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EFFICACY OF D-Q-TOCOPHERYL POLYETHYLENE GLYCOL SUCCINATE (TPGS) TO MINIMIZE ATONIK TOXICITY IN MALE RATS

Aqeel Khaleel Ibraheem

Environmental Studies, Ministry of Education, Babylon, Iraq

Abstract

This study was designed to assess the influence of D-Q-tocopheryl polyethylene glycol succinate (Vitamin E TPGS or TPGS) on cytogenetic and DNA damage induced by atonik in male rats. (T1) represents a negative control, (T2) administrated by atonik 0.1 mg/kg as a positive group, (T3) administrated by atonik 0.1 mg/kg and treated by 30mg /kg, Fourth group, (T4) animals in this group were TPGS 100mg/kg. b.w for six weeks.TPGS showed reduction of Mitotic index, Blastic index %, Chromosomal aberration % and DNA comet assay parameters in the bone marrow. The study concluded that TPGS, showed cytoprotective lead to support drug to protective bone marrow cell from DNA damage.

Key word: Atonik, Ovary, Uterus, Nitro phenol.

Introduction

Atonik define as artificial biostimulant incorporates water and 3 phenol compounds. Na para-nitrophenolate PNP (0.3%), Na ortho-nitrophenolate ONP (0.2%) and Na 5 nitroguaiacolate 5NG (0.1%). The used of Atonik was to plant growth development and stimulant, generative significantly (Černý *et al*., 2008, Calvo *et al*., 2014). Inflated of the recent dry matter, weight and production, so, the leaf space is higher, with made pigment content, supported by an associate in nursing enhanced of chlorophyl visible light parameters. The competing of appliance Atonik simulative in the role of beneath optimum conditions and protecting against frost of spring, metal stresses and drought (Djanaguiraman *et al*., 2009). The significant impact of Atonik is far a lot of desirable once the plants are growing beneath stress states. The mechanism of action by raise uptake of nutrient through the increase of protoplasm streaming and prolongation activity of plant hormone by the indolylacetic acid and abscissic acid inhibition, that increased activity of the nitrate enzyme (Przybysz *et al*., 2014). Though importance of some residue of agent in plant regulator, also, could be not excreted from plant particularly for fruit given that will result in toxicologic impact on Associate in Nursingimal and physical structure tissue Atonik once orally taken, good absorbed from stomachic internal tissue organ and blood circulation reach within thirty minute accompanied with high of the quantitative relation blood/ plasma at a pair of hours. Cosmopolitan, with the very best concentrations in a dead body, liver and kidney, also the internal organ tract, excreted through urine. Na orthonitrophenolate (OPP) additionally utilized as once harvest fungicide treatment of citrus fruits and as preservatives and disinfectants (FAO). Disinfectants in food implement are used vastly in agriculture (Kwok and Marilyn, 2013), according to that mice and rats treated with multi-dose (0, 25, 10, 25) of OPP showed craniate weight diminish and delayed skeletal ossification occurs in a high rate. Moreover, SOPP treated of craniate mice revealed malformation (rabbits: resorptions, mice: cleft plate). P-Nitrophenol (PNP) might a major element of some organophosphorus such as insecticide and alkyl group insecticide (Abu-Qare *et al*., 2000). PNP is Associate in Nursing intermediate agent wildly utilized in the production of some medication, fungicides, dyes and think about one organic Pollutant (Zhang *et al*., 2010). Bioaccumulation of PNP results from organ phosphorus (Ahmed *et al*., 2015). D-Q-tocopheryl polyethylene glycol succinate (TPGS or Vitamin E TPGS) was FDA approved mostly used in drug delivery systems and as a safe adjuvant. The physicochemical and biological characterization of TPGS gives various benefits for drug delivery uses such as high biocompatibility, selective antitumor activity, an increase of drug solubility and

***Author for correspondence :** E-mail: aqeel8077@yahoo.com

enhancement penetration of drugs (Ahmed *et al*., 2015).

Vitamin E exists in nature in eight natural isoforms that have exhibited various therapeutic properties in numerous investigations. The tocopherol isoforms have highlighted their inhibitor in clinical studies, *in vivo* and *in vitro*, medicinal drug, neuroprotective and antithrombotic talents, among others (Li *et al*., 2006, Ahmed *et al*., 2015). Significantly, natural tocopherol isoforms are shown to act in a very preventive and therapeutic manner against many forms of cancer and area unit still widely investigated for his or her potential effectuality during this sickness (Bartosz, 2008).

Materials and Methods

Animal study

Experiment performed on the fifty male rats of albino strain, weighing 250-300 g b.w. each and 12-14 weeks old obtained from the Laboratory Animal Colony, Al-Qasim Green University. Kept the rats in plastic cages under good hygienic conditions with free access to food and water Ad libitum for two weeks before starting the experiment for acclimatization. Atherosclerosis was induced in all groups except control group, as well as rats randomly divided into five equal groups.

First group (T1): animals in this group still normal without any treatment as negative control.

Second group (T2): animals in this group was administrated with 0.1 mg/kg.b.w of Atonik and treated with distilled water as positive group .

Third group (T3): animals in this group was administrated with 0.1 mg/kg.b.w of Atonik and treated with TPGS 100mg/kg.b.w for six weeks .

Fourth group (T4): animals in this group were TPGS 100mg/kg.b.w for six weeks.

The chromosomes aberration were prepared *via* direct method (Sharma and Talukder, 1987, Giri *et al*., 2002, Sharma *et al*., 2013) with some modifications, the procedure as follow:

Preparation the following solutions: Colchicine was freshly done by dissolving one colchicine tablet (1 mg) in 1 ml of sterilized physiological saline.

Table 1: Effect of Vitamin E TPGS on atonik compound induce cytogenic defect.

Cytogenic parameters %	C	T1	T2	T3
Micronuclei	4.6± 0.57B	8.77± 0.9.6 A	5.18± 1.44B	4.43±1.76B
Mitotic index	3.86± 0.89C	7.13±1.55A	5.97±0.56 B	2.99±0.88C
Mitotic index	3.86± 0.89C	7.13±1.55A	5.97±0.56 B	2.99±0.88C

The value represents mean± S E, N=5 for each group, Different capital letters indicated significant (P≤0.05) among groups.

Table 2: Effect of Vitamin E TPGS on atonik compound induce DNA damage.

Groups of experiment	% DNA in tail	Tail length mm
C	3.33±1.13C	7.5±0.66 C
T1	7.5±1.3 A	13.3±7.28 A
T2	5.10±1.15 B	9.85±0.54 B
T3	2.72±1.4 C	6.15±1.14 C

The value represents mean± S E; N=5 for each group, Different capital letters indicated significant (Pd" 0.05) among groups.

Fixative solution was freshly elaborate by added 3 portion of absolute methanol to 1 portion of glacial acetic acid (Lee and Elder, 1980).

The rats were administrated by intraperitonially with colchicine (0.5 mg/kg) B.W. to hold the divided cells at metaphase and break down the spindle fibers and left for (0.5-1) hours.

The rats were sacrificed by cervical dislocation, directly the femurs were separated and muscles were freed, sharp scissor cut the two ends of the femur bones until apparent of small opening in the bone marrow canal. Collected the cellular contents in a test tube using a disposable syringe 23 gauge and drag 5ml of PBS to collect cells of bone marrow with PBS until bone was become clear and incubated at 37°C for 10 min. then centerfugate at 1000 rpm for 5 min.

Discarded the supernatant, the cell sedeminate was suspended in warm (37°C) of 5 ml hypotonic solution (KCl, 0.075 M). Then incubated the tubes in a water bath (37°C) for 30-60 minutes with a gentle shaking every 5 minutes.

Centrifuged the tubes at 1000 rpm for 5 min., removed the supernatant and 5 ml of cold fixative slowly added to the sedeminate. The suspended of cells was left for 10 min. at room temperature, then again centrifuged at 1000 rpm for 5 min.

Removed the supernatant and slowly added 5 ml of cold fixative then left in ice bath for 5-10 min., the final step was repeated 2-3 time with attention last centerfuge discard and still 1 ml only of cell. 3-5 drops of the chromosome suspension using Pasteur pipette, were dropped in the height 75-120 cm NN over a clean, moist slide, then the slides, air dried and stained with Giemsa stain for 20 minutes and rinsed with tap water.

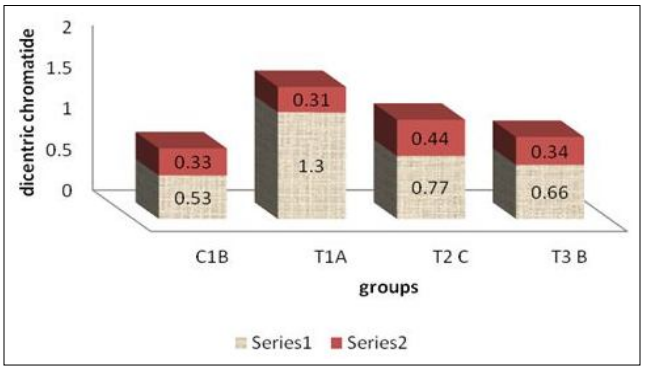

Fig. 1: Refer to effect of TPGS compound to reduced chromosomal aberration of atonik (long chromosome type).

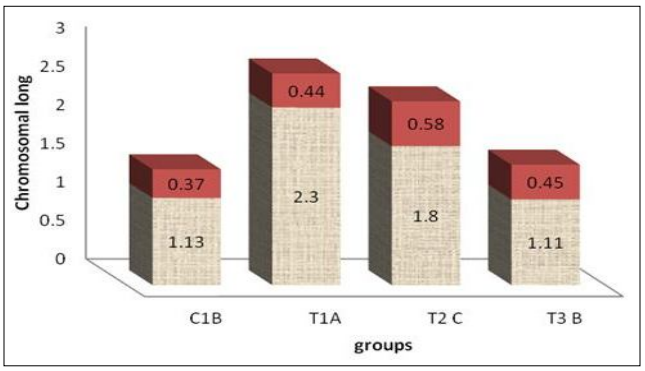

Fig. 2: Refer to effect of TPGS compound to reduced chromosomal aberration of atonik (dicentric chromosome type).

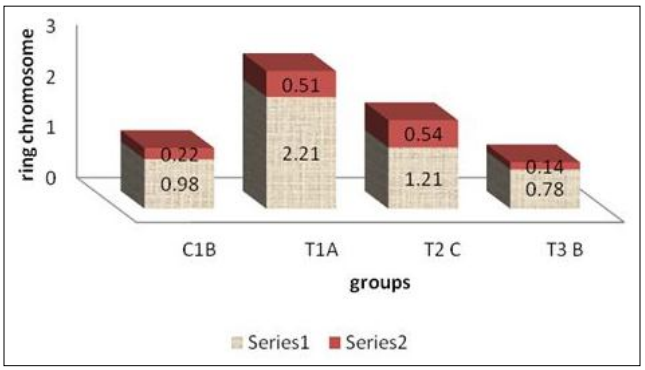

Fig. 3: refer to effect of TPGS compound to reduced chromosomal aberration of atonik (ring chromosome type).

Micronucleus Formation Assay

The present study was perform the appreciation of micronucleus formation, the procedure of MN (Çelik *et al.*, 2005) was apply, which is summarized in the following steps: cervical dislocation of the rats as sacrificed and dissected to get the femur. After both ends of the bone cutting, gathering the cellular content with a (3 ml) of heat inactivated human AB plasma using a disposable syringe 23 gauge. Beyond mixing gently, centrifuged (1000 rpm) the test tube for 10 minutes and removed the supernatant. The cellular sedeminate was mixed gently, perform a thin smear on a clean slide, then air-dried at

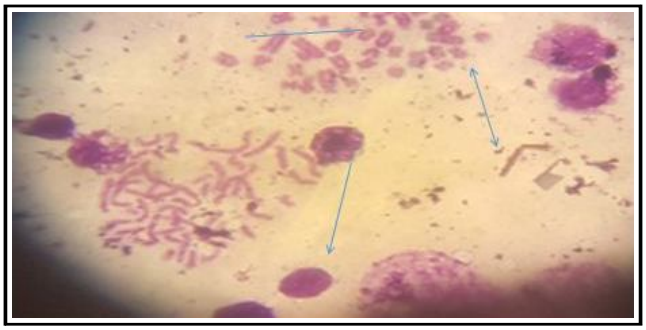

Fig. 4: Refer to long chromosome (→), ring chromosome (↔) and dicentric chromosome of bone marrow rats.

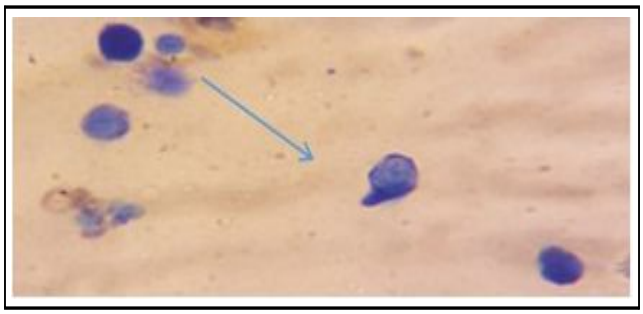

Fig. 5: Refer to micronuclei deformity of bone marrow rats.

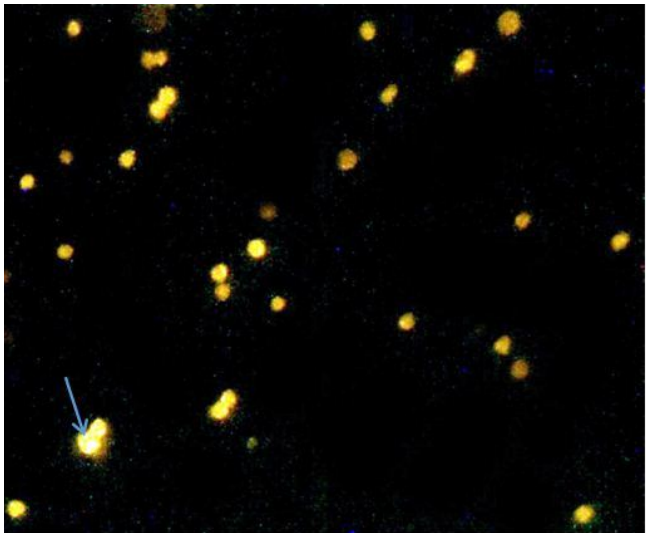

Fig. 6: Little number of bone marrow cell showed DNA damage from animal received TPGS X_{20}.

room temperature. Fixed the smear for 5 minutes by absolute methanol, then at room temperature, air-dried, finally Giemsa stain staining of the slides for 20 minutes, and tap water rinsed. Examined the slides under oil immersion, examined at least 1000 polychromatic erythrocytes (PCE) for the presence of micronucleus formation. The micronucleus index was calculated using the following equation (Kliesch *et al.*, 1981).

Micronucle us Index (micronucl eus/cell) = (Number of Micronuclei/Total Count of PCE) × 100

Results

The Micronuclei % and Mitotic index, % in rats received Atonik showed a significant increase ($P \leq 0.05$) in mean values (4.6 ± 0.57 and 3.86 ± 0.89) respectively when compared with the negative control group and all other treated groups. While the Micronuclei % and mitotic index in group (T2) that received atonik and vitamin ETPGS showed a significant reduction in mean value ($P \leq 0.05$) in mean values (5.18 ± 1.44 and 5.97 ± 0.56) respectively as compared with the negative untreated group, table 1. The percentages of long chromosome, dicentric chromosome and ring chromosome in bone marrow of atonik administrated rats after six weeks therapy were demonstrated in fig. (1, 2 and 3) respectively, showed increase significantly ($P \leq 0.05$) when compared to the negative control group, as well as the animals treated with TPGS (T2) and received TPGS alone (T3).

T1, T2 and T3 illustrated rats received atonik, received atonik and treated with Vitamin E ETPGS and animal received TPGS only.

T1, T2 and T3 illustrated rats received atonik, received atonik and treated with Vitamin E ETPGS and animal received TPGS only.

The present study revealed there is a significant reduction of $\leq$ in mean of % DNA in tail and DNA in tail in rats received atonik and treated by TPGS (T2) to record mean value (7.5 ± 1.3, 13.3 ± 7.28) respectively and (T3) that's rats treated by TPGS alone that reach to (2.72 ± 1.4, 6.15 ± 1.14) respectively.

Fig. 4-29: little number of bone marrow cell showed DNA damage from animal received reference Vorapaxar X20.

Discussion

In vivo, bone marrow cells, chromosome analysis has become a standard method to examine of the potential mutagenic effects of viruses, radiation chemical pollutants and drugs (Bakare *et al*., 2011). A dicentric chromosome is an aberrant chromosome having two centromeres. Forms of ring chromosome occurs when a portion of a chromosome has broken off and formed a ring or circle. The data in fig. (1, 2, 3 and 4) indicate the significant increasing in all types of chromosomes aberrations means in bone marrow cells and moreover, elevation in the percentage of chromosome aberrations in T1 group, compared with negative with groups of control, animal received atonik with TPGS showed a significant reduction ($P \leq 0.05$) in all chromosomal aberration than T1 group. The animal in the group (T3) that received TPGS alone showed there is no effect on bone marrow chromosomal aberration when compared with control. Our results showed high percent of chromosomal damage in all types of chromosome, this increased due to atonik herbal growth stimulant may be induced oxidative stress and hence breaks of DNA strand, loss and fragmentation of integrity of chromosoms. Animal that received treatment in both T2 that given atonik at 0.1 mg/kg b.w. showed clear prominent in among types of chromosomal aberration. Bone marrow is a completely vascularized tissue and it includes an inhabitance of rapidly cycling cells that can be easily processed and isolated, this test of chromosome aberration is particularly important to determine mutagenic hazard in that it enables respect of factors *in vivo* metabolism, DNA repair processes and pharmacokinetics, moreover these might differ amongst tissues and species (Kannan *et al*., 2014). The repairing in most types of chromosomal aberration after using TPGS due to antioxidant activity that mediated the defect as well as that act as scavenger activity of free radicals. Ascorbic acid and alpha-tocopherol can be used effectively in therapy either alone (antioxidants) or in combination with other agents like Vanadium Pentoxide to reduce its genotoxicity (Tarber and Atkinson, 2007, Brtosz, 2008, Jiang, 2014). Micronucleus test (MNT) has been widely used to discover a genotoxic effect of ionizing radiations and environmental pollutants in the mammalian system (Begum *et al.*, 2012). Micronuclei (MN) are bodies extra-nuclear, that damaged chromosome fragments contain or/and whole chromosomes, that after cell division, were not incorporated into the nucleus. Defects in the machinery of cell repair and accumulation of chromosomal aberrations and DNA damages can be induced MN (Luzhna *et al.*, 2013). Atonik administrated to animal induces an intense and acute damage (Authority, 2009). In the present study, showed that atonik-induced genotoxicity, in agreement with our data from the comet assay and chromomosomal aberration that may be due to oxidative stress as well as disturbance of enzymes and body imbalance of hormones that confirmed by Adnan *et al.,* (2016) that reported atonik at dose 0.1 mg/kg minimize reproductive activity. Confirmed act of TPGS as an anticancer agent, to induce apoptogenic activity against several types of cancer. TPGS lead to mitochondrial destabilisation for stimulation of mitochondrial mediators of apoptosis (Mrco *et al*., 2004, Neuzil *et al*., 2007). Many reports showed that alpha-tocopherol polyethylene glycol 1000 succinate (TPGS) has ability in preventing the retinal injury followed by ischemia-reperfusion (Aydemir *et al*., 2004).

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