



## Cytogenetic and cytotoxic evaluation of *Quercus infectoria* extract in somatic and germ cells of male albino mice

Bushra M.A. Mohammed<sup>1</sup> and Dilger M. Khdr<sup>2</sup>

<sup>1</sup>Department of Biology, College of Science, University of Duhok, Duhok, Kurdistan Region of Iraq; <sup>2</sup>Department of Animal resource, Agriculture College, Salahaddin University, Erbil, Kurdistan Region of Iraq

### Abstract

Aqueous extract of *Quercus infectoria* was used to investigate the cytotoxicity and possible damaging effects on genetic material on somatic and germ cells of Swiss albino mice. Three sub lethal doses 0.075, 0.037 and 0.018 mg/kg body weight were evaluated for preliminary toxicity. With respect to the extract toxicity, mice exhibited some behavioral change like loss of appetite, restlessness, diarrhea, loss of body weight and temporary blindness. The doses (0.075, 0.037, 0.018 gm/kg b.w) exhibited significant mitotic arrest in a dose dependent manner when administered to mice as a single subcutaneous injection per day, while extending treatment periods (24, 48 and 72 hrs) did not show any significant increase in mitotic index percentage. The treatment of a single subcutaneously injections per day of the three doses of *Q. infectoria* extract for three consecutive days induced significant different types of chromosome aberrations. The most types of aberrations were chromatid breaks and chromoasome breaks. The extending of treatment period (24, 48 and 72 hr) did not show any significant increase in the number of aberration. Abnormal mice sperm morphology was examined thirty five days after the first extract injection. The result revealed that the leaves extract showed a significant induction of sperm abnormalities at the three concentrations used compared with the untreated animals. The most frequent were amorphous head, pin head, hooked sperm, long and broad hook, coiled tail defect, bent midpiece defect, corkscrew defect, psudrobot defect, double tail and sperm without head. This result suggests that a mixture of compound found in the aqueous extract could cause the cytotoxicity and genotoxicity in both somatic and germ cells of male albino mice.

**Key words:** *Quercus spp*; mutagenicity; toxicity

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### Introduction

Oak tree is one of the important trees, distributed in many regions of temperate zone in the world. Oak forest community in Kurdistan region of Iraq is one of the important trees distributed in northern and north-eastern mountainous part of the country. The species of oak tree (*Quercus genus*), are classified under the family Fagaceae. Four species of oak trees are grown in Iraqi Kurdistan Forest: *Q. aegilop*, *Q. infectoria*, *Q. libani* and *Q. Marcantherea* (Chapman, 1994). Acorn and leaves of *Quercus* additionally provide fodder for animals, food for wildlife and wildstock (Cypert and

Burton, 1948). Acorn have been used as food by human beings for thousands of years virtually every where oak is found (Bainbridg, 1986). With few exceptions, forest areas in the north of Iraq are grazed over by flocks of sheep, goats, and cattle. The villagers at the previous areas have the habit of pollarding the oak forest for the supplying of foliage used in winter as flock fodder.

The potential toxicity of oak production has long been recognized and managed in most part of the world, although we are unable to find any information available or published literature on the cytotoxicity and/or genotoxicity of *Quercus spp*. in farming animals in Iraq.

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**Corresponding author:** Bushra M. A. Mohammed, Department of Biology, College of Science, University of Duhok, Duhok, Kurdistan region of Iraq

Moreover, there is no attempt made to manage Oak trees for the provision of fodder for ruminant livestock. Therefore, the present study is the first to focus on the risk of utilizing oak trees productions as a seasonal feeds. In this study, we choose *Q. infectoria* as an example of oak trees, because it is widely available and it is a small tree with leave and acorn which are easy to consume by cattle, sheep, goat and horses.

## Materials and Methods

Healthy, disease free, mature leaves of *Quercus infectoria* was harvested by hand locally from Qalasnj village on Safeen Mountain in Erbil governorate in September and December 2010. The leaves were randomly removed from branches, air dried in the shade to minimize changes in tannin content and activity (Makkar and Singh, 1991). The plant was identified by Professor Saleem Shahbaz, Duhok University, Faculty of Agriculture, Department of Forestry. Then a modified method of Muskazli et al. (2008) was used for the preparation of the aqueous extract of *Q. infectoria*. The dried leaves of the plant were ground to a fine powder using electric grinder and then the powder were suspended in distilled water for 12 hours, at the rate of 30 gm/200 ml. The obtained extract has been twice filtered through Whatman No. 42 filter paper and a clean brown filtrate was obtained, the filtrate was concentrated to semi dryness under reduced pressure and controlled temperature (37°C) using rota evaporator, a deep brown colored residue was obtained, the residue was incubated in a clean Petri dish overnight at 37°C to obtain about 25% yield of extract. Finally the extract was kept in a dark closed container at (-4°C) and was used during 1-5 days.

Experiment was carried out on 8-10-week-old male Swiss albino mice (*Mus musculus*), weighing 25-30 g. They were maintained in a controlled atmosphere of 12 h dark/light cycle, 25±2°C temperature with 50-70% humidity and had free access to food and water supply. The animals were kept in the animal house of Agriculture College, University of Salahaddin. An extract of *Q. infectoria* leaves was administered in a single dose of 1 ml subcutaneously, at concentrations of 0.075, 0.037 and 0.018 g/kg/day, chosen on the basis of LD50 value. The negative control group received 1 ml phosphate buffer saline (PBS). The positive control group received subcutaneous injection of Gemcitabine at 15 mg/kg for bone marrow cells experiments and 10 mg/kg for sperm morphology assay. The Swiss albino mice were injected subcutaneously with 0.5 ml of 10 mg/kg colchicin 2-3 hrs, prior to sacrificing after each injection. The LD50 was determined using the classical LD50 method Behrens and Karbers (1953). Graded single doses of *Q. infectoria* 2, 1, 0.5, 0.25, 0.125 g/kg of body weight were administrated subcutaneously to

five groups of mice, each of which consisted of six mice. Mortality was recorded after 24 hours. The mitotic index (MI) was obtained by counting the number of mitotic cells in the 1,000 cells analyzed per animal. Bone marrow from the femur bones were used for the analysis of chromosome aberrations in metaphase cells, using the technique of Evans and Ford (1964). One hundred metaphases per animal (600 metaphases per group) were analyzed in order to determine the number of chromosomal aberrations for this test. The *Q. infectoria* extract was administered daily for three consecutive days and the animals were sacrificed by cervical dislocation at 24, 48 and 72 hrs after the third medication. For cytological analysis of spermatozoa abnormalities, the mice were sacrificed five weeks after the last day of sub-acute treatment (Wyrobek, 1979). The spermatozoa were obtained by making small cuts in epididymis and vas deferens and were placed in 1 ml of normal saline. The sperm suspension obtained was stained with 0.05% of eosin-Y. The spermatozoa morphology was examined by bright-field microscopy with an oil immersion lens. At least 600 sperms were counted from each animal to determine sperm morphology abnormalities. The method of WHO (1998) was used to determine the total amount of tannins in *Q. infectoria* leaves.

## Statistical analyses

The data obtained were submitted to the statistical program SAS (2005). Sperm abnormalities and mitotic index were analyzed using Completely Randomized Design (CRD). Chromosome Aberrations were analyzed using factorial design. P value less than 0.05 was considered statistically significant.

## Results and Discussion

After 24 hrs of the subcutaneous administration of graded single dose/day of a series of concentration of the aqueous extract of *Q. Infectoria* in albino male mice, the dose 0.25 gm/kg produced 33.33% mortality which was increased to 100% mortality at the dose of 2gm/kg (Table 1). The experimental calculated LD<sub>50</sub> value in this study was 0.75 gm/kg body weight. The cytotoxicity effect of different concentration of *Q. infectoria*, periods and their interaction on mitotic index is shown in Table 2. There were significant differences (P<0.05) among the three experimental doses, and also there were significant effect between the doses of the aqueous extract of *Q. infectoria* and the untreated control on mitotic percentage, all the treated doses had significant inhibition of mitotic index rate when compared to negative control. However, there was no significant effect between time period (24, 48, 72 hr). In contrast, the interaction between doses and periods had significant inhibition (P<0.05) on mitotic index value

for the three doses for different periods as shown in Table 2. In order to compare the toxicity effects of *Q. infectoria* extract with other reports, Watthanachaiyingcharoen et al. (2009) used the dried galls of *Q. infectoria* to test the acute toxicity in Whistar rat and Swiss albino mice. *Q. infectoria* galls extract showed high toxicity at LD<sub>50</sub> (8.82 mg/ml) within 24 hr after administration. The main constituents found in the galls of *Q. infectoria* which can cause growth inhibition to microorganisms are tannins (50-70%) beside the small amount of tree gallic acid and ellagic acid (Basri and Fan, 2005). Another investigation of the cytotoxicity of *Q. infectoria* extract was reported by Hasmash et al. (2010). They also found that the extract inhibits various functions of macrophages and neutrophils relevant to inflammatory responses. They separated flavonols as the major compound in this plant and could inhibit cell proliferation *in vitro*. Moreover, Iwashita et al. (1981) revealed that flavonoids such as kaempferol and

quercetin could induce apoptosis in B16 mouse melanoma 4A5 cells. Also Spencer et al. (2003) showed that quercetin have cytotoxic effects, such as induction of apoptosis and cell cycle arrest beside the antiproliferative effects. In addition to flavonols cytotoxicity, many other reports were found advocating the toxic principle of oak is tannins which are naturally occurring in plant polyphenol found in all species of oak (Markker, 2003). Feeng and Bostock (1968) in their investigation of the total tannin content from a single tree of *Q. robur* found that the total tannin content increased from less than 1% in early spring to 5% in late summer, the increase being largely due to an increase in the level of condensed tannin (Nunez-Hernandez et al., 1989). Holechek et al. (1990) found that the total amount of tannin 7.9% in the immature leaves of *Q. grisea* and 5.4% in its mature leaves. Ghafour et al. (2010) determine tannin concentration among the acorn, pericarp and cupules of Iraqi oak and observed that these parts of *Q. infectoria* accumulated 66.492 mg/g level of tannin, although the result of HPLC revealed the presence of ellagic acid (5.168 mg/g) in cupules of *Q. Infectoria*.

To the best of our knowledge, no data exist on the level of tannin in the leaves of *Q. infectoria* to be compared with and concerning the total tannin amount in this study which could cause cell cytotoxicity. We may suggest that there might be other secondary compound share tannins causing cytotoxicity and genotoxicity on mice somatic and germ cells. Total amount of tannin have been associated with animal toxicity in northern California in April 1985, an estimated of 2700 cattle died from oak toxicity. The toxic principles have not been definitively established but occur when cattle ingest immature leaves or freshly fallen acorn which contains the highest levels of tannic acid (Spier et al., 1987). Moreover, tannins occur in shin oak bark has been reported to depress growth and cause toxicity of cattle, rabbits, rat and chick (Jonslyn and Glick, 2011). Both leaves and acorn especially spouted acorn contain the toxin and toxicity is not diminished by freezing or drying. However, all chemicals in oak that may cause toxicity have not been clearly identified (Doce et al., 2007). In fact very rarely have the tannins actually present been isolated, characterized and fed to experimental animals such as mice (Jonslyn and Glick, 2011).

The effect of *Q. infectoria* aqueous extract on different types of chromosome aberrations is shown in Table 3. The result in this table shows a significant (P<0.05) effect of different doses of *Q. infectoria* extract on chromosomal aberration such as chromatid breaks, chromosome breaks and centromeric breaks. The data in Table 3 reveals significant differences in the total number of aberrations at the doses 0.075 gm/kg and 0.037 gm/kg. The least values of aberration

**Table 1: The Acute toxicity of *Quercus Infectoria* aqueous extract in Male Albino Mice**

Plant extract Doses gm/kg	Number of Mice	Number of dead Mice	Percent of mortality
2	6	6	100
1	6	4	66.66
0.5	6	3	50
0.25	6	2	33.33
0.125	6	0	0

**Table 2: The Genotoxic effects of various doses of *Q. infectoria* on mitotic index frequency in bone marrow cells of male albino mice**

Parameter Factors	Percentage of mitotic index (%)
Treatment	
PBS (Negative Control)	15.10±0.06 <sup>a</sup>
Gem (Positive Control) 15 mg/kg	9.00±0.136 <sup>e</sup>
A.E. 0.075 gm/kg	10.82±0.150 <sup>d</sup>
A.E. 0.037 gm/kg	11.53±0.210 <sup>c</sup>
A.E. 0.018 gm/kg	12.20±0.31 <sup>b</sup>
Time	
24 hours	11.66±0.54 <sup>a</sup>
48 hours	11.66±0.56 <sup>a</sup>
72 hours	11.86±0.56 <sup>a</sup>
Interaction	
PBS (Negative Control)	15.10±0.12 <sup>a</sup>
Gem. (Positive Control) 15mg/kg	9.00±0.25 <sup>d</sup>
A.E. 0.075 gm/kg (24h)	10.73±0.27 <sup>c</sup>
A.E. 0.075 gm/kg (48h)	10.80±0.36 <sup>c</sup>
A.E. 0.075 gm/kg (72h)	10.93±0.27 <sup>c</sup>
A.E. 0.037 gm/kg (24h)	11.66±0.34 <sup>bc</sup>
A.E. 0.037 gm/kg(48h)	11.06±0.32 <sup>c</sup>
A.E. 0.037 gm/kg (72 h)	11.86±0.41 <sup>bc</sup>
A.E. 0.018 gm/kg (24 h)	11.83±0.33 <sup>bc</sup>
A.E. 0.018 gm/kg (48 h)	12.33±0.62 <sup>b</sup>
A.E. 0.018 gm/kg (72 h)	12.43±0.76 <sup>b</sup>

Gem. : Gemcitabine; A.E.: Aqueous extract; h: hour; the different letters in the column are significantly different (P<0.05)

Table 3: Genotoxic Effect of *Q. infectoria* aqueous Extract on Chromosome Aberrations in Mal Albino Mice

Factor	Chromatid break	Chromosome break	Centromere break	Ring chromosome	Dicentric chromosome	Acentric chromosome	pulverization	Total aberrant metaphase	Total normal metaphase
Dose	PBS (N. Co)	2.00±0.00 <sup>e</sup>	2.33±0.33 <sup>c</sup>	1.00±0.00 <sup>e</sup>	1.33±0.33 <sup>b</sup>	1.00±0.00 <sup>ab</sup>	0.00±0.00 <sup>b</sup>	8.66±0.33 <sup>c</sup>	91.33±0.33 <sup>a</sup>
	Gem.(P.Co)15 mg/kg	5.33±0.33 <sup>a</sup>	4.33±0.33 <sup>a</sup>	2.66±0.33 <sup>a</sup>	2.66±0.33 <sup>a</sup>	1.33±0.33 <sup>a</sup>	2.00±0.57 <sup>a</sup>	20.00±0.50 <sup>a</sup>	80.00±0.50 <sup>e</sup>
	A.E.0.075gm/kg (24h)	3.88±0.26 <sup>b</sup>	2.88±0.20 <sup>b</sup>	1.88±0.11 <sup>b</sup>	1.44±0.17 <sup>b</sup>	0.77±0.22 <sup>b</sup>	0.00±0.00 <sup>b</sup>	11.66±0.64 <sup>b</sup>	88.33±0.64 <sup>b</sup>
	A.E.0.037gm/kg (48h)	4.33±0.23 <sup>b</sup>	3.33±0.16 <sup>b</sup>	2.00±0.16 <sup>b</sup>	1.44±0.17 <sup>b</sup>	0.55±0.17 <sup>b</sup>	0.00±0.00 <sup>b</sup>	12.22±0.66 <sup>b</sup>	87.77±0.66 <sup>b</sup>
	A.E.0.018gm/kg (72h)	2.55±0.33 <sup>c</sup>	2.00±0.16 <sup>c</sup>	1.11±0.11 <sup>c</sup>	1.66±0.23 <sup>b</sup>	0.66±0.16 <sup>b</sup>	0.44±0.17 <sup>b</sup>	8.44±0.64 <sup>c</sup>	91.55±0.64 <sup>a</sup>
Exposure Period	24h	3.80±0.38 <sup>a</sup>	3.20±0.24 <sup>a</sup>	1.86±0.191 <sup>a</sup>	1.93±0.18 <sup>a</sup>	1.06±0.11 <sup>a</sup>	0.400±0.23 <sup>a</sup>	13.26±1.10 <sup>a</sup>	86.73±1.10 <sup>a</sup>
	48h	3.66±0.37 <sup>a</sup>	3.00±0.23 <sup>ab</sup>	1.73±0.181 <sup>a</sup>	1.66±0.18 <sup>a</sup>	0.86±0.13 <sup>ab</sup>	0.400±0.23 <sup>a</sup>	12.200±1.16 <sup>b</sup>	87.80±1.16 <sup>b</sup>
	72h	3.40±0.33 <sup>a</sup>	2.73±0.26 <sup>b</sup>	1.60±0.190 <sup>a</sup>	1.53±0.19 <sup>a</sup>	0.66±0.15 <sup>b</sup>	0.400±0.23 <sup>a</sup>	11.133±1.24 <sup>c</sup>	88.86±1.24 <sup>c</sup>
	PBS (N. Co)	2.00±0.00 <sup>e</sup>	2.33±0.33 <sup>c</sup>	1.00±0.00 <sup>e</sup>	1.33±0.33 <sup>b</sup>	1.00±0.00 <sup>ab</sup>	0.00±0.00 <sup>b</sup>	8.66±0.33 <sup>c</sup>	91.33±0.33 <sup>a</sup>
Nitration (dose & periods)	Gem.(P.Co)15 mg/kg	5.33±0.33 <sup>a</sup>	4.33±0.33 <sup>a</sup>	2.66±0.33 <sup>a</sup>	2.66±0.33 <sup>a</sup>	1.33±0.33 <sup>a</sup>	2.00±0.57 <sup>a</sup>	20.00±0.50 <sup>a</sup>	80.00±0.50 <sup>e</sup>
	A.E. 0.075 gm/kg (24h)	4.33±0.33 <sup>ab</sup>	3.33±0.33 <sup>b</sup>	2.00±0.00 <sup>abc</sup>	1.66±0.33 <sup>ab</sup>	1.33±0.33 <sup>a</sup>	0.00±0.00 <sup>b</sup>	13.66±0.33 <sup>b</sup>	86.33±0.33 <sup>a</sup>
	A.E. 0.075 gm/kg (48h)	4.00±0.57 <sup>abc</sup>	3.00±0.00 <sup>bc</sup>	2.00±0.00 <sup>bc</sup>	1.33±0.33 <sup>b</sup>	0.66±0.33 <sup>ab</sup>	0.66±0.33 <sup>ab</sup>	11.66±0.88 <sup>bcd</sup>	88.33±0.88 <sup>abc</sup>
	A.E. 0.075 gm/kg (72h)	3.33±0.33 <sup>abc</sup>	2.33±0.33 <sup>cd</sup>	1.66±0.333 <sup>bcd</sup>	1.33±0.33 <sup>b</sup>	0.33±0.33 <sup>b</sup>	0.00±0.00 <sup>b</sup>	9.66±0.33 <sup>de</sup>	90.33±0.33 <sup>bc</sup>
	A.E. 0.037 gm/kg (24h)	4.66±0.66 <sup>ab</sup>	3.66±0.33 <sup>ab</sup>	2.33±0.333 <sup>ab</sup>	2.00±0.00 <sup>ab</sup>	0.66±0.33 <sup>ab</sup>	0.66±0.33 <sup>ab</sup>	14.00±0.57 <sup>b</sup>	86.00±0.57 <sup>a</sup>
	A.E. 0.037 gm/kg (48h)	4.33±0.33 <sup>ab</sup>	3.33±0.33 <sup>b</sup>	2.00±0.00 <sup>abc</sup>	1.33±0.33 <sup>b</sup>	0.66±0.33 <sup>ab</sup>	0.66±0.33 <sup>ab</sup>	12.33±1.20 <sup>bc</sup>	87.66±1.20 <sup>bc</sup>
	A.E. 0.037 gm/kg (72h)	4.00±0.00 <sup>abc</sup>	3.00±0.00 <sup>bc</sup>	1.66±0.333 <sup>bcd</sup>	1.00±0.00 <sup>b</sup>	0.33±0.33 <sup>b</sup>	0.33±0.33 <sup>b</sup>	10.00±0.33 <sup>dec</sup>	89.66±0.33 <sup>bed</sup>
	A.E. 0.018 gm/kg (24h)	2.66±0.66 <sup>ab</sup>	2.33±0.33 <sup>ab</sup>	1.33±0.333 <sup>cd</sup>	2.00±0.57 <sup>ab</sup>	1.00±0.00 <sup>ab</sup>	0.66±0.33 <sup>ab</sup>	10.00±1.52 <sup>dec</sup>	90.00±1.52 <sup>bed</sup>
Nitration (dose & periods)	A.E. 0.018 gm/kg (48h)	2.66±0.88 <sup>ab</sup>	2.00±0.00 <sup>d</sup>	1.00±0.00 <sup>d</sup>	1.66±0.33 <sup>ab</sup>	0.66±0.33 <sup>ab</sup>	0.00±0.00 <sup>b</sup>	8.33±0.33 <sup>ef</sup>	91.33±0.33 <sup>ab</sup>
	A.E. 0.018 gm/kg (72h)	2.33±0.33 <sup>d</sup>	1.66±0.33 <sup>d</sup>	1.00±0.00 <sup>d</sup>	1.33±0.33 <sup>b</sup>	0.33±0.33 <sup>b</sup>	0.00±0.00 <sup>b</sup>	7.00±0.57 <sup>f</sup>	93.00±0.57 <sup>a</sup>

N. Co: Negative control; Gem. : Gemcitabine; P. Co positive control; A.E.: Aqueous extract, h: hour,

**Table 4: The Genotoxic effect of various doses of *Q. infectoria* extract on sperm morphology in male Albino mice**

Type factor	Pin head	Swollen head	Double head	Ribbon head	Hookless head	Amorphous head	Long and broad hook	Head without tail	Total abnormal head
PBS (N.Co)	2.50±0.50 <sup>b</sup>	2.33±0.49 <sup>b</sup>	2.00±0.36 <sup>b</sup>	2.83±0.47 <sup>a</sup>	5.33±0.61 <sup>cd</sup>	5.33±0.80 <sup>b</sup>	1.66±0.33 <sup>c</sup>	4.50±0.23 <sup>ab</sup>	26.50±1.05 <sup>e</sup>
Gem.(P.Co) 10mg/kg	7.16±0.40 <sup>a</sup>	7.50±0.42 <sup>a</sup>	7.83±0.54 <sup>a</sup>	4.66±0.71 <sup>a</sup>	13.33±0.76 <sup>a</sup>	13.33±0.95 <sup>a</sup>	8.00±0.447 <sup>a</sup>	7.00±0.77 <sup>a</sup>	68.83±2.67 <sup>a</sup>
A.E. 0.075gm/kg	2.66±0.33 <sup>b</sup>	1.83±0.30 <sup>b</sup>	1.33±0.21 <sup>b</sup>	3.00±0.36 <sup>a</sup>	7.16±0.60 <sup>bc</sup>	16.66±0.80 <sup>a</sup>	2.33±0.33 <sup>bc</sup>	2.16±0.79 <sup>b</sup>	37.50±2.45 <sup>b</sup>
A.E. 0.037gm/kg	2.00±0.36 <sup>a</sup>	2.33±0.42 <sup>b</sup>	1.66±0.49 <sup>b</sup>	2.83±0.30 <sup>a</sup>	8.16±1.13 <sup>b</sup>	15.83±1.57 <sup>a</sup>	3.50±0.71 <sup>b</sup>	1.83±0.47 <sup>b</sup>	38.16±2.16 <sup>b</sup>
A.E. 0.018gm/kg	2.66±0.55 <sup>b</sup>	2.83±0.47 <sup>b</sup>	2.00±0.73 <sup>b</sup>	3.16±0.95 <sup>a</sup>	3.50±0.42 <sup>d</sup>	16.66±1.42 <sup>a</sup>	2.33±0.55 <sup>bc</sup>	5.66±1.78 <sup>a</sup>	38.83±3.07 <sup>b</sup>

**Table (4-4-B) Sperm and tail aberrant types**

Type factor	Midpiece and tail aberrant types						Total abnormal			Total Normal sperms
	Coiled tail defect	Bent midpiece defect	Corkscrew defect	Pseud drobllet	Double tail	Tail without head	midpiece and tail aberrant types	Abnormal sperms	Total	
PBS (N.Co)	1.50±0.22 <sup>b</sup>	21.66±0.98 <sup>d</sup>	8.33±0.71 <sup>c</sup>	9.16±0.54 <sup>a</sup>	2.83±0.16 <sup>b</sup>	2.33±0.57 <sup>c</sup>	45.16±1.32 <sup>c</sup>	71.66±2.04 <sup>d</sup>	528.33±2.04 <sup>a</sup>	
Gem.(P.Co)10mg/kg	95.00±1.96 <sup>a</sup>	129.50±2.43 <sup>a</sup>	8.66±0.42 <sup>c</sup>	2.50±0.50 <sup>b</sup>	5.83±0.30 <sup>a</sup>	21.16±1.275 <sup>a</sup>	262.16±1.88 <sup>a</sup>	331.00±3.97 <sup>a</sup>	268.00±4.14 <sup>d</sup>	
A.E. 0.075gm/kg	6.00±0.81 <sup>b</sup>	37.83±2.15 <sup>b</sup>	29.83±1.01 <sup>a</sup>	3.00±0.57 <sup>b</sup>	2.33±0.49 <sup>bc</sup>	4.00±1.34 <sup>bc</sup>	79.16±4.74 <sup>b</sup>	123.166±12.71 <sup>b</sup>	476.83±12.71 <sup>c</sup>	
A.E. 0.037gm/kg	5.50±0.50 <sup>bc</sup>	31.16±1.22 <sup>c</sup>	19.83±2.91 <sup>b</sup>	2.00±0.25 <sup>b</sup>	1.83±0.47 <sup>bc</sup>	2.16±0.60 <sup>c</sup>	62.0±3.18 <sup>bc</sup>	99.66±1.99 <sup>c</sup>	500.33±1.99 <sup>b</sup>	
A.E. 0.018gm/kg	2.66±0.55 <sup>cd</sup>	29.66±0.88 <sup>c</sup>	17.83±1.44 <sup>b</sup>	1.83±0.30 <sup>b</sup>	1.50±0.42 <sup>c</sup>	6.83±1.47 <sup>b</sup>	60.33±11.54 <sup>b</sup>	99.16±11.45 <sup>cd</sup>	500.83±11.45 <sup>ab</sup>	

\* N.Co: Negative control; Gem. : Gemcitabine; P.Co: positive control; A.E.: Aqueous extract. the different letters in the same column are significantly different at level , All values given in the table are Mean± SEM

were in a centric chromosome at the dose 0.018 gm/kg. The number of aberrations did not increase with the extending of the doses period as show in Table 3. A significant ( $P<0.05$ ) interaction between doses and periods was found in chromatid break chromosome breaks, centromere breaks while the interaction in the rest of the parameters was not significant compared with the negative control. Due to the absence of any data on *Q. infectoria* mutagenicity in eukaryotes, the genotoxic effect of its leaves extract was studied for the first time, and according to the resent report of Ghafour et al. (2010) revealed that oak contain about 25-28 chemical compound, flavonoids of these chemicals seems to be mutagenic. It has been reported that the mutagenicity of more than seventy naturally occurring flavonoids, of these quercetin was the strongest mutagen followed by kaemferol (Brown, 1982; Nago et al., 1981) and both of quercetin and kaemferol has been detected in oak plant (Alvi et al., 1986; Ghafour et al., 2010; Satl et al., 2011). It is suggested that quercetin mutagenicity may be due to its binding to DNA giving rise to single or double strand breaks. So, based on the data obtained, we suggest that the chromatid breaks, chromosome breaks and centromeric breaks induced in this study by *Q. infectoria* aqueous extract are due to the presence of flavonoids such as quercetin and kaemferol.

The result of this work represent diversity in the type of abnormal sperms induced by different concentrations of *Q. infectoria* aqueous extract in male albino mice which are shown in Table 4 (A and B). The values of traits in this table are the number of abnormal sperm counted from 600 sperms. It is obvious from these data that there is a significant differences ( $P<0.05$ ) between treatment groups and negative control group in all types of sperm abnormalities listed except swollen head, double head and ribbon head. The total number of abnormal sperms increased with the increase of doses concentration of *Q. infectoria* extract. Most types of misshapen sperms induced by *Q. infectoria* extract in males albino mice were amorphous head, pin head, hooked sperm, long and broad hook, colid tail defect, bent midpiece defect, crokscrew defect, psudrobot defect, double tail and tail without head sperms. The highest mean value of abnormal head sperms of treated animals compared with other types of head abnormalities were in amorphous sperm head and in hooked sperm. While the highest mean value of abnormal tail sperm and midpiece compared with other types of tail abnormalities were in bent midpiece defect. On the other hand, the least affected trait was double head sperms for all the three doses. The 0.075 gm/kg dose was the most effective in causing most types of sperm abnormalities. The high incidence of irregular or amorphous head sperm could be explained as a reflect of the nuclear chromosome damage resulted or induced

by the leaves extract of *Q. infectoria* because normal head shape of mouse sperm cell is a very distinctive and any head abnormalities might be influenced by genetic back ground (Wyrobek, 1979; Styra et al., 2003). Other reports indicated that mitochondria of mammalian spermatozoa play an important role in its function (Gur and Breitbart, 2008). On the basis of the previous information, *Q. infectoria* might affect critical mitochondria function leading to the lose of the arrangement of the helix of mitochondria which gives the appearance of corkscrew sperm shape (Arthur et al., 1996). Moreover, corkscrew sperm shape could be inherited when present at high percentages (Arthur et al., 1996) and it may cause infertility or offspring malformations as reported by Mass (2001) who notice that calves fed on oak leaves born suffering shortened for limbs, typically bowed outwards, head was enlarged and often a defect in the spine with a vertebral protrusion. In another report, mice at the dose rate of 300 and 400 mg/kg quercetin showed a profound reduction in fertility of the male in addition to the small size of their offspring (Aravindakshan et al., 1985) suggesting that the loss of fertility could be due to germinal cytotoxicity, oligospermia or impairment of fertilizing of the treated animals. Taken all together, the result obtained in the present study revealed that *Q. infectoria* aqueous extract inhibits cell proliferation, increased the frequency of chromosome aberrations and sperm abnormalities *in vivo*. These data clearly showed cytotoxicity and genotoxicity effects of *Q. infectoria* in bone marrow cell and germ cells of Swiss albino mice. More studies should be designed on grazing animals to evaluate the effect of the supplementary feed of *Q. infectoria* on their development, growth, fertility and reproduction.

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