Biokemistri
Vol. 33, No. 1, March 31, 2021
Printed in Nigeria

0795-8080/2021 $10.00 + 0.00

http://journals.niseb.org.ng
Also available online at http://www.bioline.org.br/bk

BKR 2020085/33103

Antigenotoxic and hepatoprotective activities of ethanol extract of the leaf of *Eclipta alba in* sodium arsenite-induced toxicity

Oyeronke A. ODUNOLA[1]*, Nelson O. FASHINA[2], Ifeanyi M. ILOBA[1], Michael A. GBADEGESIN[1], Ayodeji M. ADEGOKE[1], Olorunjuwon J. OLUGBAMI[1]

[1]Cancer Research and Molecular Biology Laboratories, Department of Biochemistry, College of Medicine, University of Ibadan, Ibadan. Nigeria.
[2]African Indigenous Knowledge Production Unit, Faculty of Arts, University of Ibadan, Ibadan, Nigeria.

***Correspondences to**: ronodunola@yahoo.com and aygoke@yahoo.com, **Tel.**: +234 8023387152

(Received November 27, 2020; Accepted February 4, 2021)

ABSTRACT: Arsenic pollution in developing countries poses a major health hazard to humans and animals, thus the search for potent remedies. The medicinal use of *Eclipta alba* in the management of some ailments such as ulcer, diarrhoea, constipation, and pile have been documented. We therefore scientifically explored the effects of the ethanol leaf extract of *E. alba* (*ELEA*) in sodium arsenite (SA) induced geno-hepatotoxicity using male Wistar rats. Thirty-five (35) rats were randomised into seven groups of five animals each. Group I was treated with distilled water only while groups II to VII had various levels of treatments with *ELEA* (200mg/kg body weight) and/or SA (5.0mg/kg body weight) for 14 days. We evaluated both the preventive and therapeutic effects of *ELEA*. The activities of serum transaminases, γ-glutamyl transferase and alkaline phosphatise were evaluated, liver histological analysis and histomorphometry were also monitored as additional markers for hepatotoxicity. Micronucleus induction assay was used to assess genotoxicity and kidney histology to monitor the effect of *ELEA* on the kidney. Serum transaminases/aminotransferases, γ-glutamyl transferase and alkaline phosphatise, frequency of micronucleated polychromatic erythrocytes **(**mPCEs) as well as hepatic cell/mm^2 were significantly ($p<0.05$) increased by SA, while PCV, HB and RBC counts decreased significantly ($p<0.05$) but administration of *ELEA* significantly reversed these parameters close to normal. Ethanol extract of *E. alba* leaves exhibits some protective effect and may serve as a potent remedy in sodium arsenite induced hepatotoxicity and genotoxicity.

Key words: Arsenite, Genotoxicity, Hepatotoxicity, Herbal, Micronucleus, Transaminases.

Introduction

Inorganic arsenic compounds are linked with higher risks of cancer and other health challenges[1]. Some of the common trivalent inorganic arsenic compounds include: arsenic trioxide, sodium arsenite and

arsenic trichloride[2]. Exposure to arsenic is regarded as a major public health concern due to its carcinogenic potential[3].

According to Sharma *et al.* (2014), the exposure to inorganic arsenic is most likely via drinking water and various foods[4]. Arsenic polluted drinking water from both human-induced and naturally occurring sources have affected millions of people globally[5]. Due to absence of taste, odour and colour, exposure to arsenic cannot be detected and avoided by a layman[4]. Several regions of the world have been affected by arsenic polluted drinking-water in levels ranging from tens to even thousands of micrograms per litre, primarily in Bangladesh, China, West Bengal (India)[6].

It has been demonstrated that arsenic induced genotoxicity is associated with point mutation on the HPRT locus in lymphocytes from adults chronically exposed to arsenic via drinking water for over 10 years, regardless of the presence of skin lesions and chromosomal aberrations[7]. Arsenic exposure also induces micronucleated cells both in urothelial cells and cells from oral mucosa, and most of the infertility problems and birth defects observed in human populations exposed to arsenic are associated with genotoxicity[8,9].

Plants have played very important therapeutic roles in maintaining and enhancing the quality of human health for thousands of years. In recent times, there is an increasing focus on plant research and this has gained momentum all over the world and evidence has shown the immense potential of medicinal plants in the treatment and management of various health concerns[10]. *Eclipta alba* (syn. *Eclipta prostata*) commonly known as false daisy is a specie of plant in the family asteraceae with characteristic medicinal properties. In Nigeria, *E. alba* known as ewe arojoku (Yoruba) and agbirigba ozara (Igbo) is used locally in many herbal formulations for the treatment of ulcer, insomnia, urinary tract infection, pile, labour pains, headaches, sight and hearing abnormalities, and skin disorders[11]. Extracts and other concoctions made from the plant have been reported to be used in folk and traditional medicine for alopecia, cancer/tumour, gastrointestinal disorders, skin diseases and blood/pus in urine and wound healing[12]. The plant was known to possess significant antidiabetic, hepatoprotective, anaphylaxis, analgesic, anti-inflammatory and immunomodulatory activity[13]. Several properties of *E. alba* have been studied including its effect on CCl_4-, galactosamine- and phalloidin-induced liver damage in rats. The present study was designed to investigate the antigenotoxic and hepatoprotective potency of ethanol extract of *E. alba* in sodium arsenite exposure in male Wistar rats.

Materials and Methods

Chemicals and Reagents for Analysis

Kits for aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transferase (γGT) and alkaline phosphatase (ALP) were obtained from Randox Laboratories, UK. Sodium arsenite ($NaAsO_2$; BDH Chemicals Ltd Poole England) was used. Other chemicals and reagents used in this study were of analytical grade and are products of Sigma Chemical Co. St. Louis, MO., USA.

Preparation of Ethanol leaf extract of Eclipta alba (ELEA)

Fresh leaves of *E. alba* were bought from Bode market in Ibadan, Nigeria and were identified at the Department of Botany, Faculty of Science, University of Ibadan, Nigeria by Mr. Esimekhnai, Donatus. The leaves were air dried, weighed and ground to powdered form. The powdered sample was extracted using 70% ethanol. The extracts were further concentrated with a rotary evaporator at 40 ˚C to yield a solid residue at the Department of Pharmaceutical Chemistry, University of Ibadan.

Experimental Protocols and Treatments

Thirty-five (35) male Wister albino rats weighing 100-120 g were bought from the Animal House, Department of Physiology, Faculty of Basic Medical Sciences, University of Ibadan, Nigeria. The rats were kept in the experimental animal house, Department of Biochemistry, University of Ibadan at 29 ± 2 °C and were fed with rat pellets (Vita feeds, Ibadan, Nigeria) and with water *ad libitum*, 12 h light/dark

cycle. All the animals were handled in adherence to the guide for the care and use of experimental animals, as specified by the National Institute of Health (NIH publications number 85–93 revised in 1985). The rats were acclimatized for one week and then randomly divided into seven experimental groups of five rats per group.

Group I: Serve as the negative control and received distilled water only.
Group II: Rats treated with sodium arsenite (SA) 5.0 mg/kg body weight every other day for 14 days.
Group III: Rats treated with 200 mg/kg body weight of ethanol leaf extract of *Eclipta alba (ELEA)* daily.
Group IV: Rats treated with SA 5.0 mg/kg body weight every other day and 200 mg/kg body weight *ELEA* daily simultaneously
Group V (natural recovery): Rats treated with SA 5.0 mg/kg body weight followed by water for another 14 days.
Group VI (post-treatment): Rats treated with SA 5.0mg/kg body weight followed by 200 mg/kg body weight of *ELEA* for another 14 days.
Group VII (pre-treatment): Rats treated with 200 mg/kg body weight of *ELEA* followed by 5.0 mg/kg body weight of SA for another 14 days.

Except otherwise stated, all treatments were done by gavages for 14 days. Sodium arsenite was administered at 5 mg/kg body weight ($1/25^{th}$ of the oral LD_{50})[14].

Liver function enzymes assays

Aminotransferases activities

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were investigated according to the method previously described[15] with the use of diagnostic kits. Briefly, this method involves the reaction of pyruvate, the product of transamination reaction catalysed by ALT or AST, with 2, 4 -dinitrophenyl hydrazine which give rise to deep coloured hydrazone which is read at 546 nm with the aid of a spectrophotometer (Spectronic-20).

γ-glutamyl transferase activity (γGT)

The γGT was investigated in the serum by employing the reconstituted γGT diagnostic reagent according to the previously described method[16]. Briefly, this involves the transfer of glutamyl group from a glutamyl peptide (L-γ-glutamyl-p-nitroanilide) to another peptide (glycylglycine), in a reaction catalyzed by γGT, thereby yielding a cleavage product (pnitroaniline) which is read at 405 nm thus making a direct kinetic determination of γGT activity a possibility.

Alkaline Phosphatase (ALP) Activity

The ALP activity was determined according to the optimized method previously described[17]. Alkaline phosphatase catalyzes the hydrolysis of p-nitrophenylphosphate into phosphate and p-nitrophenol which absorbs UV light at 405nm, thus making a direct kinetic determination a possibility.

p-nitrophenylphosphate + H_20 ALP $\rightarrow$ phosphate + p-nitrophenol

Micronucleus (MN) assay

The femurs from the experimental animals were removed and bone marrow was aspirated with a needle and syringe. The microscopic slides of the bone marrows were prepared following the procedure previously described[18]. The slides were thereafter fixed in methanol, air-dried, pre-treated with May-Grunwald solution, and air-dried again. The dried slides were thereafter stained in 5% Giemsa solution and induced in phosphate buffer for 30 seconds. The slides were then rinsed in distilled water and air-dried. The air-dried slides were mounted and scored with a microscope for micronucleated polychromatic erythrocytes (MPCEs) according to the established standard procedure at x40 magnification.

Liver and Kidney histological analysis

Liver sections from the rats were fixed in 4 % p-formaldehyde and thereafter washed in phosphate buffer pH 7.4 at 4 ºC for 12 h. After dehydration, the tissue was embedded in paraffin, and then cut into sections, 5 µm thickness were stained with haematoxylin and eosin staining method[19], and then evaluated under a microscope at the Pathology Department, University of Ibadan, Ibadan, Nigeria.

Hepatic Cell Analysis

Hepatic cell per mm^2 analysis was investigated by counting the numbers of cells on stained slides prepared from the liver; this was done under a Nikon light microscope at x40 with the aid of a grid and tally counter.

Haematological analysis

Prior to sacrifice, blood was collected from experimental rats via ocular puncture in heparinized bottles for haematological analysis at the Department of Veterinary Medicine, University of Ibadan according to the method previously described[20].

Data analysis

Results are expressed as mean ± Standard deviation. Differences between the groups were analysed by one-way analysis of variance (ANOVA) with the aid of Statistical Package for Social Sciences (SPSS) software, SPSS Inc., IBM, Standard version 20.0.0. P values <0.05 were considered statistically significant for differences in mean.

Results

Effect of *ELEA* on SA-induced hepatotoxicity in male albino Wister rats

The activities of serum enzymes increased significantly ($p<0.05$; Table 1) in groups II and V rats treated with SA only compared with the control (groups I). The ELEA only treated rats showed approximately the same levels of the serum enzymes compared to the control treated with water only. Almost similar levels were observed in the SA-only treated rats (groups II and V). Pre- and post-treatment with *ELEA* (groups VI and VII) reduced the activity of the liver enzymes as compared to the SA only treated rats, with the rats pre-treated with *ELEA* (group VI) alone being statistically significant ($p<0.05$; Table 1).

Table 1 Serum activities of aspartate amino transferase, alanine amino transferase, gamma glutamyl transferase and alkaline phosphatase in sera of rats treated with *ELEA* and SA.

Group	ALT	AST	ALP	GGT
I-water	13.45 ± 1.56^a	23.19 ± 2.83^a	155.48 ± 29.77^a	10.42 ± 1.16^a
II-SA	27.68 ± 3.22^b	50.58 ± 3.75^b	451.72 ± 28.73^b	25.09 ± 1.77^b
III-ELEA	13.57 ± 2.53^a	24.06 ± 3.09^a	173.88 ± 27.18^a	9.26 ± 4.01^a
IV-SA+ELEA	13.21 ± 2.06^a	36.38 ± 7.18^a	270.48 ± 152.99^a	15.82 ± 6.38
V-SA+water	26.01 ± 2.36^b	49.27 ± 5.90^b	571.32 ± 172.12^b	30.49 ± 13.72^b
VI-SA+ELEA	17.74 ± 1.49^a	38.55 ± 7.49^a	398.36 ± 14.16^a	13.51 ± 2.41^a
VII-ELEA+SA	23.57 ± 1.43	43.04 ± 3.98	440.68 ± 56.38	23.55 ± 6.69

Values are expressed as mean ± SD. a= the mean difference is significant ($p< 0.05$) when compared with group II. b= the mean difference is significant ($p< 0.05$) when compared with group I.

Effect of *ELEA* and SA on the frequency of induction of micronucleated polychromatic erythrocytes (mPCEs) in bone marrow cells.

As expected, there is a significant increase ($p<0.05$; Fig. 1) in the number of mPCEs in the bone marrow cells of rats treated with SA alone (groups II and V) as compared with the control which received water only (group I). There was no observed difference in the number of mPCEs between the *ELEA* only treated rats (group III) as compared to the control (group I). A significant reduction ($p<0.05$; Fig. 1) in mPCEs were observed in rats treated with SA+*ELEA* simultaneously (group IV), as compared with SA only group. Pre- and post-treatment with *ELEA* (groups VI and VII) showed significant reduction ($p<0.05$; Fig. 1) in the frequency of mPCEs by at least 2.14-fold decrease when compared with the SA-only treated groups (groups II and V).

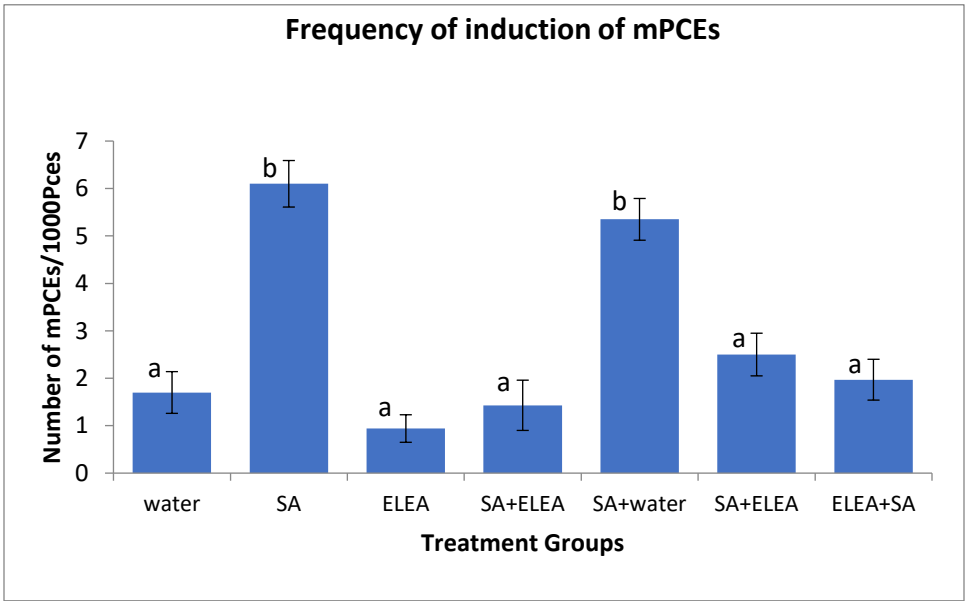

Fig 1 Frequency of micronucleated polychromatic erythrocytes induction in the bone marrow cells of rats exposed to *ELEA* and SA.
a= the mean difference is significant ($p< 0.05$) when compared with group II
b= the mean difference is significant ($p< 0.05$) when compared with group I

Histomorphometry (Hepatic cell/mm^2)

The hepatic cells per mm^2 increased significantly ($p<0.05$; Fig. 2) in SA-only rats (groups II and V) when compared with the control and *ELEA*-only treated rats (groups I and III). No statistical significant difference was observed between the positive control groups II treated with SA only and the recovery group V. There was an observed significant decrease ($p<0.05$; Fig. 2) in the rate of cell proliferation of rats treated simultaneously with *ELEA*+SA (group IV) when compared with the SA-only group. Pre- and post-treatment with *ELEA* (group VI and VII) show a slight decrease in the hepatic cells per mm^2 compared to the SA-only groups, though not significant.

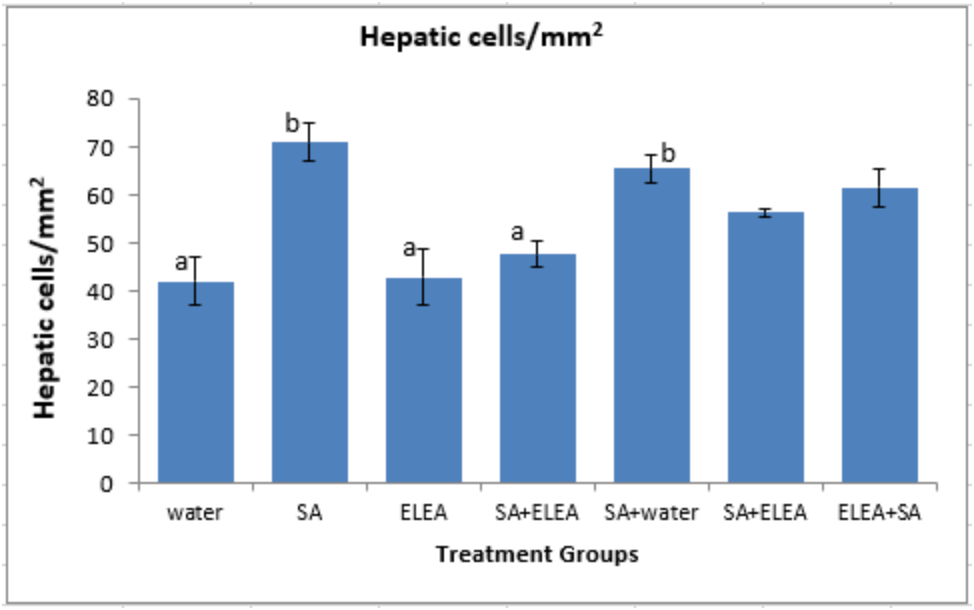

Figure 2: Hepatic Cell per millimeter sq. analysis of rats treated with *ELEA* and sodium arsenite
a= the mean difference is significant ($p< 0.05$) when compared with group II
b= the mean difference is significant ($p< 0.05$) when compared with group I

Effect of ELEA on haematological parameters

SA was observed to significantly decrease ($p<0.05$; Table 2) the RBC, Hb and PCV levels in the SA-only treated rats compared to the control (group I) fed with water only, while conversely, the lymphocyte count was significantly increased ($p<0.05$; Table 2) in SA-only rats compared to the control. *ELEA* administration significantly increased ($p<0.05$; Table 2) the RBC, Hb and PCV levels and at the same time, significantly reducing ($p<0.05$; Table 2) the lymphocyte count.

Table 2 Evaluation of PCV, HB, LYMPH, WBC, and RBC levels of rats treated with *ELEA* and sodium arsenite (SA)

Group	PCV (%)	HB (g/dl)	RBC (million cells/µl)	WBC (cells/µl)	LYMPH (%)
I- H_2O	37.50±1.73[a]	14.43±0.56[a]	7.14±0.49[a]	5437.50±912.13	50.25±9.64[a]
II-SA	31.00±3.37[b]	11.58±0.86[b]	5.25±0.54[b]	4925.00±830.25	70.25±6.85[b]
III-*ELEA*	39.25±1.50[a]	14.48±0.46[a]	7.52±0.33[a]	6162.50±1058.60	54.75±6.34[a]
IV-SA+*ELEA*	40.75±1.50[a]	15.83±0.57[a]	7.85±0.40[a]	3250.00±727.37	59.25±5.62[a]
V-SA+water	33.25±4.43[b]	11.78±1.62[b]	6.09±0.87[b]	3750.00±907.62	73.25±3.50[b]
VI-SA+*ELEA*	41.25±2.22[a]	14.08±0.99[a]	7.95±0.44[a]	4687.50±949.91	60.25±12.58[a]
VII-*ELEA*+SA	42.5±1.29[a]	13.68±0.93[a]	7.61±0.26[a]	3025.00±843.9	64.75±6.40[a]

Values are expressed as mean ± SD.
a= the mean difference is significant ($p< 0.05$) when compared with group II
b= the mean difference is significant ($p< 0.05$) when compared with group I

Packed cell volume (PCV), Heamoglobulin (HB) Lymphocytes (LYMPH), white blood cells (WBC), and Red blood cells (RBC) levels of rats treated with *ELEA* and sodium arsenite (SA).

Effect of *ELEA* on the histology of the liver cells during SA toxicity

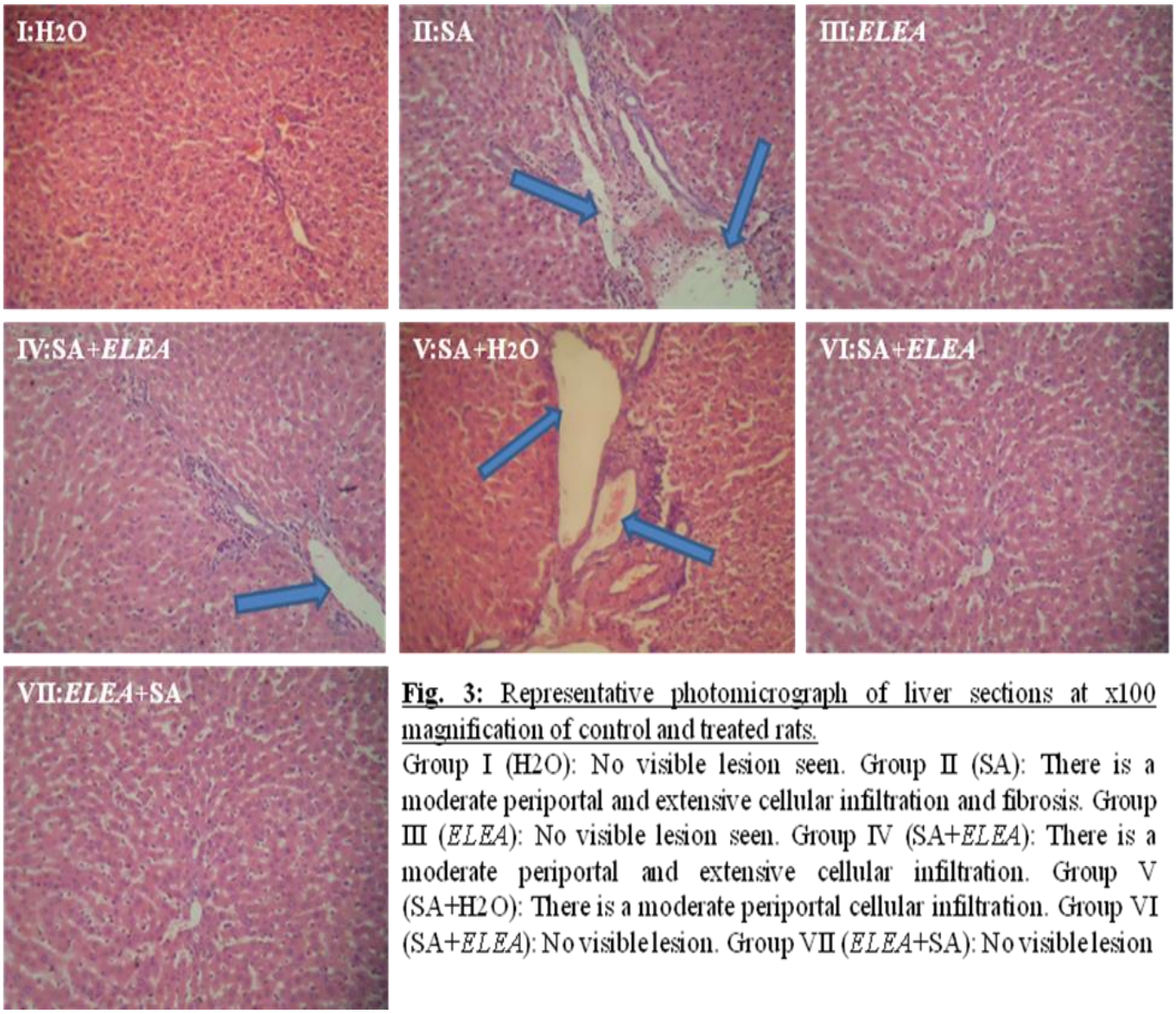

Fig. 3: Representative photomicrograph of liver sections at x100 magnification of control and treated rats.
Group I (H2O): No visible lesion seen. Group II (SA): There is a moderate periportal and extensive cellular infiltration and fibrosis. Group III (*ELEA*): No visible lesion seen. Group IV (SA+*ELEA*): There is a moderate periportal and extensive cellular infiltration. Group V (SA+H2O): There is a moderate periportal cellular infiltration. Group VI (SA+*ELEA*): No visible lesion. Group VII (*ELEA*+SA): No visible lesion

Effect of *ELEA* on the histology of the kidney during SA toxicity

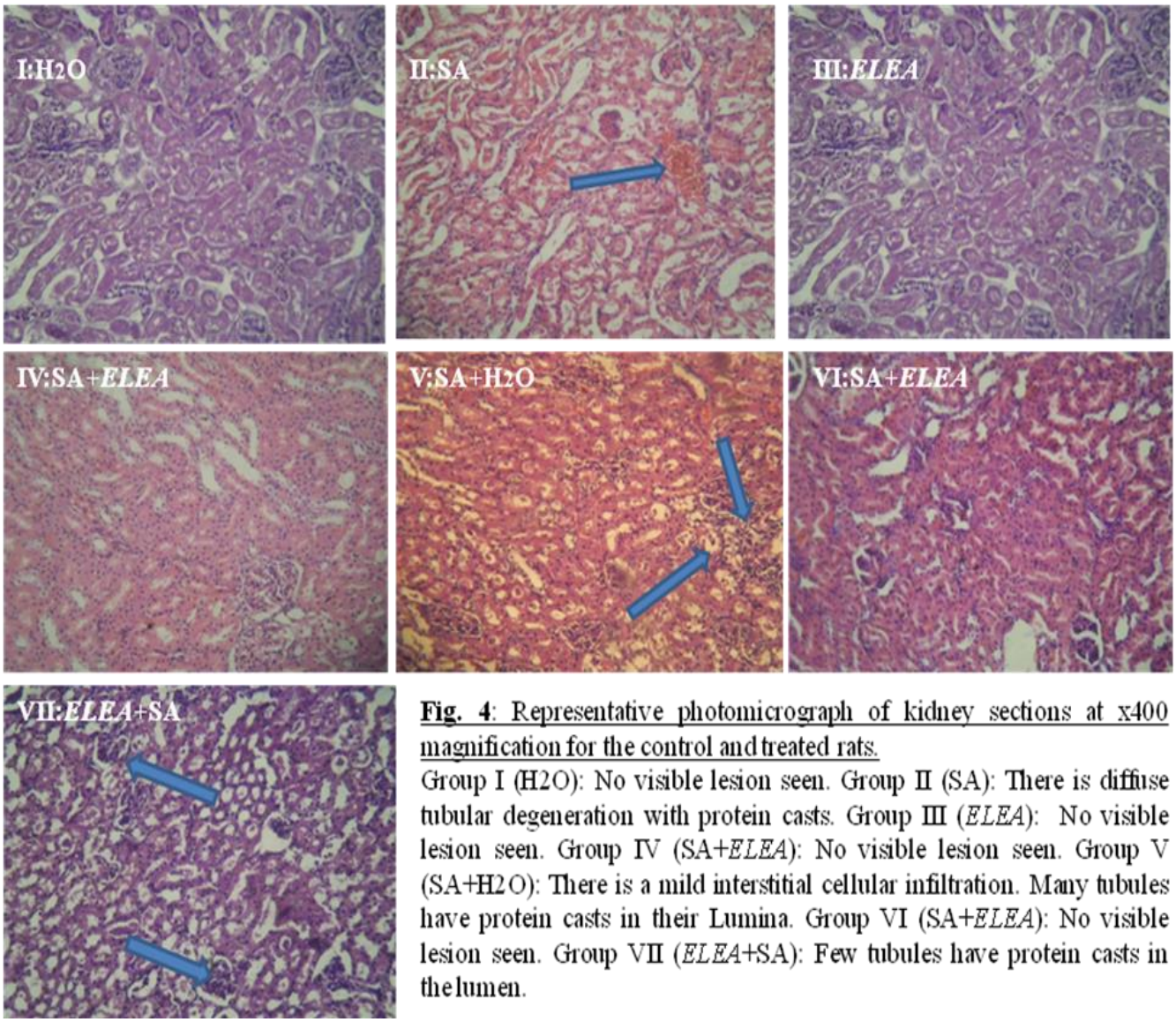

Fig. 4: Representative photomicrograph of kidney sections at x400 magnification for the control and treated rats.
Group I (H2O): No visible lesion seen. Group II (SA): There is diffuse tubular degeneration with protein casts. Group III (*ELEA*): No visible lesion seen. Group IV (SA+*ELEA*): No visible lesion seen. Group V (SA+H2O): There is a mild interstitial cellular infiltration. Many tubules have protein casts in their Lumina. Group VI (SA+*ELEA*): No visible lesion seen. Group VII (*ELEA*+SA): Few tubules have protein casts in the lumen.

Discussion

The understanding of the physiological processes of arsenic metabolism and the biochemical pathways, can be used to design treatments of arsenic toxicity[21]. The effects of ethanol leaf extract of *Eclipta alba* (*ELEA*) were studied as a preventive and treatment alternative for sodium arsenite (SA) toxicity. Because cells demonstrate the ability for repair upon insult, we attempted to make a distinction between the natural cellular recovery mechanisms on exposure to SA and pre- and post-treatment effects of *ELEA*. Exposure to inorganic arsenic compounds results in adverse health effects including renal and hepatic diseases[22]. The increased activity of AST, ALT, ALP and γGT are indicators of liver injury[23,24]. The activities of serum enzymes were increased significantly ($p<0.05$; Table 1) in the positive control (group II) and natural recovery (group V) that were treated with SA only as compared with the negative

control which received water only and *ELEA* treated rats. This observation is consistent with observations from our laboratory on the induction of hepatotoxicity and oxidative stress upon exposure to SA[25,26].

Pre-treatment with *ELEA* before exposure to SA (i.e. groups VI) significantly reduced liver enzymes activities when compared to the SA only/positive control group. This shows the potential protective effect the *ELEA* on SA induced hepatotoxicity in albino rats. This decrease in the activities of serum enzymes may be due to the presence of wedelolactone and associated intrinsic phytochemicals in *ELEA* with potential antihepatotoxic properties[27]. Wagner *et al.* (1986) also confirmed that the coumestan component of *E. alba*, wedelolactone and demethylwedelolactone, are most likely responsible for the potent anti-hepatotoxic activities[28]. The significant reduction in the levels of ALT, AST, γGT and ALP are good indicators of hepatoprotective functions[29] suggesting that *ELEA* contains active phytochemicals with the potential to reduce the hepatotoxic effects of SA and possibly restore the hepatocytes physiology in arsenic toxicity. No significant difference was observed in groups II and V (natural recovery) rats treated with SA only, showing the likelihood of the absence of natural recovery upon exposure to SA.

Chronic arsenic toxicity which occurs as a result of drinking arsenic polluted water is one of the worst health hazards in history[30]. Exposure to SA polluted drinking water has been shown to cause alteration in chromosomal segregation which may lead to cancer[30]. The micronuclei assay was developed to easily detect *in vivo* chromosomal aberration in bone marrow cells than the traditional cytogenetic methods.

The approximately 2-fold increase observed in the frequency of mPCEs in the SA-treated rats is an indication of chromosomal damage. This observation is consistent with earlier reported observation in our laboratory on the genotoxic properties of sodium arsenite[26,31]. The increased frequency of induction of mPCEs observed in the SA-only treated rats may be due to arsenic generated free radicals that can attack DNA leading to chromosomal breakage. However, most studies have reported SA as a potent mutagen or carcinogen[32,33]. In all, *ELEA* caused a decrease in the frequency mPCEs showing the potential of the *E. alba* extract to repair chromosomal damage and protect against carcinogenesis and suggesting a potential protective and therapeutic effect of the *ELEA* treatment. This may be because *E. alba* possesses antioxidant activities that helped in scavenging the free radicals and reactive oxygen species generated by SA[34].

Herbs that are rich in important phytochemicals have become a reference point for treatment of various toxicities. This has brought about novel concepts have appeared with the trend, such as nutraceuticals, phytonutrients, and phytotherapy[35]. The phytochemical present in *E. alba* will likely contribute positively to maintaining wellbeing, promoting health, and modulating immune function for disease prevention. The *E. alba* has a great potential in clinical therapy due to its potential to reduce side effects that accompany chemotherapy or radiotherapy[36].

The haematological parameters were used to assess the efficacy of *ELEA* in the prevention and treatment of SA-induced toxicity. The RBC, Hb and PCV levels were significantly reduced ($p<0.05$; Table 2) in the SA-only treated rats (both positive control and natural recovery groups) compared with the negative control group fed with water only. This observed erythrocytopenia is possibly due to depression of bone marrow activity which had been reported previously[37,38,39]. No significant recovery was observed for the blood parameters in the recovery group. Our findings showed that the administration of *ELEA* enhanced the levels of RBC, PCV and Hb concentration, thereby reversing the alterations in erythropoiesis observed during SA intoxication.

The increase in PCV, Hb and RBC levels because of the administration of *ELEA* conforms to previous reports[40,41] of restoration of blood parameters in animals exposed to toxicants. In addition, pre- and post-treatment with *ELEA* increased the PCV, Hb and RBC levels while reducing the lymphocyte count, showing the potential protective and therapeutic properties when compared to the SA-only group. The observed increase is possibly an indication of the release of the lymphocytes from lymphoid tissues as a protective response when challenged with SA. The *ELEA* showed the potential to suppress the toxic effects of SA and thus, probably protecting against impairment or cellular transformations because of the toxicant.

The biochemical observation above is supplemented with histopathological examination of the liver and kidney sections. The results show a moderate periportal and extensive cellular infiltration and fibrosis, and diffuse tubular degeneration with protein casts in the experimental animals treated with SA only (groups II and V) in both liver and kidney, respectively. This illustrates the possible contribution of SA toxicity to the induction of hepatic and adrenal tumours as previous reported[30]. No visible lesion was seen in the kidney of the rats treated with *ELEA* and SA simultaneously (group IV), while a moderate periportal and extensive cellular infiltration was observed in the liver of animals that had SA+*ELEA* simultaneously.

Pre- and post-treatment with *ELEA* (groups VI and VII) showed no visible lesion in the liver showing its potential protective and curative effect in SA toxicity most especially in the liver cells. The cells/mm^2 assay was carried out to assess the rate of cell proliferation. This was used as an index for measuring the tumorigenic potential of a compound since unregulated cell proliferation can be a marker for carcinogenesis. The significant increase by at least 1.53 folds observed in the cells as a result of the SA toxicity was probably due to the promotion of cell proliferation in the experimental rats treated with SA only. Post-treatment with *ELEA* (group VII) reduced the rate of cell proliferation when compared to the SA-only treated rats. This further supports the earlier findings that *ELEA* may serve as a potent remedy in SA induced cell proliferation.

Conclusion

Arsenic pollution of drinking water in developing countries is a major health concern for man and animals. Ethanol leaf extract of *Eclipta alba* exhibited some potential protective effect and can probably suppress sodium arsenite-induced hepatotoxicity and genotoxicity in rats. Further studies are recommended on the molecular mechanisms of *E. alba* on sodium arsenite toxicity.

Conflicts of Interest

There is no conflict of interests associated with this study.

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