

## Protective Role of *Acacia Nilotica* Extracts And Silymarin against Mutagenic and Hepatic Injuries Induced by 2-Butoxyethanol in Male Mice

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### ABSTRACT

2-Butoxyethanol (2-BE) is a clear colorless liquid that smells like ether. It is used as a solvent in spray lacquers, varnishes, varnish removers, herbicides, liquid soaps, cosmetics, industrial and household cleaners, and dry-cleaning compounds. 2-BE causes cellular damage via formation of reactive oxygen species. *Acacia nilotica* (*A. nilotica*) leaf extract exhibited significant antimutagenic and DNA-protective effects against oxidative damage due to the presence of alkaloids, volatile essential oils, phenols and phenolic glycosides; it is considered an excellent free radical scavenging antioxidant owing to the high number of hydroxyl groups. Silymarin (SIL) is a standardized mixture of antioxidant flavonolignans (silybin and silibinin).

*Silybum marianum* (Milk thistle) family *Asteraceae* is an ancient medicinal plant from which SIL is extracted. It is a free radical scavenger and a membrane stabilizer that prevents lipid peroxidation. In the present study, we investigated the effects of extracts of *A. nilotica* leaves and SIL on the toxicity of 2-BE.

**Materials and Methods:** 2-BE was given orally to male albino mice for 28 days at dose (450 $\mu$ l/kg b.wt). *A. nilotica* leaf extract (25 mg/kg b.wt) was dissolved in water and was administered orally for 14 days prior to 28 days treatment of 2-BE and during the 28 days. Also SIL (20 mg/kg b.wt) was administered orally for 14 days prior to 28 days treatment of 2BE and during the 28 days.

**Result:** In the present work, genotoxic effects were induced by 2-BE through oral administration, and the protective effect of *A. nilotica* and SIL are studied. 2-BE induced a significant increase in the structural as well as numerical chromosomal aberrations. The frequency of chromosomal aberrations showed significant decrease when mice treated with *A. nilotica* extract and SIL. Also, there were significant increases in micronuclei. *A. nilotica* extract and SIL administration significant decreases micronuclei induced by 2-BE. However 2-BE induced a significant decrease in mitotic index. Administration of both *A. nilotica* extract and SIL significant increase mitotic index in mice treated with 2-BE. Exposure of mice to 2-BE caused significant changes in the hematological parameters as well as significant increases in the activities of serum enzymes alanine aminotransferases (ALAT), aspartate aminotransferases (ASAT) and alkaline phosphatase (ALP). Also, 2-BE induced a significant decrease in the content of liver reduced glutathione (GSH), however, induced a significant increase in the level of hepatic lipid peroxidation end product (MDA) of male mice. Co-administration of both *A. nilotica* extract and SIL to 2-BE-intoxicated mice ameliorated the above-mentioned parameters. **Conclusion:** 2-BE induced mutagenic and liver injury in male mice. *A. nilotica* and SIL are found to reduce the percentage of chromosomal aberration and micronuclei cells as they are a powerful antioxidant, they are able to scavenger reactive oxygen species (free radicals) formed by 2-BE in the cells, these free radicals damage DNA and hence cause defects in the chromosomes. *A. nilotica* extract and SIL could be used as a protective agent against mutagenic and hepatic injuries resulting from 2-BE. The protective action of SIL is more effective than *A. nilotica*.

**Keywords:** 2-Butoxyethanol, *Acacia nilotica* leaves extracts, Silymarin, Chromosome aberration, Micronucleus, hematological parameters, liver functions and oxidative stress.

### INTRODUCTION

2-Butoxyethanol is glycol ether with the molecular formula: C<sub>6</sub>H<sub>14</sub>O<sub>2</sub>. It is a volatile organic compound (VOC) and is not known to occur as a natural product but is emitted into the atmosphere due to its use as solvent, mainly during surface coating and cleaning activities<sup>1</sup>. Human exposure to 2-BE can occur through skin contact, inhalation, or ingestion<sup>2</sup>. There are several studies reporting that 2-

BE causes cellular damage via formation of reactive oxygen species (ROS). ROS are believed to cause lipid peroxidation resulting in damage to biological membranes<sup>3</sup>. Herbal extracts with their proven potential and less side effects in therapeutics has replaced the synthetically derived drugs in modern allopathic medication system<sup>4</sup>. *Acacia* species (Mimosaceae) are rich in phenolics and have strong antioxidant activities<sup>5</sup>. *Acacia nilotica* (L.) Willd

also known as Gum Arabic tree is an imperative multipurpose plant <sup>3,6</sup>. *A. nilotica* is abundant throughout Asia, Australia, Africa and America. Several biologically active compounds were isolated from different parts of *A. nilotica* include umbelliferone, gallic acid, niloticane, catechin, kaempferol, rutin, apigenin and two steroids include androstene and  $\beta$ -sitosterol <sup>7</sup>. *A. nilotica* showed a marked anti-mutagenic effect, furthermore, it was more effective against indirect acting mutagens <sup>8</sup>.

Silymarin is the extract of *Silybum marianum*, or milk thistle. SIL is a flavonoid complex extracted from the seeds of *Silybum marianum* (milk thistle). It contains the isomeric flavonoids silibinin, silydianin, isosilibinin, and silychristine<sup>9</sup>. SIL has been well demonstrated to exert multiple beneficial effects and thus used as a natural remedy for the treatment of hepatitis, jaundice, and cirrhosis. It protects against liver injury induced by radiation, alcohol abuse, ischemia, iron overload, environmental toxins, and CCl<sub>4</sub>.<sup>10,11</sup>. The antioxidant, anti-inflammatory, anti-apoptotic, and immunomodulating effects of SIL have also been reported<sup>12</sup>. Also, It has the capability to lower the induction of sister chromatid exchanges, giving an evidence that it plays an important role in repairing primary DNA damage<sup>13</sup>. So, the aim of the present work was to study *A. nilotica* leaf extract and SIL against mutagenic and hepatic injuries induced by 2-Butoxyethanol in male mice.

## MATERIALS AND METHODS

### 1. Chemicals

a- **2-Butoxyethanol**: ethylene glycol monobutyl ether (EGME) (E0883), >99% purity was purchased from Sigma Chemical Co. USA.

b- **Aerial parts** of *Acacia nilotica* (*A. nilotica*) was purchased from El- Orman culture in El- Giza, Egypt. The collected plant sample was washed thoroughly with running tap water and completely shade dried under room temperature. soaked in boiled water for half hour, dried and weighed. The viscous dried part was dissolved in water (25mg/kg b.w.).

c- **Micronized silymarin** (silybin > 45%) was taken as Hepaticum drug (beta-cyclodextrin enhanced formula) suspension 20mg/ 5ml. Silymarin purchased from SEDICO for Drugs Industries Company, South-Egypt, Egypt.

### 2- Animals

Swiss male albino mice (6–8 weeks of age), body weights ranging from 25-30g were purchased

from animals breeding station. Animals were housed in polycarbonate cages. The animals were fed with standard pellet diet and water *ad libitum*. They were maintained in controlled environment (12:12 h light/dark cycle) and temperature (21±2 °C). All the animal experiments were performed according to the guidelines of the Institutional Animal Ethical Committee, Al-Azhar University, Egypt. Mice were acclimated for two weeks before the start of the treatment.

### 3- Experimental design

Mice received all doses via daily gavage (7 times per week) through gastric tube; mice were randomly divided into 6 different groups (6 mice/group) as follows:

1- Control group: Water was administered orally for 42 days.

2- *A. nilotica* group: *Acacia nilotica* leaves extract solution (25mg/kg.b.wt) was administered orally for 42 days.

3- Silymarin(SIL) group: Silymarin (20mg/kg.b.wt) was administered orally for 42 days.

4- 2-Butoxyethanol (2-BE) group: 2-BE (450 mg/kg .b.wt) was administered orally for 28 days.

5- *A. nilotica*+2-BE group: *A. nilotica* leaves extract solution (25 mg/kg.b.wt) for 14 days then for 28 days in addition to 2-BE (*A. nilotica* +2BE).

6- SIL+2-BE group: SIL (20 mg/kg.b.wt) for 14 days then for 28 days in addition 2-BE (SIL+2BE).

### 4- Genetic Analysis

#### A- Micronucleus Test

At the end of experiment, The MN slides were prepared as described by Schmid<sup>14</sup>.

The bone marrow was extracted and smear was prepared, air dried and fixed in absolute methanol for 10 min and stained with 5% buffered Giemsa (pH 7.0) in the following day. Polychromatic erythrocytes were scored for micronuclei under the microscope.

#### B- Chromosomal preparation

The chromosomal preparation assay was carried out as described by Yosida and Amano<sup>15</sup>. Experimental animals were injected with 2mg/kg b.wt of colchicine 1.5 h prior to sacrifice. Bone marrow cells were collected by flushing with 0.56% KCl (pre-warmed at 37 °C) from femur bone and incubated for 20 min at 37 °C. The material was centrifuged at 1000rpm for 5min, fixed in freshly prepared aceto-methanol (acetic acid and methanol in the ratio 1:3, v/v) followed by refrigeration for 30 min. The material was centrifuged and re-suspended

in aceto-methanol. The slides were prepared by dropping the sample on chilled slides and run over the flame. Staining was done in 5% buffered Giemsa stain (pH 7.0) after 24h, air dried and covered with cover slips. 50 well spread metaphase plates were studied per animal (6 animals/group).

### C- Mitotic index

Mitotic figures were identified according to the criteria proposed by <sup>16</sup>. Only cells with clear morphological features of metaphase, anaphase, and telophase were counted. The percentage of mitotic cells out of a total of 1000 cells (mitotic index; MI).

### 5- Physiological studies

**Blood Samples:** on the day of sacrifice, all the animals were euthanized using chloroform. Blood samples were drawn from the heart of each sacrificed mouse. The blood samples were divided into two portions. One portion was used for the determination of hematological parameters. The other portion was allowed to stand for 3 hours to ensure complete clotting. The clotted blood samples were centrifuged at 3000 rpm for 10 min and clear serum samples were aspirated off and stored frozen at -20° C for enzyme assays of the liver function. The livers were carefully dissected out, weighed and preserved for oxidative stress analysis.

**Determination of hematological parameters:** Red blood cells count (RBC), white blood cells count (WBC), hemoglobin (Hb), packed cell volume (PCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV) and platelets (PLT) were determined in whole blood with EDTA anticoagulant using Auto Hematology Analyzer and Light Direct Action Analytic. Made in China in Tumor Marker Oncology Research Center, Faculty of Pharmacy, Al- Azhar University.

**The biochemical parameters** were determined on the sera including aspartate aminotransferase (ASAT), alanine transaminase (ALAT) and alkaline phosphatase (ALP), using the Olympus 640 Chemistry AutoAnalyser, Tumor Marker Oncology Research Center. Faculty of Pharmacy, Al -Azhar University. Liver GSH level was assessed spectrophotometrically according to the method of **Beutler *et al.***<sup>17</sup>. GSH content was expressed in mg/g wet tissue. Liver lipid peroxidation (LPO) as thiobarbituric acid reactive substance (TBARS) was used to estimate malondialdehyde (MDA) content <sup>20</sup>. The study was approved by the Ethics Board of Al-Azhar University.

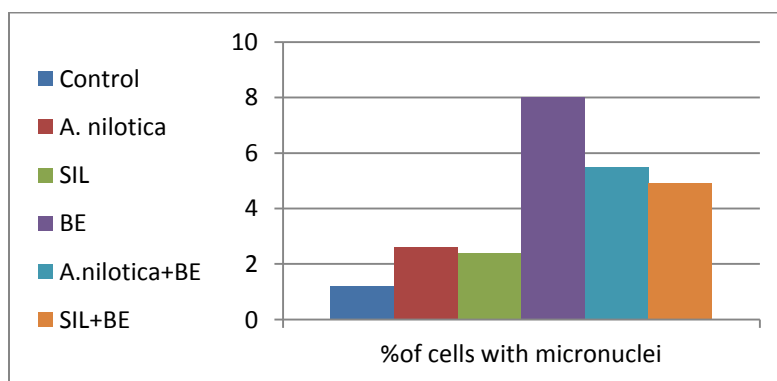
### 6- Statistical analysis

The data obtained in the present work were represented in tables as mean  $\pm$  standard error. Statistical analysis was carried out using Statistical Package for Social Science (SPSS) software version 18 for windows, using one-way analysis of variance (one-way ANOVA). Significant differences among groups were evaluated at  $P < 0.05$ .

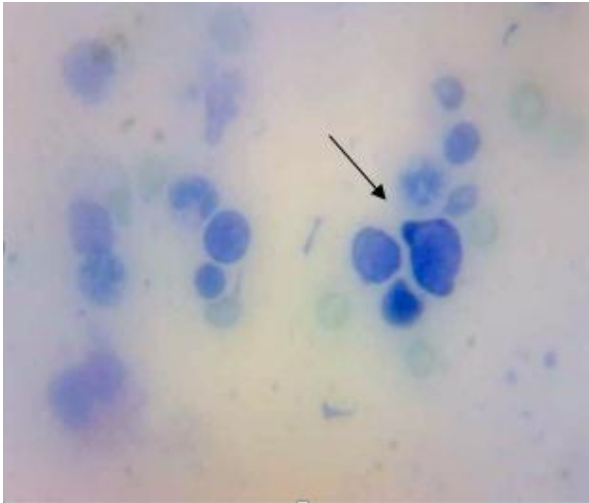
## RESULTS

### Micronucleus Test

The effect of 2-BE on micronucleus formation in bone marrow cell of male albino mice is summarized in figure 1. The results revealed that micronucleus formation in control mice was lower than those in all treated groups. Treatment mice with *A. nilotica* and SIL increased slightly micronucleus formation compared with control group. However, 2-BE increased significant the incidence of micronucleus compared with control group (fig. 1&2). On the other hand, the administration of *A. nilotica* or SIL significantly decreased micronucleus induced by 2-BE (fig. 1).



**Fig (1):** Micronucleus percentage in bone marrow cells in control and different treated groups of adult male albino mice.



**Fig (2):** Photomicrograph of bone marrow smear of male albino mouse after treatment by 2-BE (450 mg/kg .b.wt) for 28 days, *A.nilotica* (25mg/kg.b.wt) for 42 days and and SIL (antioxidant) (20mg/kg.b.wt) for 42 days showing micronucleus. (X:1500)

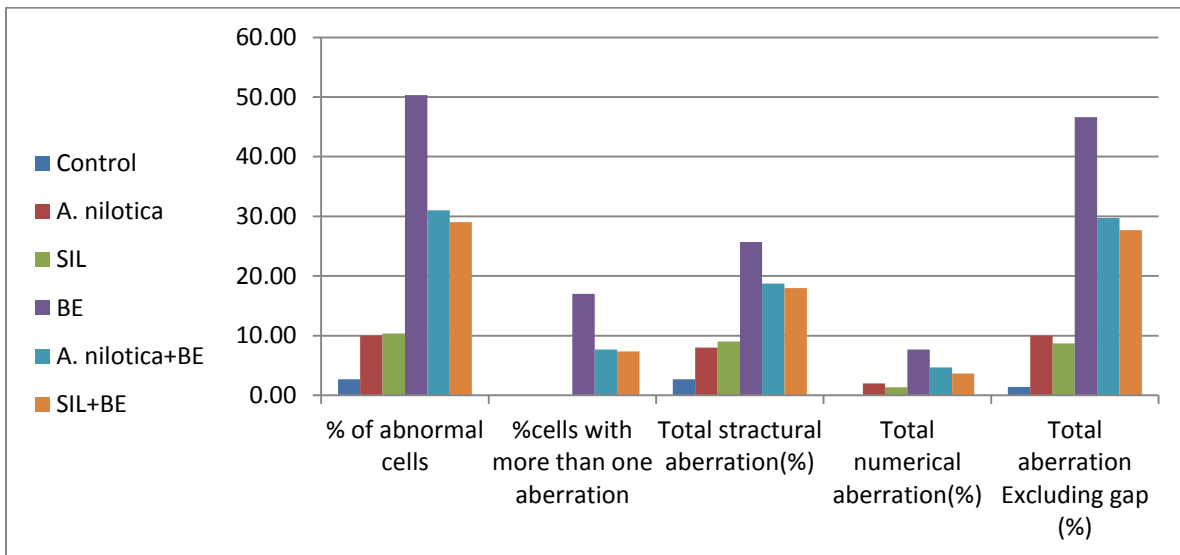
**Effect on chromosomal aberration**

In control group, the percentage of aberrant cells was 2.7. The percentage of aberrant cells in the group of animal treated with *A. nilotica* slightly increased when compared to control (10.0).

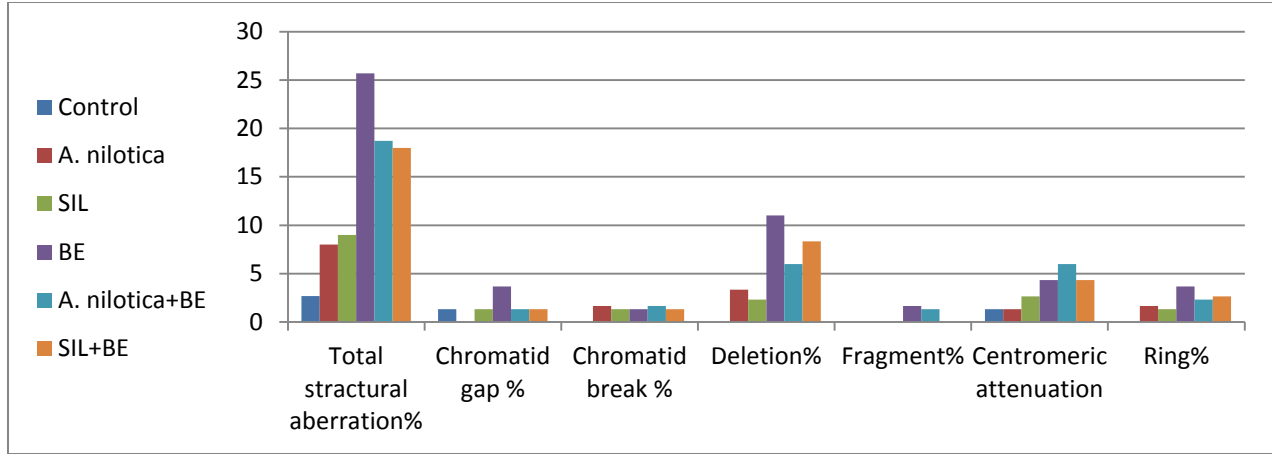
The group of animal treated with 2-BE showed highly significant increase in the means abnormal cells (50.3). The group of animal treated with *A. nilotica* and 2-BE, has shown a significant decrease in the number of cell carrying aberrations at a percentage of 31.0 (fig., 3). All types of chromosome aberration exhibited a decrease in number (fig., 4&5).

The effect of SIL on the percentage of abnormal cells caused increase in the percentage of chromosome aberration. It was observed at a level of 10.0(fig 3).

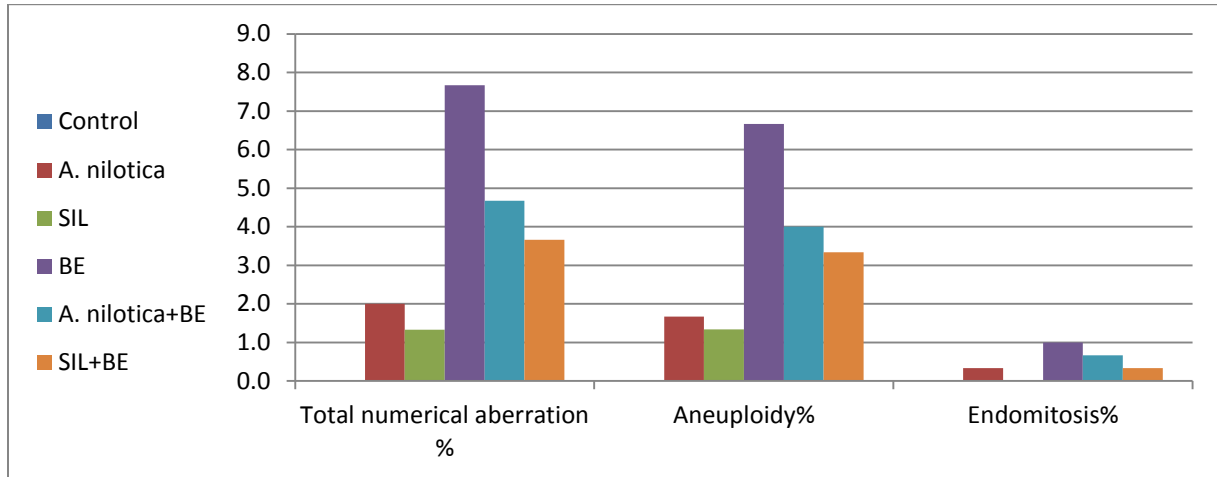
Treatment with SIL is effective against all types of chromosome aberrations appeared in mice treated with 2-BE (fig., 4&5). There is a significant reduction in the percentage of aberrant cells (29.0) as well as chromosome aberrations in the treated group compared with 2-BE group (fig., 3). The protective action of SIL is more effective than *A. nilotica*.



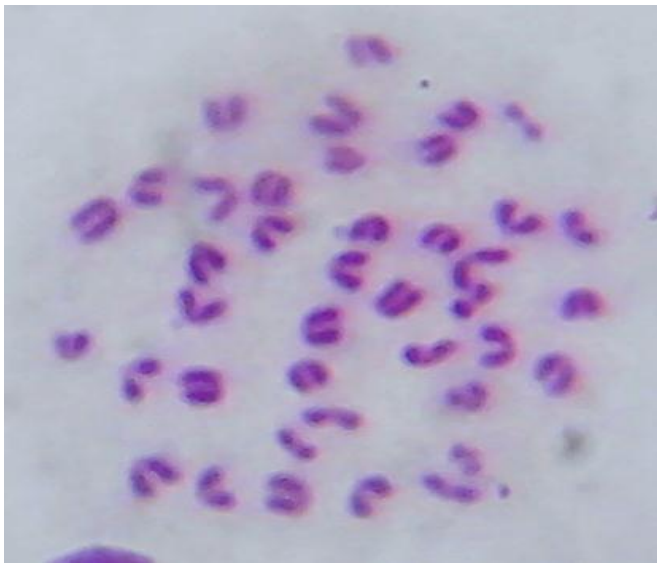
**Fig. (3):** Total chromosomal aberration percentage in bone marrow cells in control and different treated groups of adult male albino mice.



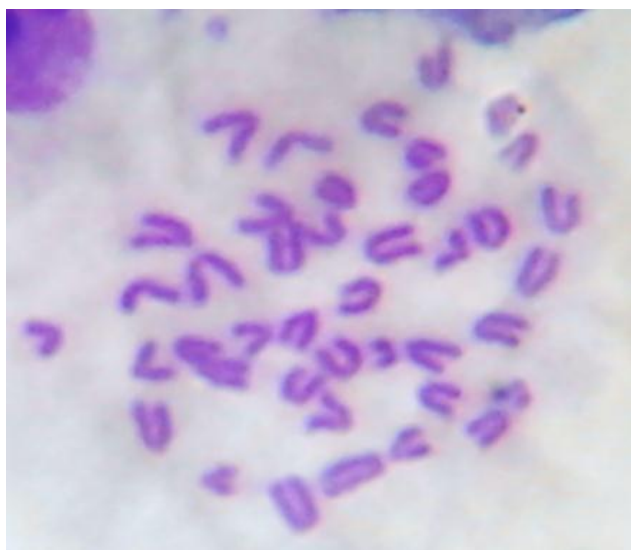
**Fig (4):** Structural chromosomal aberration percentage in bone marrow cells in control and different treated groups of adult male albino mice.



**Fig (5):** Numerical chromosomal aberration percentage in bone marrow cells in control and different treated groups of adult male albino mice.



**Fig (6):** Photomicrograph of metaphase chromosomes of the control male albino mouse *Mus musculus* showing the diploid chromosome number. (X:2500)



**Fig (7):** Photomicrograph of metaphase chromosomes of the of the male albino mouse orally treated with 2-BE (450 mg/kg .b.wt) for 28 days showing aneuploidy (41 chromosomes). (X:2500)

*A.nilotica* and SIL are found to reduce the percentage of chromosomal aberration and abnormal cells as they are a powerful antioxidant, they are able to scavenger reactive oxygen species (free radicals) formed by 2-BE in the cells, these free radicals damage DNA and hence cause defects in the chromosomes. The protective action of SIL is more effective than *A. nilotica*.

#### Effect on mitotic index

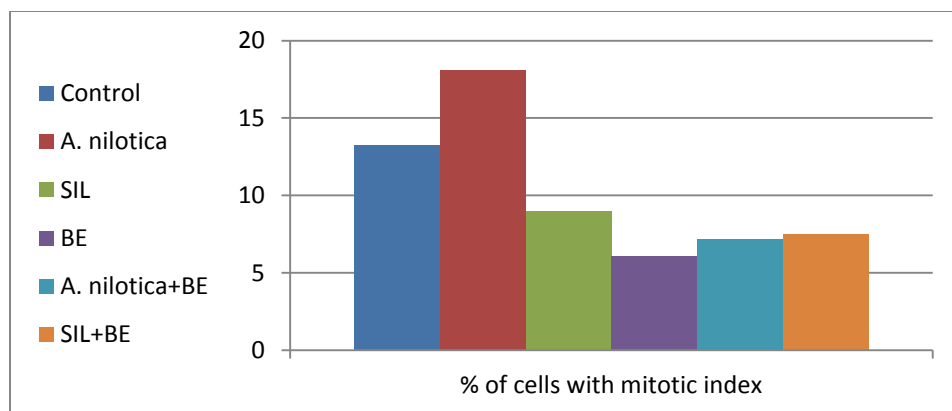
*Acacia nilotica* only induced an increase in the percentage of mitotic cell (18.1). Such percentage



**Fig (8):** Photomicrograph of metaphase chromosomes of the of the male albino mouse orally treated with 2-BE (450 mg/kg .b.wt) for 28 days showing: gap(black arrow) ,break (orange arrow) & deletion (red arrow). (X:2500)

was found to be significant compared with control group (13.2). However, the percentage of mitotic cell induced by 2-BE highly significant decreased (6.1). Administration of *A. nilotica* increased the percentage of mitotic cell in mice treated with 2-BE (7.2) (fig., 9). Silymarin is effective on the number of mitotic cell in mice at percentage of 9.0.

Silymarin is decreased mitotic cell when compared to control group. However, treatment with SIL significant increased the percentage of mitotic cell induced by 2-BE (7.5) (fig. 9).



**Fig (9):** Mitotic cells percentage in bone marrow cells in control and different treated groups of adult male albino mice.

#### 4- Hematological parameters

Mice treated with 2-BE had significant decreased RBCs count, Hb, Hct%, MCH and MCHC compared to control group.

*Acacia nilotica* alone decreased both RBCs and Hb after 42 days treatment, while SIL had no effect on the blood parameters as shown in table (1).

Co-administration of *A. nilotica* extract and standard drug SIL caused significant improvements in the RBCs count, Hb, PCV, MCH and MCHC in animals treated with 2-BE compared to 2-BE group table (1).

A significant increase in the total WBCs count was found in the mice treated with 2-BE when compared with the normal animals. Administration of *A. nilotica* extract reversed these changes and this was comparable with the results produced by the standard drug SIL table (1).

Plts decreased significantly in mice which were treated with 2-BE for 28 days in comparison with the control group. However, Plts increased in the 2-BE treated group supplemented with *A. nilotica* extract and SIL when compared to the 2-BE group table (1).

**Table (1):** RBCs count, Hb concentration, Hct%, blood indices, White blood cells (WBCs) counts and Platelets count (PIT) in control and different treated groups of adult male albino mice

Groups	RBCs (x10 <sup>6</sup> cell/mm <sup>3</sup> )	Hb(g/dl)	Hct(%)	MCV (um <sup>3</sup> )	MCH(pg)	MCHC(g/dl)	WBCs (x10 <sup>3</sup> /mm <sup>3</sup> )	Plt (x10 <sup>3</sup> /mm <sup>3</sup> )
Control	9.37±	15.08±	43.33±	46.5	16.2	34.33±	5.25±	1152.67
	0.12	0.07	0.88	±0.92	±0.12	0.67	0.16	±14.87
<i>A. nilotica</i>	8.80±	13.78±	42.33±	47.5	15.28	32.00±	5.22±	1137.83
	0.09 <sup>ab</sup>	0.17 <sup>ab</sup>	1.12 <sup>b</sup>	±1.23	±0.18 <sup>ab</sup>	0.58 <sup>ab</sup>	0.12 <sup>b</sup>	±11.26 <sup>b</sup>
SIL	9.07±	14.53±	41.33±	46.5	16.08	34.83±	5.93±	1166.5
	0.08 <sup>b</sup>	0.19 <sup>ab</sup>	0.76 <sup>b</sup>	±0.85	±0.23 <sup>b</sup>	0.48 <sup>b</sup>	0.25 <sup>b</sup>	±13.80 <sup>b</sup>
BE	7.63±	10.68±	37.00±	48.17	13.72	28.17±	17.50±	944.67
	0.11 <sup>a</sup>	0.07 <sup>a</sup>	0.45 <sup>a</sup>	±0.70	±0.23 <sup>a</sup>	0.17 <sup>a</sup>	0.96 <sup>a</sup>	±15.57 <sup>a</sup>
<i>A. nilotica</i>	8.23±	12.35±	40.00±	48.83	14.97	31.50±	8.92±	1053.33
+BE	0.08 <sup>ab</sup>	0.1 <sup>ab</sup>	0.77 <sup>a</sup>	±1.40	±0.20 <sup>ab</sup>	0.62 <sup>ab</sup>	0.29 <sup>ab</sup>	±19.52 <sup>ab</sup>
SIL+BE	8.57±	13.10±	39.33±	45.33±	15.18	33.17±	8.95±	1038.67
	0.07 <sup>ab</sup>	0.12 <sup>ab</sup>	1.20 <sup>a</sup>	1.56	±0.26 <sup>ab</sup>	0.75 <sup>b</sup>	0.24 <sup>ab</sup>	±25.05 <sup>ab</sup>

#### 5- Liver functions

As shown in table (2), serum liver enzymes including ASAT, ALAT and ALP had significant increases in mice treated with 2-BE compared to control group. Co-administration of *A. nilotica* extract or SIL to 2-BE treated mice decreased serum ASAT, ALAT and ALP activities compared to 2-BE-toxicated group.

The effect of different treatments on liver GSH and MDA activities are represented in table (3).

The GSH content in 2-BE- intoxicated mice was found to be minimum (1.86± 0.03 mg /g wet tissue) compared to control group (2.78±0.04 mg/g

wet tissue). In co-administration of *A. nilotica* extract and standard drug SIL treated animals with 2-BE groups, the liver GSH level were significantly (p<0.05) increased compared to 2-BE toxicity group.

The liver MDA level in the 2-BE toxicated mice was found to be maximum (2.84± 0.04 nM/ g wet tissue) compared to control group (0.74± 0.03nM/ g wet tissue). In 2- BE- toxicated mice and treated with extract of *A. nilotica* and standard drug, the liver MDA was decreased to 1.14± 0.03 and 0.75± 0.04 nmol/g wet tissue respectively compared to 2-BE-toxicated group.

**Table (2):** Serum aspartate amino transaminase activity (ASAT), serum alanine amino transaminase activity (ALAT) and serum alkaline phosphatase ALK in control and different treated groups of adult male albino mice.

Groups	ASAT (U/L)	ALAT (U/L)	ALP (U/L)
Control	59.83±2.77	21.17±0.31	71.67±1.65
<i>A. nilotica</i>	49.50±1.12 <sup>ab</sup>	21.00±1.00 <sup>ab</sup>	65.50±1.86 <sup>ab</sup>
SIL	59.33±2.74 <sup>b</sup>	18.17± 0.79 <sup>ab</sup>	69.50±1.41 <sup>b</sup>
BE	126.67±1.52 <sup>a</sup>	40.67±1.09 <sup>a</sup>	116.67±1.94 <sup>a</sup>
<i>A. nilotica</i> +BE	57.33±2.60 <sup>b</sup>	19.67±1.23 <sup>b</sup>	70.50±1.82 <sup>b</sup>
SIL+BE	78.00± 0.89 <sup>ab</sup>	25.67± 1.12 <sup>ab</sup>	66.00± 1.90 <sup>ab</sup>

**Table (3):** Glutathione peroxidase antioxidant (GSH) and lipid peroxidation end product (MDA) in control and different treated groups of adult male albino mice.

Groups	GSH (mg/g.wt.tissue)	MDA (nmol/g.wt.tissue)
Control	2.78± 0.04	0.74±0.03
<i>A. nilotica</i>	2.71±0.02 <sup>b</sup>	0.82±0.02 <sup>b</sup>
SIL	2.75±0.05 <sup>ab</sup>	0.74±0.04 <sup>b</sup>
BE	1.86±0.03 <sup>a</sup>	2.84±0.04 <sup>a</sup>
<i>A. nilotica</i> +BE	2.52±0.05 <sup>b</sup>	1.14±0.03 <sup>ab</sup>
SIL+BE	2.66±0.02 <sup>b</sup>	0.75±0.04 <sup>b</sup>

## DISCUSSION

Genotoxicity could be proposed to be through excessive and persistent formation of ROS inducing lipid peroxidation and decrease endogenous antioxidants in the body such as reduced superoxide dismutase and glutathione peroxidase (GSH-Px)<sup>18</sup>. There are, also, associated various gene expression changes, some of which may be responsible for oxidative stress<sup>19</sup>.

In this study, 2-BE produced oxidative stress in the liver of male mice as evidenced by enhanced levels of Lipid peroxidation (MDA) accompanied by a decrease in antioxidant glutathione peroxidase (GSH-Px).

There are several studies reporting that 2-butoxyethanol causes cellular damage via formation of ROS. ROS are believed to cause lipid peroxidation resulting in damage to biological membranes<sup>20,21,22</sup>. It appears that ROS induced by 2-BE may result from Kupffer cell activation secondary to 2-butoxyethanol-induced hemolysis and subsequent hepatic iron deposition<sup>23;20</sup>. The mode of action of 2BE involves the hemolysis of RBCs, since it induced haemolytic anaemia in rats at dose of 450 mg/kg<sup>24</sup>.

Hemolysis results in iron deposition which causes reactive oxygen species production resulting in oxidative damage, these factors may then produce oxidative DNA damage<sup>23</sup>. The oxidative stress may cause DNA, protein, and/or lipid damage, leading to changes in chromosome instability, genetic mutation, and/or modulation of cell growth that may result in cancer<sup>25</sup>. Seven-day 2-BE treatment leads to erythropoiesis in the BM were identified, including hypoxia, leading to increased Erythropoietin (Epo). A role for Epo signaling that, coupled with decreased genomic stability in hematopoietic organs (spleen and bone marrow) and increase the probability of spontaneous mutations of this cell type<sup>26,27</sup>. The present study showed that 2-BE induced high and statistically significant percentage of chromosome aberrations in bone marrow cells of male mice. 2-BE induced numerical aberrations (Aneuploidy) and structural aberrations (chromatid gap, chromatid break, deletion, fragment, centromeric attenuation, and ring).

This in agree with the data published by Hanafy *et al.*<sup>28</sup> stated that 2-BE induced high significant percentage of chromosome aberrations in mouse bone marrow cells of male mice. The mean



percentage of chromosomal aberrations was found to be dose dependently increased as the concentration of 2-BE (450µl/kg b.wt and 900µl/kg b.wt). The available studies on mice treated with 450µl/kg b.wt of 2BE showed 36.8% elevation in abnormal cell percentage than normal animals 3.6%, while mice treated with 900µl/kg b.wt of 2BE showed 64.4%.

2-Butoxyethanol induced structural CAs dose-dependently, including chromatid and chromosome gaps; chromatid breaks; ring chromosome; chromosome breaks; deletion; fragmentation and centromeric attenuation.

**El-Zein *et al.***<sup>29</sup> suggested that exposure to 2-BE (EGME) in utero could result in terminal chromosome rearrangements and shortening of telomere length, leading to the observed dysmorphic features and idiopathic mental retardation.<sup>30</sup> demonstrated a significant CAs frequency increase in painters exposed to organic solvents. Organic solvent extracts induced structural CAs dose-dependently, including chromatid and chromosome gaps; chromatid breaks; chromatid exchanges; fragmentation; chromosome breaks; chromosome exchange<sup>31</sup>. **El- zein *et al.***<sup>32</sup> provide that human exposure to 2-Butoxyethanol (ethylene glycol monobutyl ether) resulted in a significantly higher level of chromosomal aberrations and a higher percentage of aberrant cells in the in-utero-exposed subjects than in their matched controls. They had a significantly higher frequency of breaks, polyploid and endoreduplicated cells and resulted in the development of specific congenital anomalies.

In the present study, 2-Butoxyethanol induced significant increased percentage of micronuclei in mouse bone marrow cells of male mice. Micronuclei are formed from chromosome fragments and/or whole chromosomes lagging behind in anaphase, which are not integrated into the daughter nuclei<sup>33</sup>. Higher level of apoptosis implies a low recovery of viable mutants (affecting MN and CA frequency) and consequently acts as a protection against genomic instability<sup>34</sup>.

These results provide the fact that hypoxia can drive genetic instability stems from experiments that documented induction of common fragile sites in hypoxic cells. These sites are highly unstable in the human genome and are prone to chromosomal breakage and rearrangement<sup>35,36</sup>. Chromatin responses to hypoxia include methylation of histones, phosphorylation of histone ( $\gamma$ H2AX) and

premature condensation of chromosomes and abnormal chromosome mis-segregation<sup>37, 38,39,40</sup>.

The results of the present study showed that the percentage of mitotic cell was significantly decreased in animals treated with 2BE.<sup>41</sup> stated that Exposure 2-BE resulted in genetic instability characterized by a delay in cell division. The detection of polyploidy (which corresponds to an exact multiple of the haploid number of chromosomes) and endoreduplication (in which at mitosis the chromosome consists of 2 doubled chromatids instead of 2 single chromatids) in the individuals (exposed to 2-butoxyethanol), indicating defects in the G1/S and G2/M checkpoints of the cell cycle.

Genetic instability can arise as a function of hypoxia mediated resistance to apoptosis and decreased DNA repair, leading to increased rates of mutagenesis and altered chromatin biology<sup>42</sup>. Cells exposed to prolonged chronic hypoxia can also acquire genetic instability through decreased translation of DNA repair proteins leading to defective repair in proliferating cells and an increased mutation rate<sup>43</sup>.

Chromosomal aberrations in lymphocytes are an intermediate end point in carcinogenic progress and are the best validated cytogenetic biomarker to predict cancer risk<sup>33, 42, 44, 45</sup>. 2-BE induces liver hemangiosarcomas in male mice, increases the level of 8-hydroxyguanosine (8-OHdG) in mouse liver<sup>23, 20</sup>. Numerous studies have demonstrated that 8-OHdG levels are elevated in various human cancers<sup>46,47, 48</sup> and in animal models of tumors<sup>49</sup>.

Therefore our study gives a better cancer risk insight associated to organic solvents exposure in order to avoid future cancers. CAs frequency increase results are consistent with previous studies, in out-door and in-door painters occupationally exposed to organic solvents<sup>50, 51, 52</sup>.

**Amal *et al.***<sup>53</sup> documented that several OSs are potent carcinogens among population at risk. Their genetic effects have important implications for cancer induction.

Chemopreventers often have preferentially an antioxidant activity; however, they are also able to exert anti-proliferation and anti-inflammation actions. Indeed, they can directly modulate several proteins that are involved in cell cycle and cellular homeostasis and whose deregulation can play a role in carcinogenesis, such as p53, p73, p21, Bax, Bcl-2,

COX-2, NF- $\kappa$ B, catalase, glutathione (GSH)-peroxidase<sup>54, 55</sup>.

The antioxidant activity of chemopreventers is nowadays gaining more importance because of the observations, both *in vitro* and *in vivo*, that the deregulation of free-radical homeostasis can be involved in carcinogenesis<sup>56</sup>.

*Acacia nilotica* (Linn.) gum, flower and leaf extract may play an additional and synergistic antimutagenic role in the presence of tannins (ellagic acid, gallic acid and tannic acid), crude protein, crude fiber, arabin, calcium, magnesium, selenium, stearic acid with three phenolic components (viz. Kaempferol-3-glucoside, isoquercetin, leucocyanidin) Vit.C (ascorbic acid), carotene, alkaloids and saponins. *A. nilotica* showed a marked anti-mutagenic effect. Furthermore, it was more effective against indirect acting mutagens<sup>8</sup>.

In the present study, *A. nilotica* was effective against the frequency of micronuclei appeared in mice treated with 2BE. There was a significant reduction in the percentage of aberrant cells as well as chromosome aberrations in the treated group compared with 2BE. These results in agreement with **Meena et al.**<sup>57</sup> found that a significant reduction in the frequency of micronuclei was observed in mice treated by oral gavage with the aqueous extracts of *A. nilotica*. There is also a significant decrease in total chromosomal aberrations in the form of chromatid breaks, chromosome breaks, centric rings, dicentrics, acentric fragments and exchange as compared with the group given 7, 12-dimethylbenz (a)anthracene (DMBA). **Ghaly and Hanafy**<sup>58</sup> showed that *A. nilotica* was effective against all types of chromosome aberrations appeared in mice treated with sodium arsenate. There was a significant reduction in the percentage of aberrant cells as well as chromosome aberrations in the treated group compared with arsenic group. The administration of *A. nilotica* significantly decreased DNA fragmentation induced by sodium arsenate.

**Chang et al.**<sup>59</sup> indicate that both bark and heartwood extracts of *Acacia confusa* clearly have strong antioxidant effects. Furthermore, the heartwood extract protected PhiX174 supercoiled DNA against strand scission induced by ultraviolet photolysis of H<sub>2</sub>O<sub>2</sub>, and it reduced the amounts of intracellular hydrogen peroxide, a reactive oxygen species, when it was co-incubated with human promyelocytic leukemia (HL-60) cells under

oxidative stress. **Bouhleb et al.**<sup>60</sup> showed that *Acacia salicina* extracts decreased the DNA damage caused by H<sub>2</sub>O<sub>2</sub>.

The present work revealed that SIL was effective against all types of chromosome aberrations appeared in mice treated with 2-BE. There was a significant reduction in micronucleus and the percentage of aberrant cells as well as chromosome aberrations in the treated group compared with 2-BE. Also SIL significantly increased the percentage of mitotic index induced 2-BE. **Khatab**<sup>13</sup> found that SIL has anticlastogenic activity upon somatic as well as germinal cells as shown as the analysis of chromosomal aberration; micronucleus test; and analysis of diakinesis stage in mice primary spermatocytes. It has the capability to lower the induction of sister chromatid exchanges, giving an evidence that it plays an important role in repairing primary DNA damage.

Effects of 2-BE on the blood appear to be the most sensitive parameter in experimental animals following acute, short-term, subchronic or chronic exposure via oral, inhalation and dermal routes, based on an extensive database<sup>20</sup>. It is clear that RBCs are a target for 2-BE and this causes a series of other types of organ toxicity, including damage to the liver, kidney, spleen, and other organs. Alterations in haematological parameters that are consistent with haemolytic anaemia have repeatedly been observed in multiple species, including mice and rats<sup>61</sup>. The same results were recorded in the present study in male albino mice indicating macrocytic hypochromic anemia.

The liver has the fundamental role of deactivating all substances produced by toxic substances<sup>21</sup>. The liver neoplasms in male mice do not seem to be caused by 2-BE directly, but indirectly via its hemolytic effects. 2-BE acts hemolytically in rodents, producing iron deposits in the liver. On the one hand, iron can form ROS via Fenton or Haber-Weiss reactions, which can result in tumour formation via LPO or oxidative DNA damage. On the other hand, excess iron can activate Kupffer's cells, and thus release ROS and other biologically active molecules such as cytokines, which can also contribute to carcinogenesis<sup>23</sup>.

Enzymes ASAT and ALAT were found in serum and various body tissues but are mostly associated with liver parenchymal cells. The elevated activities of ASAT and ALAT were observed in acute liver damage condition. In

addition, the level of ALP rises with intrahepatic cholestasis and infiltrative diseases of the liver<sup>62</sup>. The leakage of large quantities of enzymes into the blood stream was associated with centrilobular necrosis of the liver. Similarly in the present study, increases in serum enzyme activities ASAT, ALAT and ALP after exposure to 2-BE were observed. Also, increases in liver enzymes in serum following 2-BE administration has earlier been reported<sup>63</sup>. The activities of these enzyme levels have been restored up to normal range by both *A. nilotica* and SIL treatment indicating its hepatoprotective action. The reliable criteria for judging the quality of any hepatoprotective drug are to preserve the normal hepatic physiological functions that have been disturbed by hepatotoxin<sup>64</sup>.

In this study, 2-BE produced oxidative stress in the liver of male mice as evidenced by enhanced levels of MDA accompanied by a decrease in antioxidant GSH. Depletion of GSH has been shown to be associated with enhanced toxicity to chemicals, including acetaminophen<sup>65</sup>. The results revealed that *A. nilotica* leaves extract exhibited antioxidant capacity manifested by decreasing MDA and increasing GSH in the liver tissue. This may be attributed to its antioxidant activity by promoting formation of the reduced forms of other antioxidants such as ascorbic acid and detoxification of xenobiotics, carcinogens and free radicals and maintenance of immune functions<sup>66</sup>. Similar observations have been made by<sup>67</sup>. They also reported a decrease in LPO and an increase in GSH as one mechanism underlying anticarcinogenic properties of ellagic acid<sup>57</sup>. MDA appears during peroxidation of biological membrane polyunsaturated fatty acids. The estimation of level of MDA is a measure of alterations and damage in structure of cellular membranes<sup>68</sup>. In the present study, increased levels of MDA in liver tissue of mice treated with 2-BE suggested enhanced LPO leading to tissue damage and failure to prevent formation of excess free radicals. Treatment with *A. nilotica* extract and SIL significantly reversed these changes. Also, the hepatoprotective effects of *A. nilotica* undoubtedly indicates that the treatment with *A. nilotica* was effective on inhibiting the hepatotoxicity induced by 2-BE in albino mice, most likely because of high content of flavonoids, alkaloids, phenolics, steroids, terpenoids, saponins, and tannins and may be due to synergistic action of specific constituents present in the extracts such as

umbelliferone, gallic acid, niloticane, and kaempferol. On the other hand, SIL might improve the liver enzymes activities and oxidative stress by decreasing the iron precipitation in the kupffer cells since it was previously used to treat iron overloading and thalassemia<sup>69</sup>. Also, the hepatoprotective effects of *A. nilotica* and SIL may be due to the modulation of antioxidant enzymes activities and inhibition of LPO and CYP2E1 activation<sup>70</sup>. These results collectively suggested that the imbalanced antioxidant system in liver treated with 2-BE is normalized by the protective effect of *A. nilotica* extract and SIL.

In conclusion, People who have to use organic solvents as a part of their daily work are experiencing the effects of 2-butoxyethanol exposure. *A. nilotica* and SIL were found to be very excellent protective plant extract and antioxidants protect against free radicals formed as a result of metabolism of 2-BE, these free radicals that harm genetic material leading to dangerous diseases on the long duration. *A. nilotica* and SIL were found to reduce the frequency of chromosome aberrations and they limit the number of micro-nucleated cells. The protective action of SIL is more effective than *A. nilotica*.

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