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Therapeutic Efficacy of *Nitraria retusa* Fruit Against Hematological and Mineral Profile Disorders in Penconazole-Exposed Rats

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Abstract: Our study investigated the protective effect of *Nitraria retusa* (*N. retusa*) fruit against penconazole-induced changes in blood hematological and mineral profiles in rats. Rats were treated either with penconazole (67 mg/kg body weight), *N. retusa* fruit aqueous extract (300 mg/kg body weight) or with penconazole associated with *N. retusa*. Penconazole was administered intraperitoneally every 2 days from day 7 until day 15, the sacrifice day, while *N. retusa* extract was administered daily by gavage during 15 days. Compared to the controls, the penconazole-treated group showed significant differences in several hematological parameters, including a decrease in red blood cells' count, hemoglobin concentration and hematocrit value, and an increase in white blood cells and platelets' counts. Moreover, iron, calcium and magnesium plasma levels decreased. *N. retusa* administration to penconazole-exposed rats improved the blood hematological and mineral profiles. Our data indicated that *N. retusa* fruit might be favorable to avoid changes in blood hematological and mineral parameters associated with penconazole exposure.

Keywords: Penconazole, Rats, Hematological and Mineral Profiles, *Nitraria retusa*

1. Introduction

Blood is the most important body fluid governing vital functions such as respiration, circulation, excretion, osmotic balance and the transport of metabolic substances. It is known to exhibit pathological changes before the onset of any external toxicity symptoms [1]. Many studies have demonstrated significant changes in fish and rodents' blood variables as a result of exposure to environmental pollutants like pesticides [2-4]. Among them, penconazole, a systemic triazole fungicide, is commonly used in horticultural, agricultural and forestry industries for powdery mildew control [5]. Due to its widespread use, its residues have been detected in multiple environmental and human matrices, raising the issue of possible health risks to the general population. Toxicological studies have shown that penconazole exerts harmful effects, involving free radicals damage, in several mammalian soft tissues such as the liver [6, 7], the testes [7, 8], the kidney [7, 9], the heart [10], the brain [11], and the lung [12]. Adverse effects of this fungicide on some organic and enzymatic components in blood of adult rats have been reported [6, 7, 9, 10]. Indeed, penconazole induced in the blood of exposed rats significant changes in protein, glucose, triglyceride, creatinine, urea and

uric acid levels. Moreover, alterations in aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, gamma glutamyltranspeptidase and lactate dehydrogenase activities have been observed. However, there are no data concerning penconazole impact on blood hematological and mineral parameters.

It is well documented that medicinal plants have long served as a natural and useful source of therapeutic agents against blood parameters alteration [13-15]. *Nitraria retusa* (*N. retusa*), used as a traditional medicinal plant, is a salt-tolerant and drought resistant shrub commonly known as "Ghardaq" in Tunisia. Its fresh leaves in decoction are employed in the treatment of poisoning, upset stomach, ulcers, gastritis, colitis and colonic abdominal pain [16], while its dry leaves serve to make cataplasms owing to their anti-inflammatory properties. Besides, the ashes of this plant have the ability to remove liquids (blood, lymph) of infected wounds [17]. *N. retusa* also produces red fleshy edible fruits from which a tasty and refreshing juice may be extracted [18]. Previous researches on the fruit extracts of this species have shown that they contain bioactive phytochemicals, like polyphenols, with interesting biological properties such as antioxidant effects [19, 20].

In the present study, we aimed to elucidate the adverse effects of penconazole exposure on blood hematological and mineral parameters in adult rats. The protective role of *N. retusa* fruit aqueous extract on these parameters in penconazole-exposed rats was also investigated.

2. Materials and Methods

2.1. Chemicals

In the present study, we used the commercial formulation Topas 100 EC, containing 100 g/L (w/v) of the active ingredient penconazole (Syngenta, Bâle, Switzerland).

2.2. Plant Material

N. retusa fresh ripe fruits were collected from Kerkennah Island (Sfax, Tunisia) and identified in botanic laboratory (Faculty of Sciences, University of Sfax, Tunisia), according to the Flora of Tunisia [21].

2.3. *N. retusa* Aqueous Extract Preparation

The aqueous extract of *N. retusa* fruit, prepared as previously described [9], was used in the present study to evaluate its beneficial effects on blood hematological and mineral parameters in penconazole-treated rats.

2.4. Animals

Twenty four male Wistar rats, with an average weight of 250 g, were purchased from the Central Animal House (SIPHAT, Tunisia) and kept under controlled temperature (22 ± 2°C), humidity (40%) and light conditions (12 h light/dark cycle). A standard laboratory pellet diet and distilled water were given *ad libitum*. Experimental procedure was approved by the Ethical Committee of Sciences Faculty of Sfax, with ethics approval number 1204, and in accordance with the International Guidelines for Animal Care [22].

2.5. Experimental Design

The rats were divided into the following four groups of six each:

a. Group 1 (Controls): serving as negative controls.
b. Group 2 (PEN): No treatment was performed during the first six days, then rats received intraperitoneally penconazole at 67 mg/kg body weight (bw) every two days from day 7 until day 15, the sacrifice day.
c. Group 3 (NRE+PEN): Rats received daily by gavage during the first six days 300 mg/kg bw of *N. retusa* extract. Then, from day 7 until day 15, they received both the aqueous extract of *N. retusa* (300 mg/kg bw) daily by gavage and penconazole (67 mg/kg bw) as explained previously.
d. Group 4 (NRE): Rats received daily by gavage *N. retusa* aqueous extract (300 mg/kg bw) for 15 days.

The dose of penconazole used in the present treatment represented 1/30 of LD_{50}. It was selected on the basis of the previous study of El-Sharkawy and El-Nisr [8]. These authors have shown that penconazole induces testicular dysfunction in adult rats when administered at 50 mg/kg bw and 100 mg/kg bw. Thus, we have chosen an intermediate dose of penconazole which produced toxicity in rats without lethality. A dose higher than 67 mg/kg bw provoked hemorrhage and diarrhea.

As for *N. retusa* extract, we have shown in a preliminary dose-response study performed in our laboratory that a dose higher than 300 mg/kg bw causes diarrhea in exposed rats, which could be due to the presence of fibers in the fruit pulp, as demonstrated by Hegazy et al. [19].

At the end of the treatment period (15 days), all rats were sacrificed by cervical dislocation to avoid stress. One fraction of blood was immediately collected into EDTA-containing tubes for full blood count. Another fraction, collected in heparin-coated tubes, was centrifuged at 2200 *x g* for 10 min to obtain plasma samples for analysis of mineral parameters.

2.6. Evaluation of Hematological Parameters

Total red (RBC), white (WBC) and platelet (Plt) blood cells count, hemoglobin (Hb), hematocrit (Ht), mean cell volume (MCV) and mean corpuscular hemoglobin (MCH) values and mean corpuscular hemoglobin concentration (MCHC) were determined with the use of an automatic hematological assay analyzer (Beckman Coulter, USA).

2.7. Evaluation of Mineral Parameters

Plasma iron, calcium, phosphorus and magnesium levels were assayed spectrophotometrically using commercially available diagnostic kits (Biomaghreb, Tunisia, Ref 20064, 20051, 20084, 20074, respectively).

2.8. Statistical Analysis

The results obtained were presented as means ± standard deviation (SD). They were analyzed using the statistical

package program Stat view 5 Software for Windows (SAS Institute, Berkley, CA). Statistical comparisons between groups were made by means of one-way analysis of variance (ANOVA) followed by Fisher protected least significant difference (PLSD) test as a post hoc test. Differences were considered statistically significant if $p<0.05$.

3. Results

3.1. Hematological Parameters

Compared to the controls, a significant decrease by 5% in RBC count, Hb concentration and Ht value was observed in penconazole-treated rats. However, no significant changes were recorded for MCV, MCH and MCHC following penconazole exposure. Meanwhile, a significant increase was observed in total WBC (+47%) and Plt (+27%) counts in penconazole-exposed rats, compared to the controls (Table 1). Administration of *N. retusa* extract attenuated alterations induced by penconazole by increasing RBC (+10%), Hb (+6%) and Ht (+12%) values and decreasing total WBC (-29%) and Plt (-6%) counts, when compared to those of PEN group.

Table 1. *Hematological parameters of control and treated rats with penconazole (PEN), N. retusa (NRE) or their combination (NRE+PEN).*

Parameters and treatments	Controls	PEN	NRE+PEN	NRE
RBC (10^6/µL)	9.18 ± 0.20	8.68 ± 0.43*	9.51 ± 0.38^{++}	9.27 ± 0.43
Hb (g/dL)	15.17 ± 0.36	14.35 ± 0.75*	15.28 ± 0.46^{+}	14.97 ± 0.53
Ht (%)	47.27 ± 0.70	44.70 ± 1.46**	50.10 ± 1.95^{**+++}	48.40 ± 1.98
MCV (mm^3/RBC)	51.47 ± 0.35	51.45 ± 1.83	52.66 ± 0.62	52.23 ± 1.14
MCH (g/dL)	16.57 ± 0.74	16.53 ± 0.64	16.10 ± 0.28	16.15 ± 0.58
MCHC (g/dL)	32.13 ± 1.22	31.98 ± 0.83	30.53 ± 0.30	31.13 ± 0.48
WBC (10^3/µL)	13.72 ± 1.44	20.23 ± 2.81***	14.40 ± 2.98^{++}	14.35 ± 2.64
Plt (10^3/µL)	880.67 ± 25.76	1121.33 ± 66.07***	1051.00 ± 36.03^{***+}	865.83 ± 17.45

RBC: red blood cells; Hb: hemoglobin; Ht: hematocrit; MCV: mean cell volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; WBC: white blood cells; Plt: platelets.
Values are means ± SD for six rats in each group.
PEN and NRE+PEN groups vs control group: $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$.
NRE+PEN group vs PEN group: $^{+}p<0.05$; $^{++}p<0.01$; $^{+++}p<0.001$.

3.2. Blood Mineral Parameters

Following penconazole treatment, we noted a marked decrease in blood inorganic components like iron (-39%), calcium (-15%) and magnesium (-37%), while phosphorus level showed no significant difference, as compared to the controls (Table 2). Interestingly, *N. retusa* extract was found to alleviate the changes of mineral profile in penconazole-exposed rats by enhancing plasma levels of iron (+38%), calcium (+12%) and magnesium (+32%), without reaching control values.

Table 2. *Iron, calcium, phosphorus and magnesium plasma levels in control and treated rats with penconazole (PEN), N. retusa (NRE) or their combination (NRE+PEN).*

Parameters and treatments	Controls	PEN	NRE+PEN	NRE
Iron (µmol/L)	25.25 ± 2.13	15.33 ± 1.84***	21.10 ± 2.5^{**+++}	27.32 ± 2.58
Calcium (mmol/L)	2.34 ± 0.17	1.98 ± 0.13**	2.22 ± 0.22^{+}	2.45 ± 0.12
Phosphorus (mmol/L)	2.24 ± 0.25	2.40 ± 0.16	2.38 ± 0.18	2.24 ± 0.10
Magnesium (mmol/L)	0.75 ± 0.08	0.47 ± 0.11***	0.62 ± 0.12^{*+}	0.67 ± 0.10

Values are means ± SD for six rats in each group.
PEN and NRE+PEN groups vs control group: $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$.
NRE+PEN group vs PEN group: $^{+}p<0.05$; $^{+++}p<0.001$.

4. Discussion

To the best of our knowledge, the present study constitutes the first attempt to evaluate penconazole effects on blood hematological and inorganic components. Our results regarding the erythrocyte profile revealed a significant decrease in RBC, Hb and Ht values in penconazole-treated rats, indicating the ability of this fungicide to induce anemia. This could be explained by the inhibition of erythropoiesis and the increase in the rate of erythrocytes destruction in hemopoietic organs. Indeed, pesticides residues have been demonstrated to cause anemia by interfering with Hb biosynthesis and shortening the life span of circulating erythrocytes [23].

Additionally, we observed a marked fall in plasma iron level after penconazole administration. This reduction could explain in part the observed anemia. In fact, approximately 65% of total body iron is associated with Hb [24]. Iron deficiency in the body limits the synthesis of heme (a prosthetic group of Hb), which in turn reduces Hb synthesis and decreases RBC production in the bone marrow, resulting in anemia.

Other minerals like calcium and magnesium were significantly reduced. Calcium and magnesium are critical indicators of osmotic pressure alteration [25]. Their reduction indicated that penconazole affected the osmotic and ionic regulation in rats.

On the other hand, we observed a significant increase in

WBC count following penconazole treatment, establishing leucocytosis. This might be the consequence of direct stimulation of immunological defense due to the fungicide exposure. The rise in WBC count corroborated our earlier histological observation of inflammatory leucocytes infiltration in the hepatic and renal tissues of penconazole-exposed rats [6, 9]. Penconazole also increased platelet counts (Plt), probably suggesting a secondary thrombocytosis, as reported by Kanu et al. [4] in rats exposed to dichlorvos based insecticide formulation. Generally, platelets play a pivotal role in health and disease, given their central involvement in haemostasis and thrombosis. According to Abramson and Melton [26] and Schafer [27], leucocytosis and secondary thrombocytosis may be caused by several conditions such as infections, inflammation-tissue necrosis, stress and hemolytic anemia. Similar findings, as described in the present study, have been reported in the previous work of Kasmi et al. [28] in rats exposed to difenoconazole, a triazole fungicide.

All modifications shown after penconazole treatment were alleviated by *N. retusa* fruit. Indeed, *N. retusa* increased significantly RBC, Hb and Ht values. The erythropoietic effect of this plant could be attributed to its richness in iron [9]. Moreover, other compounds present in *N. retusa* fruit, like the polyphenols, could contribute to its therapeutic effect [9]. In fact, kaempferol has been reported to prevent, through its antioxidant activity, the decrease in Hb, Ht and RBC in noise stress-exposed rats [29]. Chlorogenic acid has been demonstrated to exert also beneficial effects against anemia and mineral disturbances occurring in 4-tert-octylphenol toxicity [30].

Meanwhile, *N. retusa* extract decreased WBC and Plt counts in penconazole-treated rats, reflecting an anti-inflammatory property that could probably be attributed to its active ingredients, particularly the flavonoids [9]. For instance, p-coumaric acid has been shown to act as an immunomodulatory and anti-inflammatory agent in experimental inflammation in rats [31]. Kaempferol and cyanidin 3-O-rutinoside have been shown to exert *in vitro* anti-inflammatory effects in lipopolysaccharide-activated macrophages [32, 33].

In addition, *N. retusa* fruit contains significant amounts of minerals including sodium, calcium, magnesium, iron, zinc and copper, as previously demonstrated [9]. In the present work, administration of *N. retusa* extract to penconazole-exposed rats improved the plasma levels of iron, calcium and magnesium in rats, which could be ascribed to its richness in these minerals. Other compounds like propolis and bee pollen enhance iron, calcium and magnesium serum levels in rats subjected either to nutritional ferropenic anemia [34] or to sodium fluoride toxicity [35].

5. Conclusion

Our investigation clearly demonstrated that *N. retusa* fruit extract showed a beneficial effect against penconazole-induced changes in blood hematological and mineral parameters in rats. Hence, this medicinal plant might be used as a therapeutic agent against damage associated with penconazole exposure, especially in agricultural workers.

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