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Evaluation of genotoxic potential of *Hypericum triquetrifolium* extract in somatic and germ cells of male albino mice

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Abstract

Hypericum triquetrifolium aqueous extract were studied for the first time for its toxic and the possible clastogenic effects *in vivo* on the bone marrow and spermatozoa cells of Swiss albino mice. The lethal dose of the aqueous extract was considered to be 10.33 g/kg of the body weight, injected subcutaneously. The doses which were chosen for treatments were 2, 1, and 0.25 g/kg. H. *triquetrifolium* extract induce statistically significant increases in the average numbers of micronucleus(MN) at the dose 2 g/kg and chromosome aberrations at the doses 2 and 1 g/kg ,the majority of aberrations observed were chromatid breaks, centromeric breaks, acentric fragments. The extract was found to inhibit mitotic index (MI) in a dose-dependent manner. Moreover the plant extract showed a significant induction of sperm abnormalities in all concentrations used comparing with the untreated animals. The most frequent types of sperm abnormalities of the treated groups were; amorphous, pseudo-droplet defect, bent mid piece defect and corkscrew mid piece defect. However, the lowest dose 0.25 g/kg body weight was the most effective one which markedly increased the corkscrew midpiece defect. The results indicated that the mixture of the compounds found in the aqueous extract caused cytotoxicity and induced different cytogenetic effects in both somatic and germ cells of male albino mice.

Keywords: *Hypericum triquetrifolium* Turra, Chromosome Aberrations, LD50, Micronucleus Assay, Sperm Abnormalities

Introduction

Hypericum species are herbaceous plants known to have medicinal properties and are widely used in phototherapy in many countries. The genus Hypericum comprised more than 400 species (Robson, 2006), but only sixteen are found in Iraq, the most abundant herbs of those are *Hypericum perforatum* and *Hypericum triquetrifolium*Turra (Al-Mukhtar, 1975).

The Hypericum genus is known to contain wealth of secondary metabolites, many of which are biologically active. Of the main constituents are naphthodianthrones (hypericin, pseudo-hypericin, proto hypericin, and protopseudo-hypericin), phloroglucinols (hyperforin, adhyperforin, hyperfirin, and adhyperfirin), and a broad range of flavonoids (hyperoside and rutin) (Nahrstedt and Butterweck, 1997).

Hypericum triquetrifolium Turra (*H. crispum* L.) belongs to the Hypericaceae family and is a wild growing weed in the northern part of Iraq; locally known as Roja (Al-Rawi, 1988), the classic Arabic name for this plant species is Dathi or Nabtat Yohanna. It has been used in traditional Arab herbal medicine to treat various inflammatory diseases and as sedative, astringent, anti-spasmodic, for intestine and bile disorders and poisonous (Karim and Quraan, 1986; Saad et al., 2007) Antioxidant,Antiviral ,Antimicrobial and antinociceptive activities have also been reported in the literature for *H. triquetrifolium* (Apaydin et al., 1999; Tawaha et al., 2007; Couladis et al., 2002; Suzuki et al., 1984; Alali et al., 2007).

A few studies are reported in the literature about the medical usage of *H. triquetrifolium* (Apaydin et al., 1999; Apaydin et al., 2001; Couladis et al., 2002a, 2002b; Conforti et al., 2002).

Since a survey of the literature revealed that no studies on *H. triquetrifolium* mutagenicity in eukaryotes had been undertaken, the aim of this study was to evaluate the genotoxic activity of this herb on bone marrow and sperm cells of Swiss albino mice.

Materials and Methods

Fresh plants of *Hypericum triquetrifolium* Turra from Khanik (a village, located in the northern part of the Iraqi Kurdistan region) were used. Mainly, the

aerial parts of the plants that have a high proportion of buds and flowers were selected during the early stage of flowering time in June 2009. The plant was identified by Professor Saleem Shahbaz, Duhok University, Faculty of Agriculture, and Department of Forestry. Then the plant was air dried indoors at room temperature to protect it from direct light. A modified method of Subhan et al. (2009) was used for the preparation of the aqueous extract of the fine powdered plant. The dried milled aerial parts were suspended in distilled water for 10-12 hours, at the rate of 50 g/400 ml, and then the material was extracted aqueousley by Soxholet extractor for 1 hour. The aqueous extract was concentrated using a rota evaporator (45^{o}C) under reduced pressure. The weight of crude extract resulted from that amount of powdered plant was kept in a dark bottle in (4^{o}C) and used during 1-5 days.

Experiments were carried out on 8-10-week-old male Swiss albino mice (*Mus musculus*), weighing 25-30 g. kept in polyethylene boxes (n = 5) .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 acquired from the animal house of Agriculture College; University of Duhok.The mice were divided into experimental groups of 5 animals. An extract of *H. triquetrifolium leaves* was administered in a single dose of 0.5ml subcutaneously, at concentrations of 2, 1 and 0.25 g/kg/day, chosen on the basis of our LD50 value obtained in this study. The negative control group received 0.5ml phosphate buffer saline (PBS) The positive control group received subcutaneously 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 0.5ml of 10 mg/kg colchicin 2-3 hours, prior to sacrificing after each injection. The animals used in this study were sacrificed by cervical dislocation.

The LD50 was determined using the classical LD50 method according to Behrens and Karbers (1953).All the animals were exposed to bright sun light for 30 min /day immediately after administration to plant extract. Graded single doses of *H. triquetrifolium* (1 , 2 ,4 , 6 , 8 , 12 , 16 , 20 , and 24 g/kg of body weight) were administrated subcutaneously to nine groups of mice, each of which consisted of six mice. Mortality was recorded after 24 hours. Sun light was considered in all experiments used in this work.

For the micronucleus (MN) assay, both femur bones were excised and the bone marrow flushed into test tubes using a syringe, the bone marrow cells from one femur were prepared as recommended by Schimid (1975). The slides were coded, fixed with methanol and stained with Giemsa solution. 500 polychromatic erythrocytes (PCE) from each animal were scored for micronucleus (MN) presence. The mitotic index (MI) was obtained by counting the number of mitotic cells in the 1,000 cells analyzed per animal.

Bone marrow preparations from the femur bones were used for the analysis of chromosome aberrations in metaphase cells, using the technique of (Sharma and Sharma, 1980). 100 metaphases per animal (600 metaphases per group) were analyzed, in order to determine the number of chromosomal aberrations, for this test *H. triquetrifolium* extracts were administered daily for three consecutive days and the animals were sacrificed by cervical dislocation at 24, 48 and 72 hours after the third medication.

For cytological analysis of spermatozoa abnormalities, the mice were sacrificed 5 weeks after the last day of sub-acute treatment (Wyrobek, 1979).The spermatozoa were obtained by making small cuts in epididymis and vas deferens, placed in 1 ml of normal saline .The sperm suspension obtained was stained with 0.05% of eosin-Y; smears were made on slides, air-dried and made permanent. 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.

Statistical analysis

The data obtained were submitted to the statistical program SAS (2005), Micronuclei, Sperm abnormalities, and Mitotic Index were analysed using Completely Randomized Design (CRD). Chromosome Aberrations were analysed using factorial 3x5(CRD) to study the effect of treatments and $P<0.05$ was considered statistically significant.

Results and Discussion

Administration of *H. triquetrifolium* subcutaneously showed some behavioural changes. Those changes included the loss of appetite, restlessness, with decreasing in response to outside stimuli. Server skin lesion and adhesion between skin and abdomen also were observed. The appearance symptoms observed in the treated mice might be the result of the effects of hypericin in the skin and central nervous system. When hypericin molecules are photoenergised they are able to lyse cells in the dermis and cause the photosensitisation associated cellulitis (an infection of deep skin dermis) that develops in field cases of *Hypericum* poisoning. (Bourke, 1997). The result of the exposure of nine different concentrations of the aqueous extract produced 17% mortality at 6 gm/kg which was increased to about 83-100% mortality at the dose of 16-24 g/kg. The calculated LD50 value in this study was 10.33 gm/kg. Result expressed in Table (1)**.** Concerning *H. triquetrifolium* toxicity, a very few data on acute and

subchronic toxic effects are available, Only Saad et al. (2006) determined the LD50 of the aqueous extract of the dried leaves of *H. triquetrifolium* (14.7 g/kg) in bone marrow cells of Rats, in a review of the toxicity of some traditional Arab herbs. A number of reports included *H. perforatum* and hypericin toxicity in sheep was established. Hyperthermia is a consistent and rapidly developing clinical sign in sheep intoxicated by *Hypericum*. It was demonstrated that the rectal temperature rise in affected sheep, this response is thought to involve central nervous system inhibition of monoamine oxidase and catechol-o-methyltransferase and a consequent accumulation of noradrenaline in the hypothalamic body temperature control centre (Bourke, 2003).

Table 1: The acute toxicity of *H. triquetrifolium* extract in male albino mice

Plant extract doses gm/kg	No. of mice	Exposed time to sun light (minutes)	Percent of mortality
24	6	30	100
20	6	30	83
16	6	30	83
12	6	30	67
8	6	30	50
6	6	30	17
4	6	30	0
2	6	30	0
1	6	30	0

Toxicity studies with hypericin or *H. perforatum* extract in laboratory animals have produced few clinical signs, but these studies were conducted indoors. Conversely, the administration of hypericin to rats followed by exposure to sun light resulted in death. That may have been direct result of the hyperthermia caused by photo-activated hypericin (Bourke, 2000). In Iraq The pathology of two toxic plant species of the genus *Hypericum* (*H. perforatum* and *H. crispum*) was studied using Rabbits as laboratory animals' model. The results revealed that the *H. crispum* was more toxic and exerted more severe, seen earlier and last longer period pathological effects than those seen with *H. perforatum*. The two plants caused a type of haemolytic anaemia and haemoglobinuria (Youkhana, 1995; AL-Farwachi, 1997).

Cytogenetic analysis of micronucleus and chromosome aberrations are summarized in Table (2) and Table (3) respectively, in bone marrow cells of Swiss albino mice following treatment with different concentration of *H. triquetrifolium* extract and controls. Administration of *H. triquetrifolium* extract indicate (2 mg/kg) dose had significant differences on single MN, di MN, total abnormal micronucleated cell, total normal cell and could induce the most MN frequency, which mean that the *H. triquetrifolium* extract has dose dependent effect on MN percentage. This result disagree with that obtained by Espósito et al. (2005), which revealed that the administration of *H. brasiliense* extract showed no significant difference between the treated rat groups and untreated animals. While the photochemical genotoxicity which investigated by Kersten et al. (1999) in Chinese hamster cell line, indicates that hypericin increased the percentage of MN in the presence of UV light.

The polyphenolic compounds of *H. triquetrifolium* consist of the flavonoids, kaempferol and quercetin (Conforti et al., 2002) the kaempferol and quercetin could induce the MN ratio in a dose dependent manner in Chinese hamster V79 cell (Snyder and Gillies, 2002; Silva et al., 1997) concerning the role of flavonoids clastogenicity as DNA intercalating agents, is still an open question (Snyder and Gillies, 2002).

The MI values obtained from the analysis of 1000 cell/animal showed there were significant differences among the three doses of *H. triquetrifolium* and the untreated control on MI percentage. The effective mean value dose among the treated groups was found at dose 2 gm/kg (11.620±0.710), as shown in Table (2) .The significant decreases in mitotic index value, might ensure the cytotoxic effect at the doses tested. The *H. perforatum* extract also shows a significant concentration-dependent and long-lasting inhibition of cell growth, which might induce apoptotic cell death, or programmed cell death (Roscetti et al., 2004), According to molecular cytology view, the rapid collapse of mitochondrial transmembrane potential which induced by hypericin, promoted apoptotic pathways (Kubin et al., 2005).

The frequencies of chromosome aberrations were increased with increasing doses. Single subcutanusly exposure of *H. triquetrifolium* induced chromosome type aberration (dicentric chromosome, acentric fragments and ring formation) and chromatid aberration (centromere break, chromatid breaks and gaps) and occasionally pulverization in mouse bone marrow.

Table (3) summarized The data obtained from 500 metaphases analysed per treatment (100 metaphase cells/animal) which showed that there were statistically significant differences between the mean number of chromosome aberrations of the two treated groups which administrated the doses (1 and 2 g/kg) and of the negative control. The most frequent types of aberrations were chromatid breaks, centromere break and acentric fragments at the highest dose (2 g/kg). Moreover, centromere breaks was the most abundant type of chromosome aberrations that recorded significant differences between the doses (1 g/kg and 0.25 g /kg). Periods (24, 48, 72 hours) after treatment showed no

Table 2: Genotoxic effects of *H. triquetrifolium extract* on micronucleus and mitotic index frequency in bone marrow cells of male albino mice

No. of MN Factors	Single MN	Di MN	Tri MN	Tetra MN	Total abnormal micronucleated cell	Total normal cell	Percentage of mitotic index (MI %)
PBS (N.Co)	12.600±1.16 ^{c}	3.400±0.748^{b}	0.00±0.00^{b}	0.00±0.00^{b}	16.00±1.048^{c}	484.00±1.048^{a}	14.340±0.093^{a}
Gem.(P.Co) 5 g/kg	30.800±1.529^{a}	6.800±1.743^{a}	2.600±1.122^{a}	1.200±0.734^{a}	41.400±2.400^{a}	458.600±2.400^{c}	8.380±0.250^{e}
A.E. 2g/kg	18.400±0.678^{b}	3.00±0.447^{a}	0.600±0.244^{b}	0.00±0.00^{b}	22.00±0.894 ^{b}	478.00±0.894 ^{b}	11.573±0.263^{d}
A.E. 1g/kg	15.800±1.157^{bc}	3.400±0.748^{a}	0.400±0.244^{b}	0.200±0.200^{ab}	19.800±1.462^{bc}	480.200±1.462^{ab}	12.306±0.256^{c}
A.E.25g/kg	14.800±1.280^{bc}	1.00±0.316^{a}	0.400±0.244^{b}	0.200±0.20 ^{ab}	16.400±1.469 ^{c}	483.600±1.469^{a}	13.500±0.24 ^{b}

* N.Co : Negative control ; Gem. : Gemcitabine ; P.Co positive control ;A.E.: Aqueous extract ;MN : micronuclei
The different letters in the same column are significantly different at level (P<0.05).

Table 3: Genotoxic effect of *H. triquetrifolium extract* on chromosome aberrations in male albino mice

Aberrant type Factor	ctb	csb	Cemb	R	dic	Acf	pul	Total aberrant metaphase	Total normal metaphase
Treatment									
PBS (N.Co)	3.200±0.200^{d}	0.200±0.106^{b}	0.400±0.213^{c}	4.200±0.261^{b}	0.00±0.00^{b}	4.200±0.200^{c}	0.00±0.00^{b}	6.800±0.200^{c}	93.200±0.200^{a}
Gem.(P.Co) 15 mg/kg	13.200±0.518^{a}	3.200±0.489^{a}	2.600±0.213^{a}	9.400±0.465^{a}	2.400±0.272^{a}	12.400±0.575^{a}	24.00±8.552^{a}	18.00±0.585^{a}	82.00±0.585^{c}
A.E. 2g/kg	5.333±0.386^{b}	0.666±0.125^{b}	1.800±0.261	3.600±0.400^{b}	0.333±0.125^{b}	6.066±0.383^{b}	0.00±0.00^{b}	8.600±0.272^{b}	91.666±0.303^{b}
A.E. 1g/kg	4.733±0.266^{bc}	0.800±0.200^{b}	1.600±0.235^{b}	3.533±0.466^{b}	0.133±0.090^{b}	4.666±0.318^{c}	0.00±0.00^{b}	8.200±0.326^{b}	91.933±0.330^{b}
A.E. 0.25 g/kg	3.866±0.273^{cd}	0.400±0.130^{b}	0.666±0.125^{c}	3.666±0.318^{b}	0.266±0.118^{b}	3.733±0.266^{c}	0.00±0.00^{b}	6.866±0.191^{c}	93.133±0.191^{a}
Time									
P1 (24h)	6.040±0.800^{a}	0.880±0.301^{a}	1.480±0.258^{a}	5.200±0.541^{a}	0.520±0.216^{a}	6.280±0.717^{a}	4.800±3.517^{a}	9.760±0.907^{a}	90.480±0.925^{a}
P2 (48h)	6.360±0.765^{a}	1.120±0.296^{a}	1.400±0.216^{a}	5.040±0.524^{a}	0.720±0.212^{a}	6.400±0.678^{a}	4.800±3.517^{a}	9.720±0.895^{a}	90.280±0.895^{a}
P3 (72h)	5.800±0.795^{a}	1.160±0.286^{a}	1.360±0.215^{a}	4.400±0.574^{a}	0.640±0.215^{a}	5.960±0.731^{a}	4.800±3.517^{a}	9.600±0.896^{a}	90.400±0.896^{a}

*N.Co: Negative control; Gem. : Gemcitabine; P.Co positive control; A.E: Aqueous extract; ctb: chromatid break, csb: chromosome break, cemb: centromer break, r: ring chromosome; dic: dicentric, acf: acentric fragment, pul: pulverization, p: period , h: hour; The different letters in the same column are significantly different at level (P<0.05).

Table (4-A and B): The Genotoxic effect of various doses of *H. triquetrifolium* extract on sperm morphology in male albino mice

Table (4-A)

Aberrant type Factor	Pin head	Swollen head	Double head	Ribbon head	Irregular head defect	Hookless	Long and Broad hook	Total abnormal head
PBS (N.Co)	1.600±0.400^{b}	0.600±0.400^{a}	0.400±0.244^{b}	1.800±1.319^{a}	8.200±1.319^{c}	5.600±1.600^{bc}	0.200±0.200^{b}	18.400±3.586^{b}
Gem.(P.Co) 10mg/kg	3.200±0.583^{a}	3.200±1.319^{a}	1.200±0.374^{a}	1.600±0.400^{a}	26.00±3.687^{a}	17.200±1.113^{a}	7.800±1.593^{a}	60.200±7.102^{a}
A.E. 2gm/kg	1.400±0.748^{b}	2.800±0.663^{a}	0.00±0.00^{b}	0.400±0.400^{a}	16.600±2.541^{b}	5.200±0.860^{bc}	0.800±0.200^{b}	27.200±3.865^{b}
A.E. 1gm/kg	1.600±0.244^{b}	2.800±1.462^{a}	0.200±0.200^{b}	0.400±0.400^{a}	14.600±1.363^{bc}	7.600±1.122^{b}	1.600±0.244^{b}	28.800±2.782^{b}
A.E. 0.25gm/kg	1.200±0.200^{b}	1.00±0.547^{a}	0.00±0.00^{b}	0.600±0.400^{a}	10.600±0.927^{bc}	3.00±0.547^{c}	0.800±0.374^{b}	17.200±1.881^{b}

Table (4-B)

Aberrant type Factor	Midpiece and tail aberrant types					Total abnormal mid piece and tail defects	Total abnormal sperm	Total normal sperm
	Double tail defect	Coiled tail defect	Pseud-droblet defect	Corkscrew defect	bent mid piece defect			

significant differences for all parameters that were analysed.

Both *in-vitro* and *in vivo* tests using mouse cells and bone marrow cells obtained from the Chinese hamster yielded negative results, giving completely no indication of the mutagenic potential of *Hypericum perforatum* and *H. brasiliense* extracts (Okpanyi et al., 1990). The hexane-ethanol extract of the leaves of *H. brasiliense* , was tested *in vivo* on bone marrow cells of Wistar rats, the result also did not induce statistically significant increases in the average numbers of chromosome aberrations in the test systems employed. In addition, non-photoactivated hypericin was investigated for its genotoxicity using three mammalian cell lines. Hypericin did not alter the frequency of structural chromosome aberrations (Miadokova et al., 2010) On the other hand, It was noticed that irradiation, increased the photogenotoxic damage to human keratinocytes cell line using Comet assay which means that hypericin might induce DNA single strand breaks (Traynor et al., 2005). In addition to hypericin the *H. triquetrifolium* extract contains number of compounds which has a positive mutagenic effects such as flavonoids, among which are quercetin, kaempferol and myricetin. (Meltiz et al., 1981; Silva et al., 1996 a&b; Conforti et al., 2002; Silva et al., 1996a).which might be responsible for *H. triquetrifolium* genotoxicity.

The different types of sperm abnormalities observed are shown in Table (4) A and B It can be seen that the treated animals with *H. triquetrifolium* extract increased head abnormalities but statistically not significant, except the amorphous type at the dose (2 gm/kg). The highest mean value of abnormal head sperms of treated animals compared with other types of head abnormalities, were (16,600±2.541) at the amorphous head group. Table (4) B expresses the genotoxicity of *H. triquetrifolium* on sperm midpiece and tail. There were significant differences between the dose (2) g/kg of the extract and the untreated group on pseudo-droplet defect, corkscrew midpiece defect, bent midpiece defect, total abnormal midpiece and tail defect.

There were also significant differences between the dose (1) g/kg and the untreated control on bent midpiece, and total abnormal midpiece and tail defect. Moreover, there were significant differences between the lowest dose (0.25) g/kg and untreated control on corkscrew midpiece defect, bent midpiece defect, total abnormal midpiece and tail defects.

The results elucidate that there were significant differences among doses, negative control, and positive control on total abnormal sperm and total normal sperm as shown in Table (4-5) B. The higher incidence of amorphous head sperms confirm the genotoxicity of *H. triquetrifolium* extract on germ cells ,because the normal head shape of mouse sperm cell is very distinctive, and any abnormalities might be influenced by the genetic background (Wyrobek, 1979; Styrna et al., 2003). This result is in agreement with the observation of Aleisa (2008) who showed that St. John's Wort (SJW) supplement caused increased frequency of sperm abnormalities *in vivo*, such as banana shaped, swollen acrosome, triangular head and induced the chromosome aberrations in the testes of albino mice and the frequency of aneuploidy was found to increase significantly at 760 ($P<0.05$) and 1520 ($P<0.01$) mg/kg/day doses of SJW. At these doses, the percent polyploids and total percent aberrations of meiotic chromosomes were also significantly increased as compared to the values obtained in the control group. The genotoxic effect might be related to the *H. perforatum*, as the major ingredient in the supplement.

In fact, bent midpiece, corkscrew midpiece, and pseudo-droplet defects, may reduce fertility in one of two ways: (1) failure to reach the fertilization site; or (2) inability to fertilize the ovum once they are at the fertilization site or to sustain development of the early embryo (Chenoweth, 2005).

During the present study, *H. triquetrifolium* extract increase significantly the frequency of corkscrew and pseudo-droplet defects in midpiece of the mouse spermatozoa. The corkscrew midpiece defect is called so because the loose arrangement of the helix of mitochondrial which gives the appearance of a corkscrew to the midpiece of the sperm this defect may be inherited when present at high percentages. (Arthur et al., 1996) This defect was the most abundant shape observed in this study. The pseudo-droplets midpiece defect, which is irregular in shape and more visually dense than cytoplasmic droplet, may also be dependent on the presence of elongated and swollen mitochondria in addition to the thickened outer dense fibers (Rocha et al., 2006).

Reports indicated that hypericin can affect critical mitochondrial functions in a photodependent manner leading to inhibition of mitochondrial succinoxidase and inhibition of mitochondrial outer membrane-bound hexokinase, with a concomitant inhibition of cell proliferation, decrease in glucose phosphorylation and ATP content (Agostinis et al., 2002). The ATP that drives the sperm flagella is derived from the mitochondria located in the midpiece. Therefore, mutations in the mtDNA which increase or decrease ATP production will be reflected in increased or decreased sperm motility (Ruiz-Pesini et al., 1998; Troiano et al., 1998). Since the sperm requires motility for transport, penetration and interaction with oocyte (Ondrizek et al., 1999).

Our data demonstrated that there was a noticed reduction in sperm motility in the treated animals when comparing with the negative control of animals (data

not recorded). However, studies focused on the effects of *Hypericum* herb on the sperm cells are rare. Only Ondrizek et al. (1999) demonstrated a potent inhibitory effect on sperm motility in the presence of *H. perforatum* on the fresh human donor sperm cells. Even at the lower concentration, an inhibitory effect was observed from 24 hr onward. The mechanism involved in the reduction of sperm motility by this herb remained unknown but it did not involve changes in pH. However, Semen samples with high numbers of multiple mtDNA deletions appear to be associated with male infertility (St John et al., 2001).

In conclusion it can be said that the aqueous extract of *Hypericum triquetrifolium* with its mixture of compounds seems to induce both, point and chromosomal mutations in bone marrow cells and sperm cells at the cellular nucleus DNA or/and mitochondrial DNA level. To the best of our knowledge, no data exist on any concentrations of Hypericum *spp.* on semen or sperm *in vivo* to be compared with. Further experiments must be conducted in order to clarify the exact mutagenic mechanism of this plant and to figure out which constituent of the extract exerts this activity.

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