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The Protective Role of the Date Palm Pollen (Phoenix Dactilyfera) on Liver and Haematological Changes Induced by the Diethyl Phthalate

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ABSTRACT

Phthalic esters are an important group of chemical agents mainly used as plasticizers and solvents. This study was designed to investigate the effects of DIETHYL phthalate (DEP) which is one of the phthalatic esters on the evaluation parameters. Blood can be used to explain the blood-related functions that can act as a pathological reflector and an indicator of the physiological state of animals, and it can also be used to assess liver enzymes such as (AST) and (ALT) which are considered to evaluate the changes in liver function and therapeutic potential of palm pollen grains (DPP) by these parameters. In a group of male rabbits (DEP) which were treated orally with a dose of (330 ml / kg / day) for 30 days, toxic effects on the haematological parameters such as hemoglobin (HB), hematocrit (HCT) and erythrocytes (RBC) were decreased. It was also noticed a significant increase in rates (ALT) and (AST) in the same group. The group of rabbits which were treated with the combination of DEP and DPP by successive doses (240mg / kg / day) and (330 ml / kg / day) showed an improvement in all of the parameters studied. These findings supported the hypothesis that the two functions of the blood and liver are particularly sensitive to DEP treatment, with DPP can mitigate the deleterious effects of DEP probably by activating the endocrine and antioxidants system.

Keywords: *Rabbit, Diethyl Phthalate, Palm Date Tree Pollen, Hematology, Transaminases.*

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1. INTRODUCTION

Phthalates are a class of chemicals widely used (Saillenfait & Laudet-Hesbert. 2005), and they have been considered as a group of environmental pollutants that present dangers to public health because of their high potential for human exposure and demonstrated toxicity in rodents (Liu et al., 2012). They have been widely used as plasticizers to induce flexibility in shaping materials like polymers (Wan et al., 2015). Esters of phthalic acid (phthalates) are the product of man because they are manufactured through the reaction of anhydrous phthalic acid with alcohols, and depending on the type of alcohol used, one obtains various phthalates (Calafat & McKee, 2006). The diethyl phthalate (DEP) has been used as a plasticizer for cellulose acetate and methacrylic acid, and in particular as a solvent and fixative of cosmetics (Fujii et al., 2005). (DEP) has been currently considered as belonging to the low molecular weight phthalate ester groups, it may be considered to be toxic after exposure to short and long terms (Kent et al., 2010).

Therefore, it seems logical to assume that antioxidants could prevent or at least reduce the toxicity of hematology and hepatotoxicity induced by diethyl phthalate with herbal medicines that are used in traditional medicine. Among these drugs, date palm pollen (DPP) is a therapy-based plant and a mixture of plant base. There are the male reproductive cells of flowers from (Phoenix dactylifera) family palmea (Hassan, 2011), and about 1000 tons of (DPP) are produced every year by millions of palm trees grown in the Arab Region (El-Neweshy, El-Maddawy & El-Sayed, 2013). They are widely used as a folk remedy for curing male infertility in traditional medicine (Yasir Arfat et al., 2014). The (DPP) is a good economical source of nutrition, it has been long used as a dietary supplement (Yasir Arfat et al., 2014; Abdi, Roozbeh, & Mortazavian, 2007). The phytochemical studies on (DPP) identified the presence of amino acids which are the major constituents of this plant, and also vitamins such as B1, B2, B12, A, E, and C, minerals such as zinc, selenium, iron and copper, and a considerable amount of rutin which was obtained from the alcoholic extract of the pollen. It has also been reported that (DPP) is rich in flavonoids such as catechin and quercénine (Tahvilzadeh & Hajimahmoodi, 2011). The (DPP) is comprised of steroid components such as estrone, estradiol and estrol (Tuğba Tatar & Yasemin Akdevelioğlu, 2018).

Many data suggested the hematological changes and changes in liver function induced by the toxic products such as: diethyl phthalate in laboratory animals which can be reversed by a therapeutic treatment with the date palm pollen grains (DPP). There has been little information on the protective role (DPP), hematological parameters, and liver functions after the exposure to diethyl phthalate.

Therefore, this experiment was designed to demonstrate the hematological and hepatological roles of (DPP).

2. MATERIALS AND METHODS:

Chemicals and Reagents:

Diethyl phthalate (C12 H14 O4) is also called (ortho-phthalate diethyl).

The study was done at the laboratory of the Biology Department in the University of Annaba.

The date palm pollen grains were obtained from Biskra market in Algeria which has been known for selling such products.

Animals :

16 male rabbits of the race néo_zélandaises aged between 5 to 6 months were selected to be studied. 2,700kg of bright peas were obtained from a breeding farm at random. The animals were placed in special cages for rabbits in the animal house in the biology department at the University of Oum El Bouaghi in normal conditions of temperature and humidity, with free access to food and water. For better adaptation to the environment, the rabbits were for 10d in the animal house before beginning the experiments.

Experimental design:

The rabbits were randomly divided into four experimental groups of four rabbits per group, the treatment was administered orally, the first group was used as control (groupeC), the second group was treated with a dose of (240 mg / kg / day) (DPP) dissolved in a little distilled water (groupeDPP), the third group was treated with a dose of (330 ml / kg / day) (DEP) diluted in 5 ml of water (group DEP) ; however, the fourth group received a combined treatment (240mg / kg / day) (DPP) and (330 ml / kg / day) (DEP) (group DPPDEP). All animals received their treatment daily on the basis of once a day. After weighing during 30 day, the animals were sacrificed and dissected, the liver was removed and weighed to estimate the relative weight.

The haematological and biochemical parameters:

The blood samples with EDTA anticoagulant were analyzed for hematological parameters including: red blood cells (RBC), hemoglobin (HB) hematocrit (HCT).

For biochemical parameters, the blood samples were centrifuged at 4000 rpm for 15 min, and the serum was separated, ALT (alanine aminotransferase) and AST (aspartate aminotransferase) were measured in serum.

Statistics :

The data were analyzed using the software (mintable 16). The statistical analysis was performed by Student's T to determine the significance between two means. the multiple comparison of the means was done using the analysis of variance for classification criterion (ANOVA), and

(P<0.05) was considered statistically significant.

3. RESULTS AND DISCUSSION :

In general, all the rabbits were healthy without any clinical signs of toxicity throughout the experimental period except a decrease in body weight which was noted in the group treated with DEP.

Liver weight:

The results showed that treatment with the DEP dose (330 ml / kg / day) showed no significant increase in the relative liver weight compared to the control. A non-significant improvement relative to liver weight was recorded in the rabbits treated with the combination of the DPP (240ml / kg / day) and DEP (330ml / kg / day).

This weight increase might be due to an enlarged liver, and it has been considered when taking the reduced body weight in other rabbits. These results were consistent with those obtained by (Brown et al., 1978) which revealed increased relative liver weight in rats treated with a high dose of DEP in a short-term.

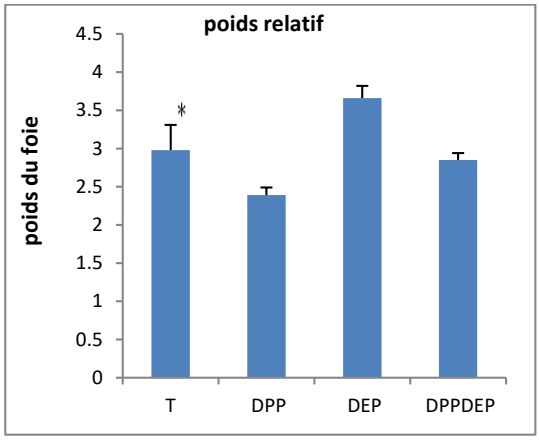


Figure 1 : Estimated (mean ± standard deviation) of relative weight of both male rabbits after 30 days treatment

(Student T)for the significant differances between the groups and the control

(ANOVA) for significant differances between groups

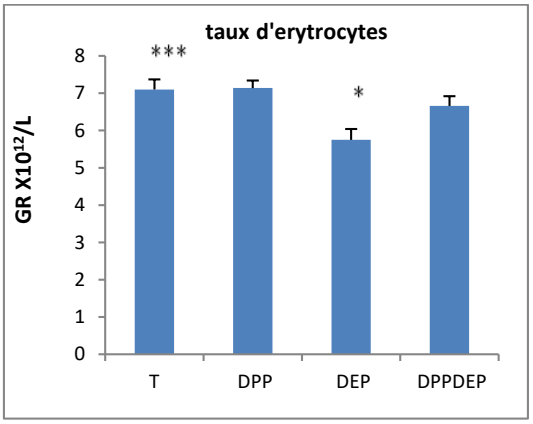
*(P< 0.05) significant

**: (p < 0.01) highly significant

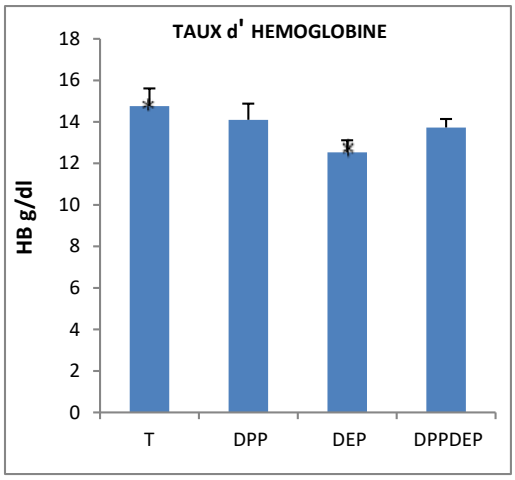
***: (p <0.001) very highly significant

Hematologic:

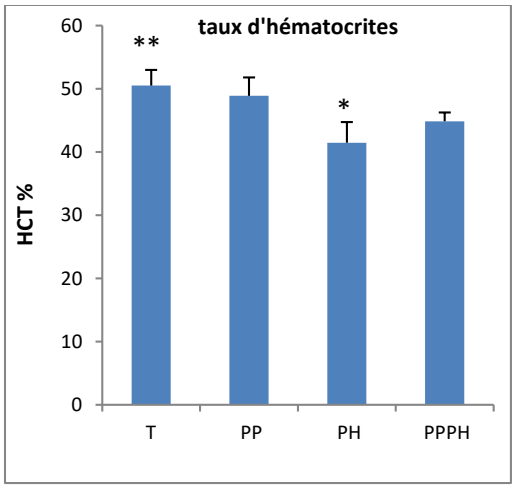
Compared to the control, a significant decrease was observed in erythrocyte group of hematological parameters (Rbc) hematocrit (HTC) hemoglobin (HB) in the rabbits treated by (DEP). The (DPP) treatment gave no significant differences with the control group.However, the rate of haematological parameters in the treated group (DEP) was improved by the supplementation of DPP (group 4).



(a)



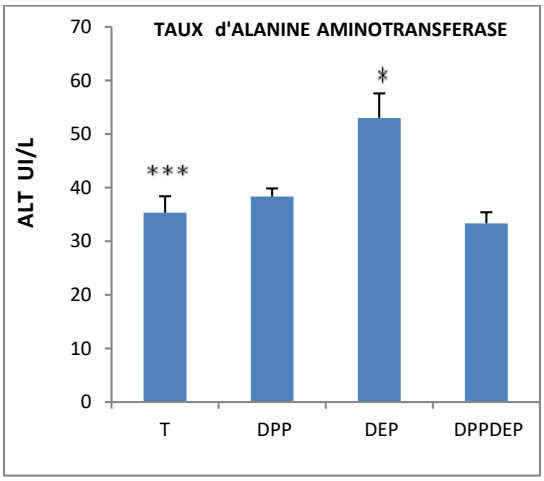
(b)



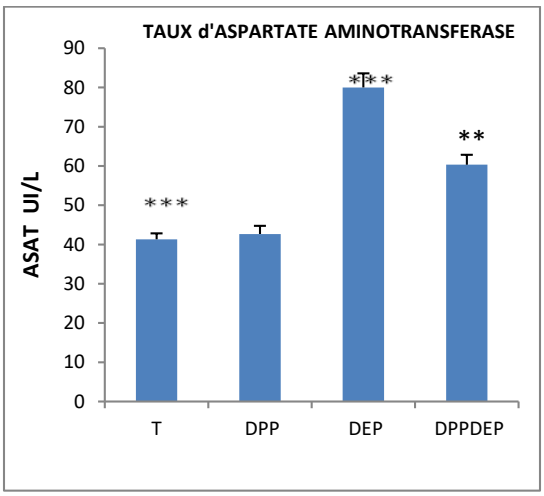
(c)

Figure 2 a, b, &c: Estimated (mean ± standard deviation) hematologic parameters in rabbits after processing 30 day statistics given in Figure 1

The decrease in hematologic parameters might be due to the toxic effect of (DEP), and the decrease in (HB) was a microcytic anemia indicator of impaired oxygen supply in tissues that could lead to a long metabolic rate and low energy production.
The decrease in the rate of (GR) indicated the stress caused by anemia status (DEP).
The results were consistent with previous reports (Poopal et al., 2017), confirming that the adition of DEP had sulétales concentrations that could cause adverse effects on hematological parameters such as (Rbc) (HTC) (HB) Fish (Cyprinus carpio). Moreover, other studies have found significant decreases in rates of (GR) and (HTC) which were observed the rats in treated with (DEHP), and 500mg (HB) was reduced in the group treated with 250mg (DMP) (Seung Jun Kwack et al., 2009).
Rates of (ALT) and (AST) were studied to assess changes in liver functions.
The concentrations of transaminases have increased significantly ($p<0.05$) in the group treated with (DEP) compared to the control; the group treated with (DPP) gave no significant differences ($p> 0.05$) compared to the control; however, an improvement in rates of (ALT) and (AST) in the (group 4) which were treated with the combination of (DPP) and (DEP) was observed, but not in the control.



(a)



(b)

Figure 3a & b: Estimated (mean± standard deviation) of transaminases in rabbits after processing 30 day statistics given in Figure 1

The increase in the transaminases indicated the liver dysfunction that can be attributed to the toxic effect of (DEP). The results were consistent with those obtained by (Nivedita Ghorpade et al., 2002) which confirmed a significant increase in transaminases after the treatment with (DEP) in fish (Cirrhina mrigala) changes in liver enzymes known to indicate liver damage (Brautbar & Williams, 2002). The activity of liver enzymes has been connected to the hepatocyte increase and their operation can be affected by an increased synthesis in the presence of increased biliary pressure (Jawad Kadhim Araak & Maisa Ala Abdulhussein, 2012).
A significant improvement in the haematological and biochemical parameters was registered in group 4 which was treated with the combination of (DEP and DPP) implying that

the DPP had an antioxidant effect. Evidence indicated that flavonoids had a stimulatory activity of erythropoietin (Jorum, Piero & Machocho, 2016). Quercetin and catechin have been the most widely known flavonoids in plants including (DPP) which had protective effects that might be due to its antioxidant capacity of hematological and hepatotoxic parameters that can stabilize membranes decreasing their fluidity (Fatma GokceUzun & Yusuf Kalender, 2013). Flavonoids improved hematological parameters, increasing the splenic tissues and the availability of iron and expression of feroportine (Maryam Mazhar et al., 2017). The deficiency of vitamin B12 can impair the erythrocytes and increase their volumes (Gülali Aktas et al., 2014).

It has been reported that vitamin (C) has an antioxidative potential that positively promotes the liver in the regulation of hematopoiesis and regeneration of erythrocytes implying the protective effect of haematotoxic (Hounkpatin et al., 2012). Research has shown that vitamin (C) has hepatoprotective properties. This has been certainly related to its antioxidant property, and vitamin (C) might reduce liver damage induced by certain chemicals in particular in animals. It can normalize aminotransferase levels and can also keep 100% of cellular integrity and modulated (ALT) and (AST) (Elias Adikwu & Oputiri Deo, 2013).

Vitamin E has been recognized as the main soluble lipid and an antioxidant that prevents free radicals from initiating tissue damage (Suna has Kalender et al., 2010).

4. CONCLUSION :

In this study, it was found that a dose of phthalic diethyle (DEP) (330m/kg/day) can induce significant damages in hematological and hepatological parameters in a remarkable way, and the treatment with DPP (240mg/k/day) administered with DEP (330ml/kg/day) for 30 days effectively prevented the harmful effects of (DEP), indicating that the therapeutic administration with DPP can improve the oxidative stress induced by the DEP.

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