Egypt. J. Chem. **Vol. 66,** No. **SI 13, 1141-1153** (2023)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Highlights on The AntimutagenecityImpact of *E. schimperiana* and *E. balsamifera* with Respect to Their Toxicity

Ahlam H. Mahmoud[a*], Doaa S. Foda[a], Asmaa K. Al-Mokaddem[b], Entesar E. S. Hassan[c] Ayman Farghaly[c], Heba-tollah M. Sweelam[d]

[a]*Therapeutic Chemistry Department, National Research Centre, Egypt.*

[b]*Department of Pathology, Faculty of Veterinary Medicine, Cairo University, Egypt.*

[c]*Department of Genetics and Cytology, National Research Centre, Egypt.*

[d] *Chemistry of Natural Compounds Department, National Research Centre, Egypt.*

Abstract

The medicinal plant is considered a double-edged sword that could be beneficial or toxic to people or animals. Accordingly, this study is concerned with the *vitro* and in *vivo* evaluation of *Euphorbia schimperiana* and *Euphorbia balsamifera* toxicity in rats. A comparison study was carried out between both extracts on THLE2 and WI38 cell lines. Furthermore, the anti-inflammatory and anti-mutagenicity influence of both target plants were studied in rats administrated monoiodoacetate. The study was supported by biochemical and histological examinations of key organs. Methanol extract of *E.schimperiana* and *E. Balsamifera* displayed IC_{50} values of 30.6±1.96 and 88.7±5.67 respectively on WI38 cell lines. The chloroform fraction of *E. schimperiana* showed the greatest safety profile with an IC_{50} value of 72±3.74. In contrast, the petroleum ether fraction of *E. balsamifera* displayed the maximum safety profile among the other fractions with an IC_{50} value of 56 ±3.74 on THLE2 cells. Male Wistar rats administered orally 2.5 mg/kg of methanol extract did not show any fatalities or ill effects. The daily dose of the two extracts demonstrated a substantial dose-dependent decrease in the percentage of MNPEs and chromosomal abnormalities; due to mono-iodoacetate administration, suggesting the valuable role of the target plants.

Keywords: *E. balsamefira*, *E.schimperiana*, Toxicity, Monoiodoacetate, Cytogenetic investigations.

1. Introduction

There are advantages over conventional discovery of novel medications from medicinal plants, whether as an extract, pure component, or derivative. Most of the natural materials included in folk remedies have strong scientific support for their biological functions. Nevertheless, a study on the toxicity of the extracts from *E. schimperiana* or *E. balsamifera* was necessary to support the therapeutic claims made from these plants. There are many matters of concern from all relevant groups, including the pharmaceutical industry, health authorities, and patients' rights, which need to be taken into consideration when it comes to medicinal research and development **[1]**. The general public, patients, and consumers are mostly concerned with safe and effective medications that can be obtained rapidly. One could anticipate plants used in traditional medicine to have low toxicity given their long-term use by humans.

However, the most recent studies have revealed that several medicinal herbs used in conventional treatment had negative consequences **[2]**.

It should be underlined that a plant's traditional use for therapeutic reasons does not **,** in any way**,** imply that the plant is safe.Because of this, there is a reason to be concerned regarding the possible harmful effects of both short- and long-term use of such medicinal plants **[3,4]**.To examine any plant extract for potential hazardous effects on both animals and humans, it is essential to consider the toxicological consequences of the extract.

The goal of the current study depends on the extensive history of the use of the genus

*Corresponding author e-mail: ahlamhosnynrc@gmail.com.

Receive Date: 11 April 2023, Revise Date: 20 May 2023, Accept Date: 22 May 2023

DOI: 10.21608/EJCHEM.2023.205483.7858

Euphorbia in traditional medicine. According to states the euphorbia varieties have medicinal properties that include anti-neoplastic, anti-viral, anti-bacterial, and anti-fungal properties. Some species that form latex are used topically in folk medicine to treat certain skin conditions and sexually transmitted illnesses like gonorrhea, migraines, and gastric parasites. **[5]**.

In the present study, ***in*** *vivo* biochemical, antimutagenic, and histopathological effects of *Euphorbia schimperiana* and *Euphorbia balsamifera* methanol extracts against monosodium iodoacetate (MIA) harmful effects were studied for the first time in addition to evaluating the anti-inflammatory impact of both plants.

2. Materials and Methods:

2.1. Plant materials

Samples of *E. schimperiana* and *E. balsamifera* aerial parts were obtained from Abha City and Alssoda Resort. The aerial portion was harvested; samples were collected in bags, labeled, delivered to the lab, cleaned with water, initially dried in the sun, and then dried in the shade. With the aid of a grinder, the dried aerial components were ground to a fine powder and stored.

2.2. Qualitative assays of the total extracts of both *E. schimperiana* and *E. balsamiphera*

Different qualitative assays were used to identify the phytochemical components of the total extracts of *E. Schimperiana* and *E. Balsamiphera* **[6]**.Alkaloids (Dragendorff's test), flavonoids (Magnesium and hydrochloric acid reduction test), terpenoids and sterols (Liebermann-test) and saponins (Foam Index test).

2.3. Extraction and fractionation

Our job began with the preparation of the plant, which entailed extraction using 80 % methanol. Rotating evaporators were used to remove the solvent at low pressure and temperatures below 45 °C. To conduct the biological experiments, a portion of the crude extract was saved and the other part was suspended in the least quantity of water and partitioned by separating funnel using solvents with increasing polarity (petroleum ether, chloroform, and *n*-butanol). The resultant fractions and crude extracts were kept at -20°C for use in the biological tests. The residue was dissolved in distilled water and tween 80 (10%) at the proper concentration before delivery. Rats were given an oral dose of this extract.

2.4. Cytotoxicity assay protocol

The MTT method for determining *in vitro* cytotoxicity of both Euphorbia plants is studied using the multiwell plate method **[7].**

2.5. Animals

In this study, healthy male Wistar albino rats weighing 130±20 g were employed. In hygienic glass fiber enclosures rats were kept in the animal house unit at, the National Research Centre, Egypt. Commercial pellets were given to the animals. These rats were used in investigations of experimental toxicity that were both acute and subchronic. Throughout the trial, the rats had unrestricted food and tap water access. The National Research Centre, Cairo, Egypt's experimental care and animal care committees gave their permission for the work to be done (Approval No: 19-276).

All animals were observed daily for signs of toxicity. All animals were sacrificed at the termination of the experiment.

2.5. Induction of inflammation

Lightly sedated rats (3% isoflurane in O2) were given a single intra-articular injection of monosodium iodoacetate (MIA) in a volume of saline totaling 50µl through the infra-patellar ligament into the joint cavity of the right knee **[8]** for induction of inflammation in rat's knee. All rats were kept under observation throughout the experiment. All animals were sacrificed after 4 weeks.

2.6. Experimental design:

2.6.1. Acute toxicity:

Exploratory trials were performed in rats by supplementation of concentrated doses of the plant extracts. The animals were divided into three groups of five rats each:

The first group: Rats were supplemented orally with *Euphorbia balsamifera* extract with a dose equivalent to 2.5 g/kg once for two weeks [9].

The second group: Rats were supplemented orally with *Euphorbia schimperiana* extract at a dose equivalent to 2.5 g/kg once for two weeks.

The third group: Rats representing the healthy group or the control.

Blood samples were obtained from their different organs. The mortality rate was recorded over two weeks.

2.6.2. Subchronic toxicity:

Rats were divided into eleven groups, ten rats each. Four groups were administered orally with 1/40 and 1/20 with respect to 2.5g/kg (62.5, 125 mg/kg respectively) aqueous solution of *E. Schimperiana* or *E. balsamifera* alcoholic extract for one month in addition to another four groups administrated the plants after induction with MIA as follows:

Group 1: Control group.
Group 2: Positive (MIA).
Groups 3,4: Control groups treated with *Euphorbia schimperiana* (62.5, 125 mg/kg) respectively.
Groups 5,6: Control groups treated with *Euphorbia balsamifera* (62.5,125 mg/kg) respectively.
Groups 7,8: Positive (MIA) treated with *Euphorbia schimperiana* (62.5,125 mg/kg) respectively.
Groups 9,10: Positive (MIA) treated with *Euphorbia balsamifera* (62.5,125 mg/kg) respectively.
Group 11: Positive (MIA) treated with oral dose of Chondrogen (67.5mg/kg daily) and Voltaren group (8.7mg/kg**)** alternately. Samples were collected 24 hours after the last treatment for biochemical, cytogenetic, and histological analysis.

2.7. Biochemical investigations

Blood samples were collected from the retro-orbital plexus of veins (5 ml per rat) placed in clean, sterile tubes, and allowed to set until clotting occurred to investigate the impact of Euphorbia methanol extracts on the serum parameters in rats. After fifteen minutes of centrifugation at 3,000 rpm, serum was recovered. The serum was kept at -20 C until used. According to the biodiagnostic company's directions, chemical analyses of serum parameters using colorimetric kits were done to evaluate the condition of the liver and kidney in the event of acute toxicity. This included: serum alanine aminotransferase (ALT), total protein, cholesterol, triglycerides, glucose, and urea nitrogen. In the subchronic toxicity, serum ALT, and aspartate aminotransferase (AST) were determined colorimetrically while glutathione reductase (GR). Regulated upon Activation, Normal T Cell Expressed and Presumably Secreted (RANTES) were determined by ELISA technique according to the manufacturing kit.

2.8. Cytogenetic investigations

2.8.1. Micronucleus determination:

The micronucleus preparation from the bone marrow of the control and treated rat was performed following the standard test protocol **[10]** and according to the Guideline (OECD) 474 for Testing Chemicals **[11]**. Briefly, the bone marrow cells were collected from bilateral femurs after separating in 3 ml of fetal bovine serum, centrifuged, and smeared on slides. The air-dried slides were fixed by submerging them in absolute methanol (for 10 ~ 20 min). Fixed slides were stained with the May Grünwald - Giemsa protocol. Micronuclei were identified as dark blue staining bodies in the cytoplasm of polychromatic erythrocytes (PEs). A total of 1000 nucleated cells were scored/animal (5 animals/group). Scoring was performed under 1000× magnification with a light microscope.

2.8.2. Chromosomal aberrations determination:

Rats from different groups were injected i.p. with colchicine 2.5 h before collecting bone marrow cells. One hundred well-spread metaphases were assessed per rat. Metaphases with gaps, fragments, breaks, deletions, and polyploidy were scored in bone marrow cells. Bone marrow chromosomes were assessed using the standard protocol **[12].** One hundred well-spread metaphases were analyzed per animal and different kinds of chromosome abnormalities (CAs) were described. CAs were identified based on criteria established by the OECD guideline 475, updated and adopted on July 21, 1997 **[11].** A light microscope (Olympus, Japan) was used for CAs scoring at 1000x magnification.

The method that followed to assess how well *Euphorbia schimperiana* and *Euphorbia balsamifera* preparations to prevent DNA damage brought on by the MIA group:

Inhibitory index (II) = [1- (Plant extract plus MIA group – control) / (MIA group - control)] X100 **[13]**.

2.9. Histological investigations

On dissection of rats, tissue specimens from the brain, lung, liver, kidneys, spleen, and stifle joints were kept at 10% neutral formalin for fixation. Then tissue specimens were processed in different grades of ethyl alcohol, changes of xylene, and lastly set into paraffin wax. Hematoxylin and eosin (H&E) staining of sections of 5 µm width was a standard procedure for light microscopy **[14]**.

2.10. Data analysis

Data were analyzed using computerized software SPSS (Statistical Package of Social Science, version 20, Armonk, New York: IBM Corp). The data were checked for normality and the homogeneity of the variance using the Kolmogorov-Smirnov's test and Levene's test, respectively. The differences among groups with normal distribution were analyzed by one-way analysis of variance (ANOVA) followed by the Tukey LSD test. The results were regarded as significant when the P-value was less than or equal to 0.05.

3. Results:

3.1. Phytochemical screening test. The phytochemical screening showed that the crude

plant extracts have a plethora of flavonoids, terpenoids, and sterols. Furthermore, they have a small amount of saponins. *E. balsamifera* extract is distinguished from *E. Schimperiana* in containing some Alkaloids (table 1).

3.2. *In vitro* Cytotoxic activity

Table (2) presented a comparison between the different fractions of *E. Schimperiana* and *E . balsamifera* extracts on the IC50 of THLE2 and WI38 cell lines.

In the case of *E. schimperiana*, chloroform fraction showed the greatest safe concentration with IC_{50} 72±3.74 on THLE2 cell lines. On the other hand, in the case of *E. balsamifera*, the petroleum ether fraction displayed the maximum safety among the other fractions with IC50 56 ±3.74. Moreover, all fractions showed IC50 greater than Staurosporine (17.7±0.92). Methanol extract of *E.schimperiana* and *E. balsamifera* displayed IC50 (28.2±1.96 and 88.7±5.67 respectively) on WI38 cell lines among the other fractions where Staurosporine showed more toxic effects than most of the different extracts used. WI38 cell lines are more sensitive cells than THLE2 cell lines. Accordingly, the methanol extract that expressed the safest doses on THLE2 cell lines in the case of *E. schimperiana* and *E. Balsamifera* was chosen to test its toxicity *in vivo* by administrating the extract to male rats.

Table (1): Phytochemical screening test

Plant Test	*E. Schimperiana*	*E. balsamifera*
Alkaloids	-	+
Flavonoids	+++	+++
Terpenoids and Sterols	+++	+++
Saponins	+	+

Table (2): Effect of different fractions of *E. schimperiana* and *E. balsamifera* extracts on THLE2 and WI38 cell lines

Extracts / Conc(ug)	*E. shimperiana*					*E. balsamifera*			
	80% total methanol extract S1	Petroleum ether fraction S2	Chloroform Fraction S3	Butanol fraction S4	Staurosp-orine	80% total methanol extract S5	Petroleum ether fraction S6	Chloroform Fraction S7	Butanol fraction S8
IC50 for THLE2	29.9±1.56	53.6±2.79	72±3.74	18.6±0.97	17.7±0.92	24±1.25	56±2.91	20±1.04	48.5±2.52
IC50 for WI38	28.2±1.8	11.9±0.76	12.1±0.77	30.6±1.96	15.9±1.02	88.7±5.67	29.7±1.9	12.9±0.83	6.37±0.41

3.3. Acute toxicity:

Methanol extract of *E.schimperiana* or *E. balsamifera* at a dose of 2.5 g/kg had no adverse effect on the behavioral responses of the tested rats up to 14 days of observation**.** Physical observations indicated no signs of changes in the skin, fur, eyes, mucous membrane, behavior patterns, tremors, salivation, and diarrhea of the rats. There was no mortality observed at the tested dose.

3.4. Subchronic toxicity: Four weeks of daily oral administration of both extracts did not result in any overt signs of toxicity in rodents. Except the breathing (respiratory problems), there were no deaths or clear clinical signs identified in the case of the administration of both doses of *E .shimperiana* extracts throughout the experimental period.

3.5. Biochemical investigations

Table (3) showed the effect of single high doses of both plants on liver and kidney functions (acute toxicity). Significant changes in the chosen parameters in case of the group administrated *E.schimperiana*. Contrarily, the group that received *E. balsamifera* revealed minimal changes in the majority of the serum parameters. Both plants showed significant noticeable decrease in glucose and urea levels compared to the control group.

Table (4) displayed the administration toxicity of both plants compared to a control group after a duration of a month and the effect of the chosen doses of the plants as a treatment for ameliorating the MIA harmful effect. The results clarified the slightly toxic effect of both daily doses of Euphorbia extracts for one month. *E. schimperiana* detected significantly decreased levels of ALT and RANTES in the case of the high dose(125 mg/kg) administrated group. *E. balsamifera* (62.5 mg/kg) administrated group showed generally non-significant changes in the chosen parameters.

Table (3): Effect of oral single doses of 80 % methanol extracts of *E. schimperiana* and *E. balsamifera* (2.5g/kg b.wt) on serum parameters in male rats (Acute toxicity).

Parameters / Groups	Cholesterol (mg/dl)	Triglycerides (mg/dl)	ALT (U/ml)	Glucose (mg/dl)	Total protein (g/dl)	Urea (mg/dl)
Control	77.39±4.9	104.16±18.4	13.6±2.7	96.44±2.8	10.48±0.24	107.18±5.3
E. schimperiana	91.42±11.1	49.66±4.16 ^{c}	16.91±1.1	70.14±4.4^{c}	8.72±0.37^{c}	66.75±4.2^{c}
E. balsamifera	78.09±9.5	80.49±12.16	13.33±1.9	69.17±2.3^{c}	10.27±0.6	64.75±6.9 ^{c}

Data are represented by mean ± S.E. P^{C} significant at p ≤0.05 compared to control group.

On the other hand, *E. balsamifera* 1/20 (125 mg/kg) administrated group detected significantly decreasing levels of ALT against control rats. The glutathione reductase enzyme levels increased significantly in the case of *E. balsamifera* administrated groups (62.5 mg/kg) and decreased non-significantly in the case of *E. schimperiana* administrated ones.

In sub-chronic toxicity, the high dose in two extracts affects significantly in different parameters. Glutathione reductase determines the most suitable conditions for redox control within a cell or for activation of programmed cell death. A single dose administration of MIA to the rats resulted in a significant decrease in total GR content in serum when compared with the normal group. Treatment with plant extracts (62.5 and 125 mg/kg) for the MIA model resulted in a significant increase in the total GR content when compared to non-treated ones.

3.6. Cytogenetic investigations

3.6.1.Bone marrow cell micronuclei investigations (MNPEs) :

The results in Table (5) indicated a significant increase ($p < 0.05$) in the frequency of MNPEs in rat bone marrow induced in the positive (MIA) group, where the percentage reached 10.86 ± 0.67 in comparison with 3.04 ± 0.45 for the control. On the contrary, *Euphorbia schimperiana* and *Euphorbia balsamifera* extracts at doses 62.5 and 125 mg/kg had a normal effect on MNPEs frequency in comparison with the control. *Euphorbia schimperiana and Euphorbia balsamifera* extracts at 2 different doses inhibit DNA damage induced in the positive group.

The percentage of inhibitory index reached 42.71 and 61.63with *Euphorbiaschimperiana*and30.94 and 57.03 with *Euphorbia balsamifera* at low and high doses respectively. On the other hand, when chondrogen and voltaren were given for a month, it significantly increased the DNA damage ($p \leq 0.05$) compared to the controls (table 6). Bone marrow MNPEs cells in MIA rat are shown in Figure (1).

Table (4): Effect of *E. schimperiana* and *E. balsamifera* daily dose on some serum parameters in male rat after MIA injection.

Euphorpia species	Parameters / Groups	ALT (U/ml)	AST (U/ml)	Glutathione reductase (pg/ml)	RANTES (pg/ml)
	Control	16.37±0.095	40.31±0.3	411.5±19.34	75.94±13.9
	Positive (MIA)	14.15±0.045^{c}	37.16±0.9 ^{c}	177.33±0.33^{c}	48.24±3.61 ^{c}
	Standard drug	17.14±0.13^{p}	40.03±0.025^{p}	335±21.36 ^{p}	67.10±1.81
	1/40 dose	15.79±0.64	37.11±0.86 ^{c}	290±25.84	55.97±13.32
E. schimperiana	1/20 dose	4.55±0.085^{c}	36.43±1.16 ^{c}	213.3±36.60	29.61 ± 11.8^{c}
	1/40 Treated	16.98±0.17 ^{p}	37.49±1.03	405±29.44 ^{p}	62.02±10.94 ^{p}
	1/20 Treated	15.85±0.38 ^{p}	36.72±0.6 ^{s}	262.5±30.88 ^{p}	34.77±7.36 ^{s}
	1/40 dose	17.09±0.206	35.78±0.46 ^{c}	429±27.13^{c}	72.31±18.35
	1/20 dose	12.33±0.45^{c}	41.42±0.915	320.5±19.91^{c}	73.41±3.192
E. balsamifera	1/40 Treated	17.09±0.26 ^{s}	37.36±0.09 ^{s}	349±26.21 ^{p}	84.32±0.79 ^{p}
	1/20 Treated	12.33±0.45 ^{s}	41.8±1.15 ^{p}	389±40.41^{p}	72.75±2.7^{p}

Data are represented by mean ± S.E. $P^{c, p,s}$ significant at p ≤0.05. Where c, p, and s represent the control, positive and standard groups respectively.

Table (5): Percentage inhibition of micronuclei polychromatic erythrocytes (MNPEs) induced by monosodium iodoacetate (MIA) in rat model bone-marrow treated with *E. schimperiana*and *E. balsamifera* extracts.

Treatment and doses	No. and percentage of MNPEs		Inhibitory index of MNPEs (%)
	NO.	Mean% ± S.E	
I- Control (non-treated)	152	3.04 ± 0.45	_
II. MIA group	543	10.86 ± 0.67	__
III. *E. schimperiana* (1/40)	163	3.26 ± 0.48	__
IV. *E. schimperiana* (1/20)	174	3.48 ± 0.55	__
V. *E. balsamifera*(1/40)	137	2.74 ± 0.58[a]	__
VI. *E. balsamifera*(1/20)	141	2.82 ± 0. 38 [a]	__
VII. MIA + *E. schimperiana* (1/40)	376	7.52 ± 0. 45 [c]	42.71
VIII. MIA + *E. schimperiana*(1/20)	302	6.04 ± 0.60 [b]	61.63
VIIII. MIA + *E. balsamifera* (1/40)	422	8.44 ± 0. 72 [c]	30.94
X. MIA + *E. balsamifera*(1/20)	320	6.40 ± 0. 48 [b]	57.03
XI. Standard drug (Chondrogen + Voltaren)	501	10.02 ± 0. 42 [d]	__

No. of examining nucleated cells = 1000/ rat (5 rats/ group). The values having different subscript letters in each column are significantly different from one another as calculated by ANOVA. The data were presented as mean ± S.E. (n=5).

3.6.2. Chromosomal aberrations in rat bone marrow cells:

According to Table (6), the MIA group caused rat bone marrow cells to exhibit a substantial percentage ($p \leq 0.05$) of chromosomal aberrations (15.60 ± 0.55*vs* 4.0±0.48for control). Breaks/fragments are the most pronounced aberrations in MIA group (Fig.2). The results also demonstrated that *Euphorbia schimperiana*and *Euphorbia balsamifera* extracts at 2 doses had approximately the same effect as control negative. Moreover, the tested concentrations of *E. schimperiana*and *E. balsamifera* extracts significantly decreased MIA-induced chromosomal aberrations in a dose-dependent manner. Administration of chondrogen withvoltaren for 1 month induced a significantly high DNA damage ($p \leq 0.05$) compared with negative control.

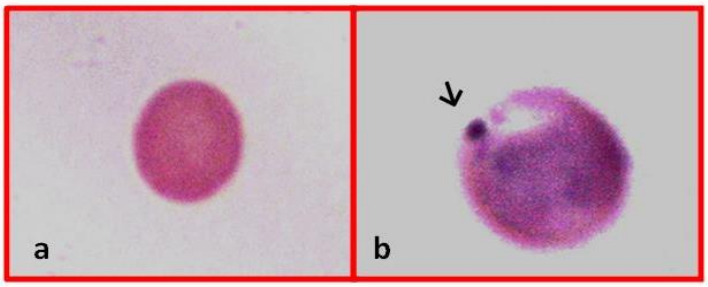
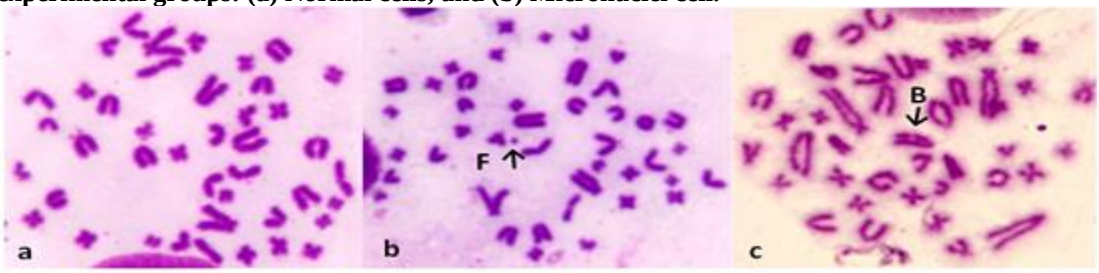


Fig.(1) Micronuclei in polychromatic erythrocyte induced in rat bone marrow cells in different experimental groups: (a) Normal cells, and (b) Micronuclei cell.



Fig(2). Chromosomal abnormalities in rat bone marrow cells in different experimental groups showing: (a) Normal, (b) Fragment, and (c)Break.

Table (6): Percentage inhibition of chromosomal aberrations induced by monosodium iodoacetate (MIA) in rat model bone-marrow treated with *Euphorbia schimperiana* and *Euphorbia balsamifera* extracts.

Treatment and doses	Total abnormal metaphases		No and (%) of metaphases with different types of chromosome aberrations				Inhibitory index (%)
	No	Mean (%) ± SE	Gap	Fragment and/or Break	Deletion	Centromeric attenuation	
I- Control (non-treated)	20	4.0 ± 0.48 a	7 (1.40)	6 (1.20)	2(0.40)	5(1.0)	-
II. MIA group	78	15.60 ± 0.55 a	15 (3.0)	45 (9.0)	7 (1.40)	11(2.20)	-
III. *E. schimperiana* (1/40)	23	4.60 ± 0. 60 a	8(1.60)	9 (1.80)	1(0.20)	5(1.0)	-
IV. *E. schimperiana* (1/20)	28	5.60 ± 0. 45a	8(1.60)	13(2.60)	1(0.20)	6(1.20)	-
V. *E. balsamifera*(1/40)	21	4.20 ± 0.72 a	10(2.0)	7 (1.40)	2(0.40)	2(0.40)	-
VI. *E. balsamifera*(1/20)	24	4.80 ± 0.38 a	11(2.20)	9(1.80)	1(0.20)	3(0.60)	-
VII. MIA + *E. schimperiana* (1/40)	53	10.60 ± 0. 58 d	10(2.0)	33 (6.60)	1 (0.20)	9 (1.80)	43.10
VIII.MIA+*E. schimperiana*(1/20)	42	8.40 ± 0.72 c	7(1.40)	26(5.20)	3(0.60)	6(1.20)	62.06
VIIII. MIA + *E. balsamifera* (1/40)	59	11.80 ± 0. 58 d	14(2.80)	35 (7.0)	3 (0.60)	7 (1.40)	32.75
X. MIA + *E. balsamifera*(1/20)	51	10.20 ± 0.60 c	11(2.20)	31(6.20)	4(0.80)	5(1.0)	46.55
XI. Standard drug (Chondrogen + Voltaren)	56	11.20 ± 0.92 d	8(1.60)	19 (3.80)	2 (0.40)	27 (5.40)	-

A total of 500 cells were analyzed (5 rats per group; 100 cells/rat). One-way ANOVA–Tukey's multiple comparison test was used. The values having different superscript letters in each column are significantly different from one another

3.7. Histopathological investigations

Microscopic examination of the cerebral cortex, hippocampus, and cerebellum from the control group (Fig.3) revealed normal histology of the different brain regions without any detectable alterations. Likewise, the administration of both *E. schimperiana* and *E. balsamifera* did not induce any histopathological alterations in the different regions of the brain.

Rats lungs (Fig. 4) from the control group showed normal histology alveoli and bronchioles without any inflammatory reaction or degenerative changes. Similarly, both tested plant extracts *E. schimperiana and E. balsamifera* did not result in pulmonary affections, and the examined lung sections were histologically normal as well.

Microscopic analysis of the liver tissue from the control group showed that the hepatic parenchyma had a typical architecture, with polygonal hepatocytes radiating outward from a central region towards the portal triads, which comprised branches of the bile duct, hepatic artery, and portal vein. Both substances under evaluation had no negative effects on liver parenchyma (Fig. 4).

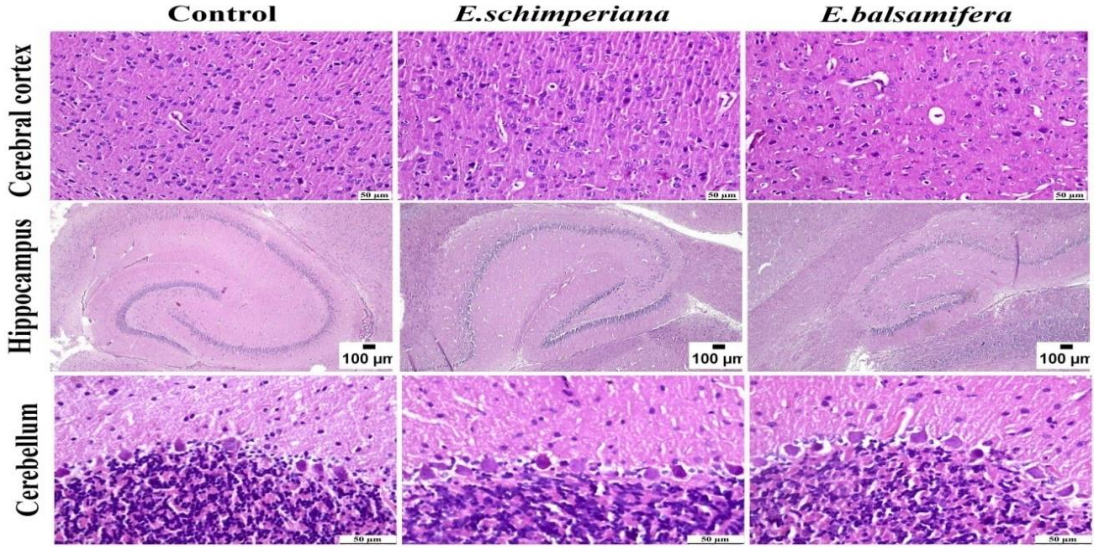


Fig. (3) Photomicrograph of the brain (H&E) showing the normal structure of cerebral cortex, hippocampus and cerebellum in the different experimental groups.

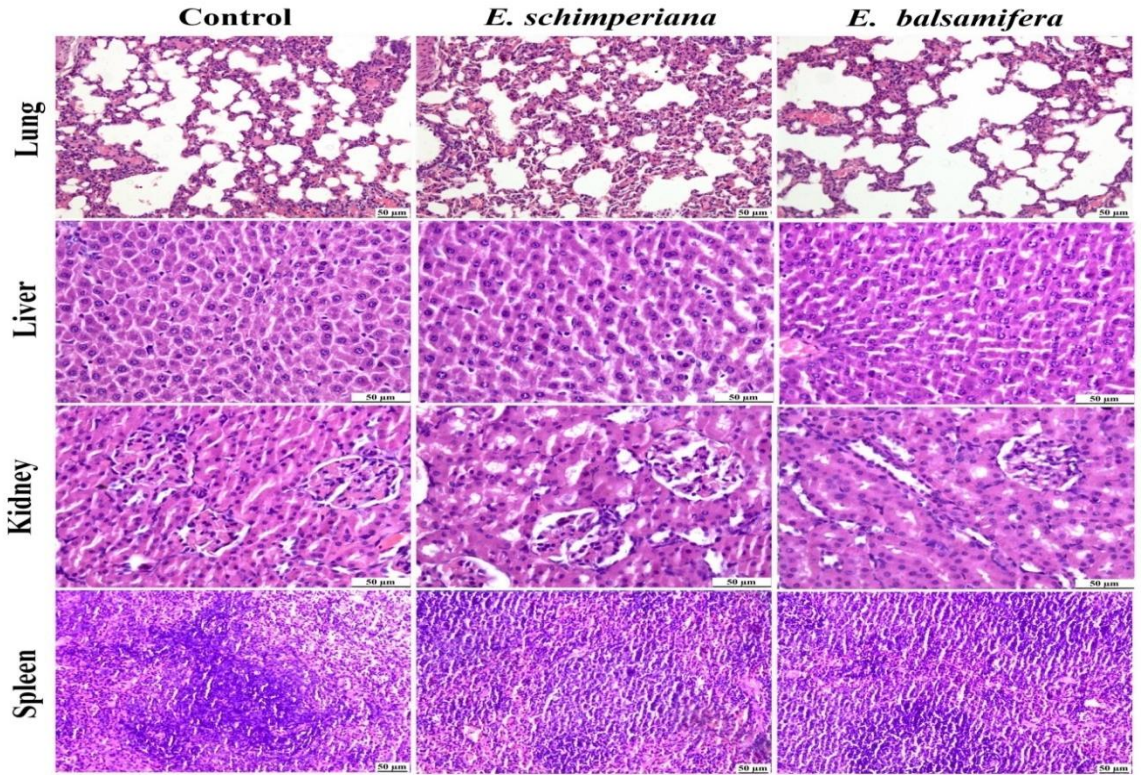


Fig. (4) Photomicrographs of lung, liver, kidney and spleen tissues from the different groups showing apparently normal structure without any detectable alterations (H&E).

Both renal cortex and renal medulla of all experimental groups were histologically normal.The renal cortex was containing glomeruli and renal tubules while the medulla was made of renal tubules only (Fig. 4). Normal spleen tissue was observed in all examined groups with normal red and white pulps.

Microscopic examinations of the stifle joint collected from the different groups revealed the normal bony structure of the head, articular cartilage and synovial capsule (Fig. 5).

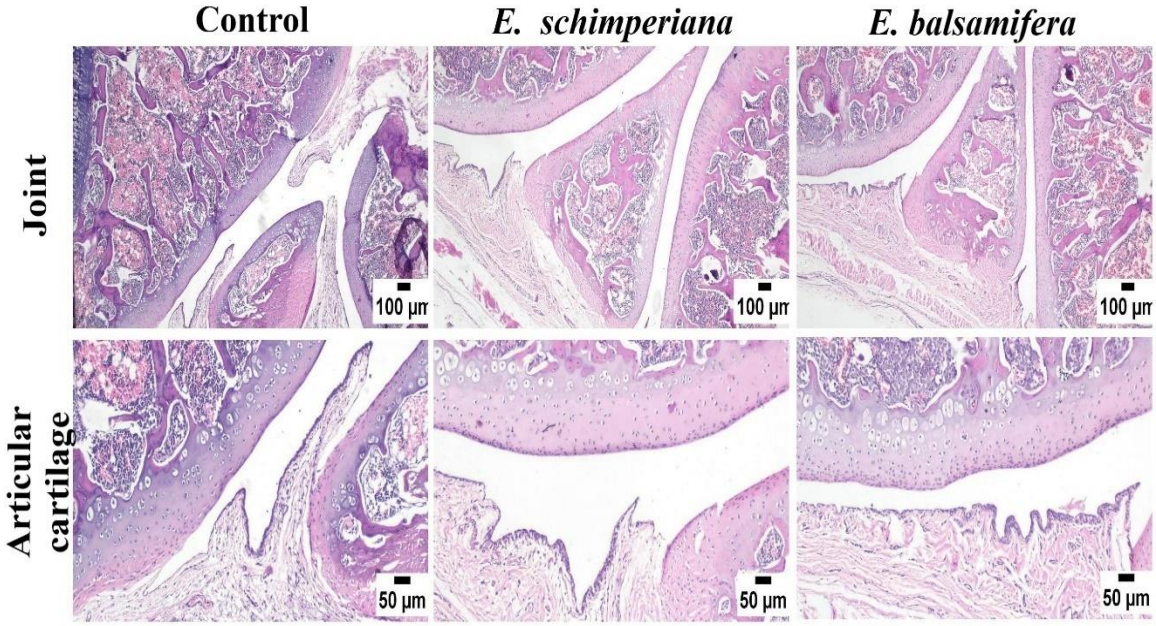


Fig. (5) Photomicrographs of joint showing normal joint structure and articular cartilage in different groups (H&E).

4. Discussion:

Natural remedies; medicinal plants and herbs, have long been the cornerstone of the management of many diseases. The initial stage of screening natural products for a pharmacological activity usually involves assessing and evaluating the hazardous properties of a natural product extract, fraction, or molecule. Detailed knowledge about the chronic toxicology of these herbs must be studied to evaluate their toxicity or safety impacts in a case using animals.

The assessment of acute toxicity during the evaluation of the toxic properties of medicinal plants may offer preliminary data in the toxic mechanism action of a substance, assist in determining the dose of a novel compound and in dose determination in animal studies, and provide numerous indices of potential drug activity types. The *vitro* cytotoxic activity revealed that WI38 cell lines are more sensitive cells than THLE2 cell lines. Accordingly, an alcoholic extract that expressed the safer doses on THLE2 cell lines in the case of *E. schimperiana* and *E. balsamifera* was chosen to test its toxicity *in vivo* by administrating the extract to male rats.

Up to 14 days of observation, both extracts in this research at a dose of 2500 mg/kg had no negative effects on the tested rats. At a dose of 2500 mg/kg in this research, none of the extracts had any negative effects on the tested rats up to 14 days of observation in bowel habits as diarrhea or constipation, they didn't exhibit any behavioral changes such as lethargy, convulsions, irritability or aggression, no hair discoloration or change in color of lips or nose, and salivation was not found. More importantly, there were no deaths among all tested groups. Cholesterol and ALT were increased non-significantly in treated rats, which was parallel with histopathology in acute toxicity in the animals intoxicated with both extracts. This non-significant increase may be due to slight toxicity **[15,16,17,18].**

The decrease of protein concentration and glucose, in the animal serum intoxicated with *E. schimperiana*, may be due to compounds in the crude extracts. It may be due to di and triterpenoid compounds in both extracts which affect the appetite of the animals for feeding [Animals abstain from food] and consequently, decrease glucose, protein, and urea concentration. However, it could be argued that these changes may not be toxicologically significant, as they were not corroborated by histomorphology findings, also it was observed that they suffered from dyspnea.

More than 5000 mg/kg body weight of the 80% methanol extract of *E. balsamifera* fed orally to rats was shown to be the median lethal dose (LD50) **[19].**

According to a previous publication, a phytochemical investigation of the extract revealed the presence of steroid/triterpenes, tannins, anthraquinone, and cardiac glycosides **[20].** Because of their connection to sex hormones, substances having steroidal activity are important and interesting to pharmacists **[21,22]**. Acute toxicity studies of the plant extract showed no sign of behavioral changes and no mortality was recorded in a maximum dose of 2.5 g/kg as reported before **[15].** Hence the LD50 is higher than 2.5g/kg and practically non-toxic.

As a result, this investigation suggests that neither extract, at the measured level, produces obvious acute harmful effects. Although the limit test method is often not used to calculate an exact LD50 value, it does provide a proposal for categorizing the crude extract based on the dose level at which animals are anticipated to survive **[23].** Data from acute and subchronic toxicity studies on medicinal plants should be collected to increase confidence in the safety of preparations made from them for use in the manufacturing of medications **[24].** A crucial element in determining human safety is selecting the tests and dosage plants that will best indicate an adequate margin of exposure. The subchronic toxicity of both extracts in rats for up to 28 days was investigated further in order to generate the whole toxicology data of this fabled medicinal plant because no toxic effects were found during the acute toxicity experiment. In subacute toxicity ALT and AST were reduced in high concentrations of both extracts significantly. Low levels are generally considered good and are usually not a cause for concern. The decrease in both enzymes may be due to deficiencies in vit B6, kidney disease, or inflammatory diseases **[25,26].** Moreover, oral administration of each extract in the normal state may affect ALT concentration**[27].**

The high levels of cytokines directly induce cellular oxidative stress by depleting the vital antioxidant substances (such as glutathione) of the body. Therefore, it would be interesting to check the effectiveness of drugs, including antioxidant enhancers or antioxidants to effectively combat the side effects of anti-cytokine therapy **[28].** Both extracts are rich with phenolic compounds which help in scavenging free radicals **[29, 30].**

Refusing the animal to eat may interrupt the immune system. Continued exposure to these plants to animals may then lead to lymphopenia, which may have an immunosuppressive effect **[15].**

In sub-chronic toxicity, the high dose of the two extracts affects significantly the different parameters. Glutathione reductase determines the most suitable conditions for redox control within a cell or for activation of programmed cell death. When compared to the normal group, the rats that received a single dosage of MIA had significantly less total GR in their serum. Treatment with plant extracts (62.5 and 125 mg/kg) for the MIA model resulted in a significant increase in total GR level when compared to the normal one. The results showed that taking plant extracts in normal conditions has a negative effect on GR and RANTS. Furthermore, they have a positive effect on the treatment of MIA-induced inflammation in rats. After receiving two distinct doses of the plant extract, an upward reversal was seen. This could be explained by the extract's direct influence on the activation of the hepatic GR. which indicates a protective effect to liver tissue due to the antioxidant compound in both extracts **[19, 29].**

According to cytogenetic research, the MIA-treated group showed a noticeably higher incidence of rat MNPEs and chromosomal abnormalities in the current study. The percentage reached 10.86 and 15.60 compared with 3.04 and 4.0 for the control normal rats respectively. Our results are in the same line with Chen et al., 2008 and Davies et al., 2008 **[31, 32].** They found that a significant increase in DNA damage in the MIA chondrocytes compared with the non-MIA chondrocytes using comet assay in pigs.

The experimental and clinical evidence showed increasing reactive oxygen species in patients with inflammation **[33, 34].** As we know exposure of the genetic material to ROS and inflammation could cause DNA damage, mutation, and cancer **[35,36,37]**.

On the other hand, *E. schimperiana* and *E. balsamifera* extract at doses 62.5 and 125 mg/kg had a normal effect on MNPEs and chromosomal aberration frequency in comparison with the control. The same results were observed by Zamith et al., 1996 **[38].** They found that no DNA damage was induced in HPRT Locus nor chromosome aberrations in V79 Chinese hamster lung cells treated with *Euphorobia millii.* Moreover, *E. schimperiana* and *E. balsamifera* extracts with the 2 different doses demonstrated a substantial dose-dependent decrease in the percentage of MNPEs and chromosomal abnormalities caused in the MIA group. The phytochemicals represent the main sources of alternative potential that could contribute successively to human health. *E schimperiana* and *E. balsamifera;* a rich natural sources of bioactive secondary metabolites (flavonoids and phenolic) that could be utilized as antioxidant antibacterial and anticancer agents **[20, 29, 39]**.

To the best of our knowledge, there aren't many studies on *E. schimperiana* and *E. balsamifera*, and there aren't any data on the secondary metabolites isolated from the plants' antimutagenic properties. Hence, in this study, we can assume that *Euphorbiaschimperiana* and *Euphorbia balsamifera* extracts may exert their antimutagenic effects on the MIA rat model not only through their antioxidant activity[39, 40] but also through their anti-inflammatory activity **[41,42,43,44].**

Many natural products such as grape seed proanthocyanidin extract **[45]** vitamin C **[46]** and dioscin extracted from the roots of *polygonatum zanlanscianense***[47]** have the ability to minimize the harmful effect induced by MIA through inhibition of ROS and inflammation.

5. Conclusion

Further studies are needed for both plants to investigate their safety on different body organs to ensure their application use.

Funding

Not applicable.

Declaration of Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

All the obtained data are documented in this article.

Acknowledgment

The authors are thankful to the National Research Centre for providing all the needed facilities and tools.

References

[1] I.G Widhiantara, I.M Jawi, Phytochemical composition and health properties of Sembung plant (*Blumea balsamifera*): A review, Vet World. 14(5) (2021) 1185-1196.

[2] K. Okaiyeto, O.O. Oguntibeju, African Herbal Medicines: Adverse Effects and Cytotoxic Potentials with Different Therapeutic Applications, Int J Environ Res Public Health, 18(11) (2021) 5988.

[3] A. N. Ukwuani, M. G. Abubakar, S. W. Hassan, B. M. Agaie, Toxicological studies of hydroethanolic leaf extract of Grewia crenata, International Journal of

Pharmaceutical Science and Drug Research, 4 (2012) 245–249.

[4] S.L.Chen, H. Yu, H.M. Luo, *et al.*Conservation and sustainable use of medicinal plants: problems, progress, and prospects, Chin Med., 11 (2016)1-10.

[5] A.L.V. Alves, L.S. da Silva, C.A. Faleiros, V.A. Silva, R.M Reis, The Role of Ingenane Diterpenes in Cancer Therapy: From Bioactive Secondary Compounds to Small Molecules, Natural Product Communications, 17(7) (2022) 1-30.

[6] B. Benzidia, M. Barbouchi, H. Hammouch, N. Belahbib, M. Zouarhi, H. Erramli, N. Ait Daoud, N. Badrane, N. Hajjaji, Chemical composition and antioxidant activity of tannins extract from green rind of Aloe vera (L.) Burm. F, Journal of King Saud University - Science, 31 (4) (2018) 1175-1181.

[7] S.P.C. Cole, Rapid chemosensitivity testing of human lung tumor cells using the MTT assay, Cancer Chemother. Pharmacol. 17(1986) 259–263.

[8] T. Pitcher, J. Sousa-Valente, M. Malcangio, The Monoiodoacetate Model of Osteoarthritis Pain in the Mouse, J Vis Exp., 16;(111) (2016) 53746.

[9] Abou Baker DH, Ibrahim BMM, Hassan NS, Yousuf AF, El Gengaihi S. Exploiting Citrus aurantium seeds and their secondary metabolites in the management of Alzheimer's disease. Toxicol Reports. 2020;7:723–729.

[10] M. A. Fahmy, A. A Farghaly, E. E Hassan , E. M. Hassan,., Z. M. Hassan, K. Mahmoud, E. A. Omara, Evaluation of the Anti-Cancer/Anti-Mutagenic Efficiency of *Lavandula officinalis* Essential Oil, Asian Pac J Cancer Prev. 23 (4) (2021) 1215-1222.

[11] OECD Guidelines for the Testing of Chemicals, Genetic Toxicology: Mammalian Erythrocyte Micronucleus Test, Organisation for Economic Co-operation and Development, 21(1997).

[12] M.A. Fahmy, A.A. Farghaly, E.E. Hassan, E.M. Hassan, N.S. Abdel-Samie, E.M. Abdel-Ghany, E.A. Omara, Fennel (*Foeniculum vulgare)* essential oil ameliorates DNA and histopathological damage induced by cyclophosphamide in mice, Bioscience Research, 16(1) (2019) 320-336.

[13] H.A.A. Al-Ashaal, A.A. Farghaly, N.S. Abdel-Samee, Antidiabetic Efficacy of *Solanum torvum*Extract and Glycoalkaloids against Diabetes Induced Mutation in Experimental Animals, J. Pharm. Sci. & Res. 10(6) (2018) 1323-1331.

[14] J.D. Bancroft, M. Gamble, Theory and Practice of Histological Techniques. 6th Edition, Churchill Livingstone, Elsevier, (2008), China.

[15] A.A. Adedapo, M.O.Abatan, O.O. Olorunsogo, Toxic effects of some plants in the genus Euphorbia on haematological and biochemical parameters of rats, VeterinarskiArhiv, 74(1) (2004) 53-62.

[16] E.S. Ahmed, N.A. Al-Zanbagi, Mammal Toxicity and Mutagenicity Assessment of the Methanol Extract of the Molluscicidal Plant *Euphorbia schimperiana*, Pakistan Journal of Biological Sciences,9(10) (2006)1911-1916.

[17] J. Xavier, K. Kripasana, Acute Toxicity of Leaf Extracts of *Enydrafluctuans* Lour in Zebrafish (*Danio rerio* Hamilton)., 2020 (2020) 1-6.

[18] K. Benrahou, H.N. Mrabti, H. M. Assaggaf, S. Mortada, N. Salhi, L. Rouas, R. El Bacha, A.Dami, A. Masrar, M. M. Alshahrani, et al., Acute and Subacute Toxicity Studies of Erodium guttatum Extracts by Oral Administration in Rodents, Toxins, , 14(11) (2022) 1-12.

[19] S. Shehu, U. Iliyasu, A.I. Barau, N.A. Muhammad, Preliminary phytochemical screening, acute toxicity and laxative activity on the leaves of euphorbia balsamifera ait (euphorbiaceae), African J Pharm. Res. Dev, 11(2) (2019), 95-99.

[20] A. Kamba, L.G. Hassan, Phytochemical screening and antimicrobial activities of Euphorbia balsamifera leaves, stems and root against some pathogenic microorganisms, African Journal of Pharmacy and Pharmacology,, *4*(9) (2010) 645-652.

[21] A. Rasheed, M. Qasim, A review of natural steroids and their applications, International Journal of Pharmaceutical Sciences and Research, 4 (2) (2013) 520 – 531.

[22] F. Labrie, Intracrinology in action: Importance of extragonadal sex steroid biosynthesis and inactivation in peripheral tissues in both women and men, The Journal of Steroid Biochemistry and Molecular Biology, 145 (2015) 131-132.

[23] K.Yuet Ping, I. Darah, Y. Chen, S. Sreeramanan, S. Sasidharan, Acute and subchronic toxicity study of Euphorbia hirta L. methanol extract in rats, BioMed research international, 2013 (2013)1-14.

[24] P.V. Christapher, S. Parasuraman, M..Z.Asmawi, V. Murugaiyah, Acute and

subchronic toxicity studies of methanol extract of Polygonum minus leaves in Sprague Dawley rats, Regulatory Toxicology and Pharmacology, 86 (2017) 33-41.

[25] P.M. Ueland, A. Ulvik, L. Rios-Avila, Ø Midttun, J. F. Gregory, Direct and functional biomarkers of vitamin B6 status, Annual review of nutrition, 35(2015) 33-70.

[26] A.M. Diehl, J. Potter, J. Boitnott, M.A. Van Duyn, H.F. Herlong, E. Mezey, Relationship between pyridoxal 5′-phosphate deficiency and aminotransferase levels in alcoholic hepatitis, Gastroenterology, 86(4)(1984)632-636.

[27] W.R. Kim, S.L. Flamm, A.M. Di Bisceglie, H.C. Bodenheimer, Serum activity of alanine aminotransferase (ALT) as an indicator of health and disease, Hepatology, 47(4) (2008)1363-1370.

[28] F. A..Mannaa, K. G. Abdel-Wahhab, Physiological potential of cytokines and liver damages, Hepatoma Res. 2 (2016)131-143.

[29] S.M. Aljubiri, K. Mahmoud, S.A. Mahgoub, A.I. Almansour, K.H.Shaker, Bioactive compounds from Euphorbia schimperiana with cytotoxic and antibacterial activities, South African Journal of Botany, 141 (2021) 357-366.

[30] S.M. Aljubiri, E. Abd Elsalam, F.K. Abd El Hady, M.O. Radwan, A.I. Almansour, K.H. Shaker, In vitro acetylcholinesterase, tyrosinase inhibitory potentials of secondary metabolites from *Euphorbia schimperiana* and *Euphorbia balsamifera*, Zeitschrift für Naturforschung C J Biosci., (2022) Online ahead of print.

[31] A.F. Chen, C.M. Davies, M.D. Lin, B. Fermor, Oxidative DNA Damage in Osteoarthritic Porcine Articular Cartilage, J. Cell Physiol., 217(2008) 828–833.

[32] C.M. Davies, F. Guilak, J.B. Weinberg, B. Fermor, Reactive nitrogen and oxygen species in interleukin-1-mediated DNA damage associated with osteoarthritis. Osteoarthritis and Cartilage, 16 (5) (2008) 624-630.

[33]F. Berenbaum, Osteoarthritis as an inflammatory disease (osteoarthritis is not osteoarthrosis), Osteoarthritis and Cartilage, 21 (2013)16-21.

[34]P.Lepetsos, A.G.Papavassiliou, ROS/oxidative stress signaling in osteoarthritis. Biochimica et Biophysica Acta, 1862 (2016) 576–591.

[35] A.A. Farghaly, Z.M. Hassan, S.M. Donya, Ameliorative potential of Myristica fragrans extract as hypoglycemic agent on oxidative stress produced by diabetes mellitus in mice, Journal of American Science, 8(1) (2012)183-189.

[36] J. Kay, E. Thadhani, L. Samson, B. Engelward, Inflammation-Induced DNA Damage, Mutations and Cancer, DNA Repair (Amst). 83(2019)1-53.

[37] N.A. Ragab, E.A. Aboutabl, M.M. Marzouk, A.M. El Halawany, A.A. Farghaly, A.A. Sleem, S.A. El Sawi, Chemical Profiling, *In Vivo* Antioxidant Effect and Antimutagenic Evaluation of *Melilotus indicus* (L.) All. Extracts. Trop J Nat Prod Res, March, 5(3) (2021) 534-542.

[38] H.P.S. Zamith, F.J.R. Paumgartten, G. Speit, Evaluation of the mutagenicity of the molluscicidal latex of Christs crown (*Euphorbia milii var. hislopii*). In mammalian cells *in vitro* and *in vivo*, Mut. Res., 368 (1996)15-20.

[39]K.H.Shaker, S.M. Al-Sawqaee, Cytotoxic and antioxidant activities of Euphorbia balsamifera with a new phenolic compound, Research Journal of Pharmaceutical, Biological and Chemical Sciences, 9(4) (2018) 969.

[40] K.H. Shaker, B.M. Al shehria, M.D.Y. Oteefa, M.F. Mahmoud, Antioxidant Compounds from *Euphorbia schimperiana*scheele in Aseer Region, Saudi Arabia, Int. J. Pharm. Sci. Rev. Res., 32(1) (2015) 117-122.

[41] M. Majid, M.R. Khan, N.A. Shah, I.U. Haq, M.A. Farooq, S. Ullah, A. Sharif, Z. Zahr, T. Younis, M. Sajid, Studies on phytochemical, antioxidant, anti-inflammatory and analgesic activities of Euphorbia dracunculoides, BMC Complementary and Alternative Medicine15(2015)1-15.

[42] M.A. Abo-dola, M.F. Lutfi, Anti-inflammatory activity of *Euphorbia aegyptiaca*extract in rats,International Journal of Health Sciences, Qassim University, 10 (1) (2016)69-75.

[43] S. Ahmed, M. Yousaf, R.A. Mothana , A. J. Al-Rehaily, Studies on wound healing activity of some Euphorbia species on experimental rats, Afr J Tradit Complement Altern Med., 13(5) (2016)145-152.

[44] M.S.M. Rahman, S. Rana, A.A.F.M. Islam, A. Kumer, A-K. Hashem, H. Rahman, Mohaimenul,T.K. Biswas, S.A.A. Sabbir, Antithrombotic and anti-inflammatory activities of leaf methanolic extract of

*Euphorbia hirta*Lin, Int J Complement Alt Med., 12(4) (2019)154-162.

[45] Y.J. Woo, Y.B.Joo, Y.O. Jung, et al., Grape seed proanthocyanidin extract ameliorates monosodium iodoacetate-induced osteoarthritis, Experimental and molecular medicine, 43(10)(2011)561-570.

[46] P-R. Chiu, Y-C. Hu, T-C. Huang, B-S Hsieh, et al., Vitamin C Protects Chondrocytes against Monosodium Iodoacetate-Induced Osteoarthritis by Multiple Pathways, Int. J. Mol. Sci.,18 (2017) 1-15.

[47] J. Lu, T. Zhang, H. Sun, S. Wang, M. Liu, Protective effects of dioscin against cartilage destruction in a monosodium iodoacetate (MIA) - induced osteoarthritis rat model, Biomedicine & Pharmacotherapy, 108 (2018) 1029–1038.