronuclei in polychromatic erythrocytes of the bone marrow. The degree of reduction of arsenite was of the order *B. ferrugenia* > O. *gratissimum*>*T. procumbens* > *L. inermis*. Phytochemical analysis carried out on the extracts revealed the presence of known chemical constituents. The phytochemical constituents might be responsible for the strong DPPH scavenging activities exhibited by the plant extract. Conclusion: This study suggests that *B. ferruginea, T. procumbens, O. gratissimum,* and *L. inermis* extracts have anticlastogenic potential and possibly due to their antioxidant properties.Therefore, the plant extracts may be useful in the stoppage of arsenite-induced toxicity in areas where arsenic is a potential environmental contaminant.

KEYWORDS:**Medicinal plants; Cancerprevention; Phytochemical; Anticlastogenicity; Chemoprevention.**

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1.0 INTRODUCTION

Traditional medicine is an important source of potentially valuable new compounds for the development of chemotherapeutic agents (Sofowora,1993). Many studies have shown that an increased intake of fruits or medicinal plants rich in antioxidants decreases the level of oxidative DNA damage (Huang *et .al.,* 2010; Yousef *et al*., 2010; Nayak *et al*., 11). Phytochemical examination of plants which have a suitable history of use in traditional medicine for the treatment of cancer has often resulted in the isolation of agents with antitumor activity (Bellini *et al*., 2006). Phytochemicals obtained from medicinal plants have also been proven to suppress experimental carcinogenesis in various organs (Omoseyindemi *et al*., 2003; Yousef *et.al.*, 2010). A number of undesired side effects sometimes occur during chemotherapy of cancer by the use of synthetic products or drugs. Natural remedies, such as the use of plant-products in cancer treatment, may be a better alternative (Sies, 1997; Bellini *et al*., 2006). Therefore, this study was aimed at investigating the anticlastogenic and antioxidant effects of four selected plants; *B. ferruginea,T. procumbens, O. gratissimum,* and *L. inermis* which are known to prevent some diseases in Africa. *B. ferruginea* (Euphorbiaceae) is commonly foundin Savannah regions. The leaves have been used to treat diabetes (Iwu, 1993) and wounds (Adetutu *et al*., 2011) in African traditional medicine. The phenolic compounds in *B. ferruginea* were demonstrated to be responsible for the therapeutic, antiseptic, antifungal or bactericide, as well as anti-viral and anti-tumor activities of *B. ferruginea* (Iwu, 1993; Cimanga *et al.,* 1999). *T. procumbens* have acted as a chemopreventive agent in the development of cancer, heart and age related diseases (Iwu, 1993; Bhagwat and Germination, 2008). *T. procumbens* have been described to be chemopreventive agents by depressing cholesterol and fixing damage cells (Duthie and Ramachandram, 2011). The hepatoprotective activity of aerial parts of*Tridax* shows significant protection in lessening of Dgalactosamine/lipopolysaccharide (D-GalN/LPS)-induced hepatocellular injury (Vilwanathan *et al*., 2005; Vishnu *et al*., 2011). *O. gratissimum* is found in the tropical and warm temperate regions such as India and Nigeria (Okigbo and Ogbonnaya, 2006). *O. gratissimum* is used by the Ibos of South Eastern Nigeria in the management of the new baby's cord, to keep the wound surfaces sterile. It is also used in the treatment of fungal infections, fever and cold (Iwu, 1993). *L. inermis* (Henna) belongs to the family Lythraceae and is cultivated in North Africa and South-West Asia and throughout the tropics as an ornamental and dye plant (Gupta *et al*., 2003). *L. inermis* has been reported to have analgesic, hepatoprotective, anti-inflammatory, antioxidant and anticancer properties (Kamal and Jawaid, 2010). Based on the widely reported health benefits of the four selected plants, this present study is planned to explore antioxidant properties and the protective effects of the leaf extracts of the plants against the toxic effects of SA, using the micronucleus assay in male albino rats.

2.0 MATERIALS AND METHODS

2.1 Collection and identification of plant materials

The leaves of the plants were collected in February 2011, from Adenike area of Ladoke Akintola University of Technology Ogbomoso, Oyo States. The plants are: *Bridelia ferruginea, Tridax procumbens, Ocimum gratissimum* and *Lawsonia inermis.* The plants were properly identified and authenticated by Dr. Ogunkunle J.A. at the Department of Pure and Applied Biology, LAUTECH, Ogbomoso.

2.2 Preparation of plant extracts

The dried plant materials were ground and then extracted in absolute methanol (BDH product) (50 g in 250 mL). The mixtures were allowed to stand at room temperature for three days with occasional agitation, the methanolic extracts were obtained after filtration using Whatman No.1 filter paper and

the filtrates were kept in the refrigerator. The filtrates were then concentrated using a rotary evaporator (RE801, made in China).

2.3 Phytochemical analysis

The following phytochemical analysis was carried out on the following plant extracts: *B. ferruguinea, V. amygdalina, T. procumbens, O. gratissimum* and *L. inermis* using the procedure of Trease *et al.* (1989).

2.4 Micronucleus test

Healthy white male Albino Wistar rats were sourced from and housed in the preclinical animal house, Faculty of Basic Medical Sciences, Ladoke Akintola University of Technology, Ogbomoso, Oyo State. The rats were kept five per cage with hust bedding and fed with pellets and water *ad libtum*.The ratsof 70 days old and weighing 188 ± 2 g were randomly allocated to groups I to VIas shown in Table 1, each group consisting of five rats. Two doses of the plant extracts (50 mg/kg per body weight) were given by gavaging to the rats in 5 groups for seven consecutive days as a dietary supplement followed by a single dose of SA (2.5 mg/kg body weight) and were administered intraperitoneally. The rats in the negative control, were given distilled water and the positive control group were given SA. The rats were sacrificed by cervical dislocation and smears of bone marrow cells were prepared on slides according to a standard method of Schmid, (1975) with slight modification. The number of micronucleated polychromatic erythrocytes (mPCE) were determined by scoring 1000 PCE per rat.

2.5 Determination of GGT and ALP concentration in serum of rats

GGT and ALP kits are products of Randox and Agappe diagnostics respectively. The concentration of the GGT and ALP were determined according to the manufacturers' protocol. For GGT, 1 mL of the working reagent was mixed with 0.10 mL of sample (serum) and the initial absorbance was taken followed by an interval of one minute for 3 minutes at 405 nm. For ALP, 1000 μL of the working reagent was mixed with 20 μL of sample (serum) and allowed to stay for 1 min.The absorbance was read at 405 nm at an interval of one minute for 3 minutes.

2.6 Statistical analysis

Statistical analysis was done using analysis of variance (ANOVA), means were compared for significance using Duncan's multiple range test ($p > 0.05$)

3.0 RESULTS

3.1 Micronucleus test

Table 1 shows induction of mPCEs in rat bone marrow cells after exposure to SA alone and when co-administered with the plant extracts. The mPCEs formed by the SA treated group were significant as compared with group treated with distilled water. The plant extracts appear to reduce micronuclei formation when administered alone with SA. In addition, the extracts appear to reduce SA induced mPCE formation and decreasing in order of *B.ferrugenia*> O.*gratissimum*>*T. procumbens*> *L. inermis* when compared with the negative control (Table 1).

Table 1
Micronucleated polychromatic erythrocyte (mPCEs) count in rats treated with plant extracts and SA

Groups	mPCE
I	21.7 ± 1.25
II	12.0 ± 4.97
III	12.7 ± 0.94
IV	14.9 ± 2.43
V(+ve control)	23.5 ± 3.5
VI (-ve control)	2 ± 0.01

Values are Mean ± Standard Deviation
Group I: rat fed with B. ferruguinea and SA
Group II: rat fed with O. gratissimum and SA
Group III: rat fed with L. inermis and SA
Group IV: rat fed with T. procumbens and SA
Group V: +ve control; the rat was fed with SA
Group VI: -ve control; the rat was fed with distilled water alone

3.2 GGT and ALP concentration in serum of rats

Table 2 shows the serum level of GGT and ALT in the sera of treated rats. SA increases the serum level of GGT and ALP when compared to the negative control significantly (p< 0.05) (Table 2). However, most of the plant extract co-administered with SA significantly reduced the level of hepatic transaminases in serum (Table 2).

TABLE 2
ALP and GGT concentrations in the serum Of the rat fed with SA and plant extracts

Group	I	II	III	IV	V	VI
ALT Activity (U/L)	12.8±8.5	6.42±3.3	7.02± 1.7	5.81±4.3	15.58±0.5	6.42±0.5
GGT Activity (U/L)	2.0±0.4	6.05± 1.3	8.70±1.4	14.23±2.9	23.16±5.9	5.32±0.3

Values are Mean ± Standard Deviation; Group as listed in Table 1

3.3 DPPH radical scavenging activity of the plant extract

Table 3 shows the EC_{50} of the plant extracts in respect to the antiradical activities of the extracts following the order: *L. inermis*>*V. amygdalina*>*O.gratissimum*>*B.feruginea*>galli c acid. It is noted that all the extract has a measure of antioxidant activity in respect to gallic acid; with *L. inermis* having in the highest antioxidant activity.

Table 3
Antioxidant properties of the plant extracts

Plant extracts	EC_{50} (mg/mL)
Gallic acid	3.9±0.3
B. ferruginea	3.4±0.2
V. amygdalina	1.3±0.1
O. gratissimum	1.9±0.2
T. procumbens	6.9±1.1
L. inermis	1.2±0.1

Values are Mean ± Standard Deviation

3.4: Phytochemical constituents of crude extracts of B. ferruginea, O. gratissimum, L. inermis and T. procumbens.

Phytochemical analysis of aqueous extract of *O. gratissimum, L. inermis* indicated the presence of flavonoids, tannins, saponins, triterpenes, steroids and cardiac glycosides. While *B. ferruginea* and *T. procumbens* indicated the absence of coumarin and saponin but showed the presence of has flavonoids, triterpenes, steroids and cardiac glycosides (Table 4).

Table 4
Phytochemical constituents of crude extracts of B. ferruginea, O. gratissimum, L. inermis and T. procumbens.

Plant extract	Flavonoids	Tannins	Alkaloids	Saponins	Triterpenes	Steroids	Coumarins	Cardiac glycosides
B. ferruginea	+	+	+	-	+	+	-	+
O. grastisimum	+	+	+	+	+	+	+	+
L. inermis	+	+	+	+	+	+	+	+
T. procumbens	+	+	+	-	+	+	-	+

(+) indicates the presence of phytochemical constituent. (-)indicates the absence of phytochemical constituent

4.0 DISCUSSION

Medicinal plants contain many compounds with antioxidant potentials and may prevent the genetic effects of mutagens and carcinogens (Sies, 1997; Rao and Rao 2001; Puntumchai *et. al.,* 2004). In this study, SA induced micronuclei in erythrocytes in the bone marrow cells of rats (Table 1). It has been reported that SA induced chromatid and chromosome breaks in rats and mice (Sokal *et al*., 1969; Abe *et al*., 1983; Erexson *et al.*, 1995; Adetutu *et al*., 2004). In this study, oral administration of the extracts resulted in no significant increase in the number of mPCE thus, indicating that the extracts produced no clastogenic effect in respect to micronuclei frequency, demonstrating that the extracts has anticlastogenic activity. The results obtained in this study showed that *B. ferruguinea, T. procumbens, O. gratissimum and L. inermis* extracts showed anticlastogenic potentials and were able to inhibit SA induced clastogenicity in rat. The degree of reduction of arsenite was in the order *B. ferrugenia>* O. *gratissimum>T. procumbens* > *L. inermis* (Table 1). Free radical damage and oxidative stress are the major reasons for liver tissue damage. The antioxidant enzymes are the first-line defense against such damage and thus provide protection against the deteriorating outcome (Rahman and Moon 2007; Yedjou *et al*., 2008). In this study oxidative injury was monitored by measuring the liver GGT and ALP activities. In the current study, oral treatment with the plant extract caused significantly decreased ALP and GGT levels compared with the SA-treated group (Table 2) which suggest that the plant extracts might have provided some protection against SA-induced toxicity in rats. The phytochemical screening carried out on the selected plants revealed the presence of flavonoids, saponins, triterpenes, cardiac glycosides (Table 4). The medicinal values of the plant leaves may be related to their phytochemicals (Yedjou *et al*., 2008; Sabir and Rocha, 2008; Yousef *et.al.,* 2010). Many studies have demonstrated that an important mechanism of hepato-protective effects may be related to the antioxidant capacity of scavenging reactive oxygen species (Tiwari and Wanasundara,, 1992; Nayak *et al*., 2011; Mukherjee *et al*., 2011). In this study, it was noted that the extracts posess a significant measure of antioxidant activity in respect to gallic acid, a known standard antioxidant. Therefore, the selected plants assayed in this study may have exhibited anticlastogenic and chemopreventive potentials because of the phenolic compounds or the phytochemicals which are key determinants of their radical scavenging activity. The presence of the identified phytochemicals and the antioxidant activity may be responsible for their usefulness in the management and treatment of various diseases in traditional medicine in Africa.

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