

Effect of Long Term Administration of Atonik Compound in Female Rabbits on Hematological and Pathological Changes in Important Functional Body Organ

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Abstract

Atonik is a synthetic bio stimulant consist of three phenolic compounds used as stimulant plant growth and development, farmers used atonik with high abuse for enhancements of fruit maturity so study done to detect the harmful effect of atonik in laboratory animal.

Eight female rabbits of weighting (1.4-1.6) kg were obtained from animal house in college of Veterinary Medicine, animal housed two weeks before beginning study and all laboratory condition was taking into consideration. Animal randomly divided in to two main groups: Control group still without treatment at all period of study, Atonik group: each rabbits orally received 0.1 mg/kg /three time weekly (TIDW). Our result disturbance of hematological parameters specially WBCS, RBCS, HB and lymphocyte. Moreover histopathological section showing dilation of the central vein and filled with fluid and granuloma beside it, with vaculation of hyapatocyte. Kidney reveals coagulative necrosis of renal tubules with distraction of glomerular tuft. Uterus of rabbits treated with 0.1 mg/kg bw atonik for 8 weeks showing necrosis of unrein glands. Ovary showed increase in the numbers of attratic follicles. Our result concluded that atonik has very harmful effect for reproduction, liver, and kidney.

Keywords: Atonik; Ovary; Uterus; Nitro phenol

Introduction

Atonik is a synthetic bio stimulant consist of three phenolic compounds: sodium para-nitrophenolate PNP (0.3%), sodium ortho-nitrophenolate ONP (0.2%) and sodium 5nitroguaiacolate 5NG (0.1%), and water. Atonik used as stimulant plant growth and development, particularly generative [1,2]. The fresh weight and dry matter, and production are increased so that there is a higher leaf area, with rich chlorophyll content, supported by an improvement of chlorophyll a fluorescence parameters (Figure 1). The application of Atonik played simulative role under optimal conditions and protective against spring frost, drought, and noble metal stresses [3]. The positive effect of Atonik is much more preferable when plants are growing under stress conditions. The mechanism of action through increase nutrient uptake by increase cytoplasmic streaming and prolongation of auxin activity via inhibiting the indolylacetic acid and abscissic acid) that increases of the nitrate reductase activity [4]. Although importance in plant regulator some residue of agent may be not excreted from plant specially if given for fruit that may lead to toxicological effect on animal and human body tissue Atonik when taken orally well absorbed from gastric intestinal tissue and reach to blood circulation within 30 minute with an high of the ratio blood/plasma at 2 hours. Widely distributed with the highest concentrations in carcass, kidney, liver and the intestinal tract, excreted through urine. Sodium orthonitrophenolate (OPP) also used as fungicides after harvest treatment of citrus fruits and as disinfectants and preservatives [FAO]. Moreover widely used in agricultural and disinfectants in food utensil [5] reported that rats and mice treated with multi dose of OPP (0,25.10,25) reveal diminish fetal body weight, high incidences in

delayed skeletal ossification and Percent postimplantation loss (12.2; 16.7; 19.2; 18.3) respectively. In addition, fetal mice treated with SOPP showed malformation (mice: cleft plate; rabbits: resorptions). P-Nitrophenol (PNP) is a major metabolite of some organophosphorus such as parathion and methyl parathion [6].

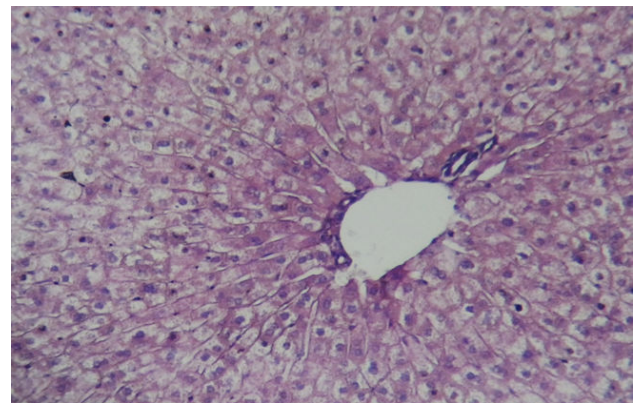


Figure 1: Histopathological section of control groups showing normal histological structures.

In fact PNP is an intermediate chemical agent wildly used in the manufacture of some drugs, fungicides, dyes, and consider one organic pollutant [7]. High abuse of organ phosphorus lead to bioaccumulation of PNP [8]. Therefore OPs insecticides abuse increases humans, livestock, and wild animals exposure to PNP toxicity, which lead to severe environmental pollution and increase

health problem [8,9]. PNP has an adverse effect on endocrine system in male via inhibition of androgen and estrogenic activity was record in vitro and in vivo. Moreover 3-methyl-4-nitrophenol and 4-nitro-3-phenylphenol at dose 100 mg/kg PPMC and 0.1 and 1.0 mg/kg respectively to ovariectomized immature female rats subcutaneously for 2 days showed significant increases in uterine weight and estrogenic activity. The purpose of the present study was to detect the effects of atonik orally administrated to rabbits on the hematological and histological of important internal organ moreover due to widely abused the compound for hastening plant growth specially Fruits such as Figs which maturity period very short then consumed by human.

Material and Method

Animal study

Twelve's female rabbits of weighting (1.4-1.6) kg were obtained from animal house in college of Veterinary Medicine, animal housed two weeks before beginning study and all laboratory condition was taking into consideration.

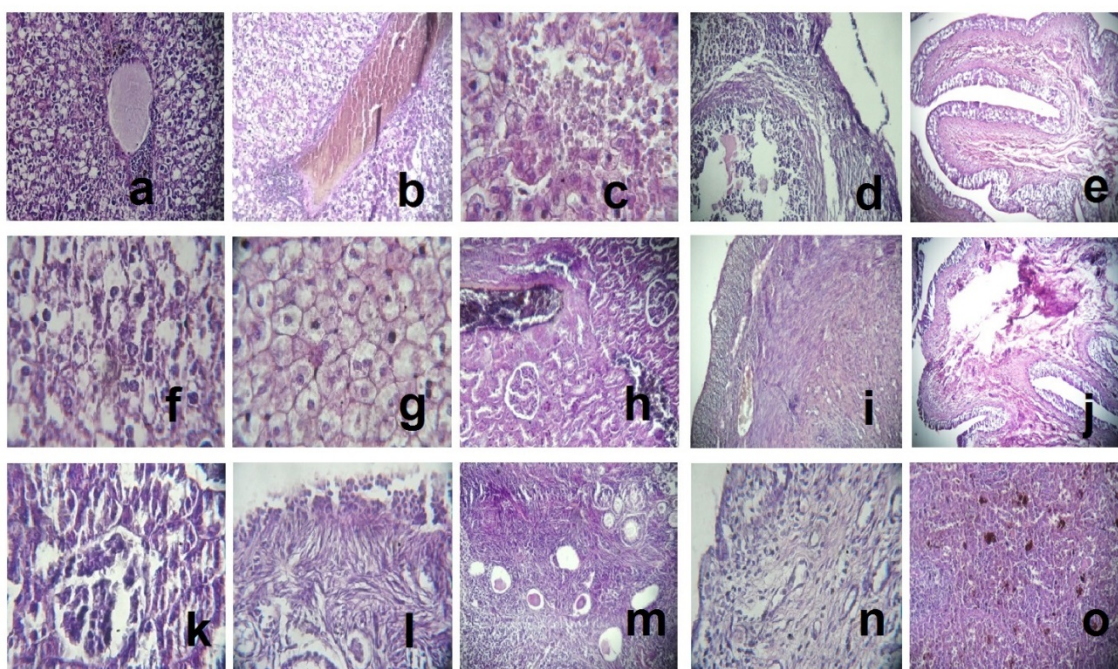


Figure 2: (a) Histopathological section of liver of rabbits treated with 0.1 mg/kg bw of atonik for 8 weeks showing dilation of the central vein and filled with fluid and granuloma beside it, with vacuolation of hepatocyte. (b) Treated with for 8 weeks showing seve 0.1 mg/kg bw atonik for dilation and congestion of the central vein with inflammatory cell infiltration. (c) Treated with 2 mg/kg bw 0.1 mg/kg bw atonik for 8 weeks showing distraction of hepatic tissue with hemorrhage. (d) Treated with 2 mg/kg bw sodium nitrophenolate for 8 weeks showing sloughing of epithelial cells lining, with degeneration of granulosa cells. (e) Histopathological section of oviduct for control groups showing normal histological structure. (f) Treated with 0.1 mg/kg bw atonik for 8 weeks showing necrosis of hepatic tissue. (g) Treated with 0.1 mg/kg bw atonik for 8 weeks showing hydropic degeneration of hepatocyte (h) histopathological section of kidney of rabbits treated with 0.1 mg/kg bw atonik for 8 weeks showing dilation and congestion of the renal artery with hemorrhage. (i) Histopathological section of uterus of rabbits treated with 0.1 mg/kg bw atonik for 8 weeks showing congestion of uterine blood vessels. (j) Treated with 0.1 mg/kg bw atonik for 8 weeks showing severe distraction of lamina propria with sloughing of epithelial cell lining. (k) Treated with 0.1 mg/kg bw atonik for 8 weeks showing coagulative necrosis of renal tubules with distraction of glomerular tuft. (l) Histopathological section of ovary of rabbits treated with 0.1 mg/kg bw atonik for 8 weeks showing squamous metaplasia of epithelial cells lining the ovary. (m) Treated with 2 mg/kg bw sodium nitrophenolate for 8 weeks increase in the numbers of atretic follicles. (n) Histopathological section of uterus of rabbits treated with 0.1 mg/kg bw atonik for 8 weeks showing necrosis of unrein glands. (o) Histopathological section of spleen of rabbits treated with 0.1 mg/kg bw atonik for 8 weeks showing depletion in lymphoid follicles and hemosiderosis.

Animal randomly divided in to two main groups:

1. Control group still without treatment at all period of study
2. Atonik group: each rabbits orally received 0.1 mg/kg /three time weekly (TIDW) at end of experiment blood sample was obtained via ear technique and xylazine-ketamine anesthesia were used for

hematological parameters with scarified animal for taken specimen (liver, kidney, ovary) were fixed immediately in 10% formalin.

Atonik solution was purchased from agricultural officer in AL-Qassim city, from japan company origin. The result was showed increase of WBCS, RBCS, Lymphocyte and Granulocyte of atonik treated group (Figure 2) (Table 1).

Discussion

In the present study the experimental groups showed disorganized hepatic architecture with marked affection of the hepatocytes due to periportal fibrosis. Liver fibrosis represents the final common pathway of almost all types of chronic liver diseases characterized by excessive connective tissue deposition in extracellular matrix (ECM). ROS can activate fibrogenic gene expression and transforming growth factor (TGF- β 1) signaling pathway, which is known to play major role in the activation of hepatic stellate cells (HSCs) in liver fibrosis [10]. The result was showed increase of WBCs, RBCs, Lymphocyte and Granulocyte of atonik treated group that may be due to activity of agent as androgenic action induces erythrocytosis by stimulating erythropoietin production. The results showing that the animals exposed to sodium nitrophenolate exhibited extensive area of coagulative necrosis with severe dilation and congestion of blood vessels in both liver, kidneys and uteri. Necrosis may result from the accumulation of sodium nitrophenolates in the mitochondria and lysosomes progressive hepatocyte organelles damage, cellular degeneration and necrosis [11]. Moreover hypoxia in the perivenular region with increase in hepatic oxygen demand without an appropriate in hepatic blood flow similar results were obtained by Machida et al. [12-15]. Previous study carried by Sikka et al. [16] indicated that necrotic lesions can be a cause of oxidative stress induced by sodium nitrophenolates. this results agree with some studies carried by Boehncke et al. [17] whom reported the congestion in liver and kidneys and ulcers of the stomach in high doses in rats others noted many effects including decreased in body weight gain, differences in organ weights, and focal fatty changes of livers, this changes related to the kind of substrates that comes to the reaction, also its localization in the hematological examination, clinical chemistry and histopathological examination of the mager organ and tissues did not give any indication of a substance- related toxic effected in compassion with control . Recent study reported that quails orally given (0.01, 0.1, 1) mg/kg b.w. of PNP for 75 days. Liver and plasma samples were collected at days 45 (45, 60, 75 day) The results of liver showed lymphocytic infiltration, congestion of blood vessels and degeneration of hepatocytes also positive increases in corticosterone levels were record . Moreover After60-days of in vivo exposure, the quills exhibited an overexpression in the liver CYP1A4, 1B1, AhR1, and HO-1. Furthermore, with 75 day, an overall down regulation of the tested genes was observed. In vitro, although a significant overexpression of CYP1A4, 1B1, and HO-1 was observed, CYP1A5 was down regulated [8] (Figures 3 and 4).

The histopathological changes of spleen tissues showing depletion of lymphoid tissue and that can be related to the effect of the compound which cause depression in immune system. Similar results were obtained by Boehncke et al. [17-18] whom reported that Administration of 4-nonylphenol to rats in doses of 125-375 mg/kg of body weight caused changes in the activity of the immunological system. The current study showed that sodium nitrophenolate toxicity leading to significant changes in both uteri and ovaries could lead to infertility however with regard reproductive toxicity testing.

The present study showed follicular atresia this may be due to sodium nitrophenolates that disturbed the reproductive hormones, this result agreed with Koerdel et al. [19-25] who reported that Phenols also affect the function of the hormonal system. Some phenols are capable of disturbing sexual hormones function, which finally may lead to sterility of animals and humans.

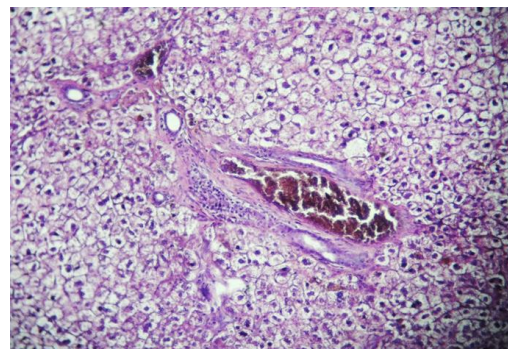


Figure 3: Histopathological section of liver of rabbits treated with 0.1 mg/kg bw atonik for 8 weeks showing congestion and thickening of portal area with inflammatory cell infiltration.

Traits	First group (Control)	Second group (Atonik)	Mean \pm SE
No. of rabbits	6	6	6
WBC ($\times 10^3$ cells/ml ³): *	6.3250 \pm 0.31 B	7.9000 \pm 0.43 A	7.1125 \pm 0.37
RBC ($\times 10^6$ cells/ml ³): NS	4.3650 \pm 0.53 A	5.0525 \pm 0.32 A	4.7087 \pm 0.42
Hb (gm): NS	8.77 \pm 0.31 A	6.80 \pm 1.70 A	7.78 \pm 1.00
PCV (%): **	23.50 \pm 1.44 B	28.47 \pm 0.62 A	25.98 \pm 1.03
Lymphocytes (%): **	37.26 \pm 2.81 B	54.57 \pm 4.08 A	45.91 \pm 3.44
Monocyte (%): NS	7.52 \pm 0.39 A	8.90 \pm 1.22 A	8.21 \pm 0.80
Granulocytes (%): *	55.07 \pm 1.54 A	47.87 \pm 2.65 B	51.47 \pm 2.09

The traits that carried different letters horizontally indicate significant differences at probability 0.05 or 0.01.
 NS non-significant
 *Significant differences at 0.05
 **High significant differences at 0.01

Table 1: Effect of atonik on some blood parameters of local rabbits.

In another experiment bisphenol A caused protein expressions in TM4 cells in mice, which play a key role in spermatogenesis. It was noted that viability of cells decreased 10 to 70% after exposure to doses of 50-250 μ M/kg of body weight over 16 hours. Obtained results showed that bisphenol A may induce infertility in mice. In an oral study with rats, 2- nitrophenol induced developmental effects in the offspring only at doses that also produced maternal toxicity [17]. Trisomboon, et al., [26] reported that 4-Nitro-3-phenylphenol possess both androgenic and anti-androgenic effect, treatment with agent showed not raise of FSH and LH secreted from the pituitary cells without GnRH activation (Figure 5). contrariwise, 10-5-10-7 M of agent for 1 day can increase hormones when the cells were stimulated with GnRH. on the other hand leydig cell culture treated with (10-5-10-9 M) of agent Appear significantly Testosterone increased, in the presence or absence hCG. Immature female rats administrated

subcutaneously with 10, or 100 mg/kg PNP daily for one week, reveal significant increases in uterine weight [9].

Our results therefore suggest that atonik can lead to accumulation of main compound specially PNP in water, and soil, so that could have serious harmful effects on wildlife and human health. histopathological section of kidney of rabbits treated with 2 mg/kg bw sodium nitrophenolate for 8 weeks showing dilation and congestion of the renal artery with hemorrhage accompanied with coagulative necrosis of renal tubules with distraction of glomerular tuft.

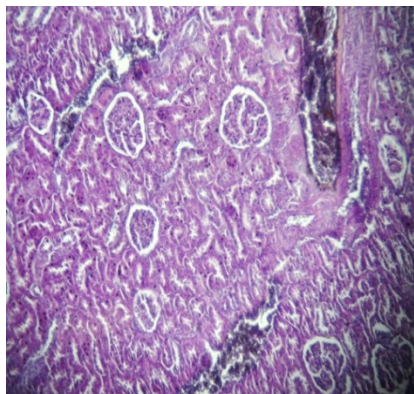


Figure 4: Histopathological section of kidney of control groups showing normal histological structure.

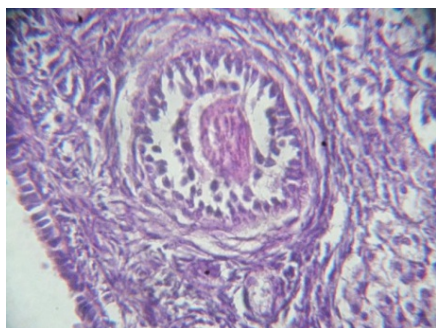


Figure 5: Histopathological section of ovary of control groups showing normal histological structure.

Dilation of the urinary space of the renal corpuscles in atonik treated rabbits because of basement. Membrane alterations and proximal convoluted tubules epithelial destruction so decrease the physiological functional leading to alteration of glomerular filtration and the accumulation of fluid in the urinary space. Our study was concord with Tootian et al. [15,25] that demonstrated high percent of creatinine, blood urea nitrogen, alkaline phosphatase which consider waste products of protein metabolism that excreted by the kidney and give indicators of kidney destruction. In other study was Conducted on rat reveal that nitrophenol compound cause deformity in the shape of mitochondria with dense deposits in the basement membranes of the glomeruli was noted in electron microscope [15,27,28].

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