EVALUATION OF ACUTE TOXICITY AND EFFECT OF PICOSITOL IN LETROZOLE INDUCED POLYCYSTIC OVARIAN SYNDROME IN ANIMAL MODELS

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This study aims to investigate the acute toxicity and the therapeutic potential of Picositol in a letrozole-induced PCOS model in Wistar rats. An acute toxicity study of Picositol by administering a single dose (5000 mg/kg) was done in rats following OECD guideline 423. For the PCOS-induced experiment, after induction of PCOS by orally administered letrozole (1 mg/kg) for 21 days, female rats were divided into six groups for treatment up to 15 consecutive days. Blood samples were withdrawn at the end of the study for the determination of sex hormone levels, malondialdehyde (MDA), glutathione (GSH), and the relative weight of fat, uterus, and ovaries was separated. The results demonstrated that treatment with Picositol at both doses significantly reduced oxidative stress markers and increased GSH. In addition, the formulation improved endocrine disturbances by lowering testosterone and showing a tendency to increase progesterone, while no improvement in estradiol was observed. Picositol also decreased fat mass and ovarian weight and tended to increase uterine weight relative to the model group. Collectively, these findings indicate that Picositol exhibited no detectable acute toxicity and provided supportive therapeutic effects on experimental polycystic ovary syndrome in Wistar rats.

Keywords: Picositol, acute toxicity, polycystic ovary syndrome, *Wistar* rats.

I. INTRODUCTION

Polycystic ovary syndrome (PCOS) is a complex endocrine disorder that profoundly impacts the reproductive, metabolic, and overall health of women of reproductive age.[1] Women with PCOS commonly exhibit insulin resistance and compensatory hyperinsulinemia, which increases their risk of early-onset type 2 diabetes mellitus and metabolic syndrome. Besides clinical symptoms such as menstrual disorders or infertility, PCOS is also associated with numerous long-term risks, including hyperinsulinemia, obesity, type 2 diabetes, and cardiovascular diseases.[3] The diversity of its manifestations and pathogenesis makes the management of PCOS a persistent challenge in clinical practice. Currently, treatment of PCOS options primarily focus on lifestyle interventions like diet and exercise, and improving endocrine disorders includes metformin, letrozole, clomiphene, or combined oral contraceptive pills and metformin. Although these drugs show certain efficacy, long-term use is often accompanied by undesirable side effects, ranging from gastrointestinal disturbances and musculoskeletal pain to mood changes. Consequently, many naturally derived preparations or those with physiological mechanisms of action closer to the pathogenesis of PCOS are receiving increasing attention as safer supportive treatment options.

PCOS is a complex disease caused by multiple etiological factors; a single drug cannot be used for its treatment. Picositol is one of

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the prominent preparations today, containing ingredients: Myo-inositol, L-arginine, folic acid, and vitamin D3 in each sachet. The formulation is a new combination based on the active ingredient's effects on the pathogenesis of PCOS. These components are expected to have the potential to improve glucose metabolism, increase insulin sensitivity, support hormonal balance, and promote ovarian function.[4-7] However, the safety and efficacy of the combination of these four active ingredients in a single formulation have not been fully evaluated in experimental models.

Based on this reality, this study was designed to determine the acute toxicity and evaluate the effects of Picositol in a model of polycystic ovary syndrome induced by letrozole.

II. MATERIALS AND METHODS

1. Materials

Ingredients

Picositol is produced at Fortex Nutraceuticals Ltd, Bulgaria, and meets GMP-WHO standards. Picositol formula ingredients include Myo-inositol 2000 mg, L-Arginine 100 mg, folic acid 400 mcg, Vitamin D (Cholecalciferol) 10 mcg, enough for 1 sachet. Intended use per person: 1 sachet/day. The test dose used in animal research can convert from a prophylactic application above 0.12 sachets/kg/day.

Chemicals

Letrozole (brand name: Oncolet), 2.5 mg tablets, manufactured by Zydus Lifesciences, India. Metformin (brand name: Glucophage), film-coated tablets, a product of Merck Santé S.A.S, France. Clomiphene (brand name: Clostilbegyt), in tablet form, supplied by Egis Pharmaceuticals Public Ltd., Co., Hungary.

Experimental animals

Female *Wistar* rats (180 - 220 g) were obtained from the Dan Phuong laboratory animal supply center in Hanoi. The animals were housed in large, clean polypropylene cages in a temperature-controlled room (22 ± 2°C) with a relative humidity of 44%-55% under a 12-h light/dark cycle. All animals were acclimated to the laboratory environment for one week before the experiment.

2. Methods

Acute toxicity study

An acute toxicity study of Picositol was performed following OECD Guidelines for the Testing of Chemicals, Test No. 423.[8] Female *Wistar* rats (n = 3) of both were fasted overnight before the experiment and were maintained under standard laboratory conditions. Before dosing, the rats were weighed. Picositol was crushed and suspended in water. A single dose of this formulation (5000 mg/kg) was administered, and animals were observed for clinical signs for 30 min, then hourly for the next 24 h, and thereafter for a total of 14 consecutive days. The animals were observed for signs of convulsions, tremors, circling, depression, excitement, and mortality.

If any rat died within 14 days from the start of administering the study product, the time of death must be recorded in detail, followed by a gross necropsy to determine the cause of death. All rats surviving after 14 days of receiving the study product were dissected (necropsied) to inspect the gross morphology of the internal organs. Any changes in gross morphology must be recorded for each rat.

Evaluation of effects on the polycystic ovary syndrome model

All experimental animals, except the control group, were orally administered letrozole at 1 mg/kg dissolved in water daily for 21 days. The control group received a vehicle only. Vaginal smears were collected daily and evaluated microscopically with a crystal violet stain to

confirm PCOS induction. The disease was confirmed by the irregularity of the estrous cycle.[9] Female *Wistar* rats were equally divided into six groups designated as Group I (served as a control group), Group II (served as a PCOS-induced group), Group III (served as a standard group), and Groups IV, V, and VI (served as treatment groups). Following letrozole administration, the standard group was administered with clomiphene. Treatment groups IV, V, and VI were administered with Picositol at 0.12 sachets/kg (low dose) and 0.24 sachets/kg (high dose) body weight, respectively, for 15 days.

At the end of the study, the rats were fasted for 12-14 hours before being anesthetized. Carotid artery blood was collected into EDTA tubes and centrifuged at 3000 rpm for 10 minutes. The separated serum was analyzed using the semi-automatic biochemical analyzer Erba Chem 5V3 (India) for the following parameters:

Concentrations of sex hormones: Estradiol, progesterone, and testosterone.Levels of oxidative stress markers: MDA and GSH.

Relative organ weights: the fat, uterus, and ovaries were excised (separated), and their relative weights were determined.

3. Statistical analysis

Research data are collected and processed using systematic methods and algorithms in biomedical statistics on Microsoft Office Excel 2013 and SPSS 20.0 software. Biomedical statistical methods were performed according to the Student's T-test and the before-and-after test (Avant-après). Worth it presented as ±SD. The difference is statistically significant when $p < 0.05$.

III. RESULTS

1. Acute toxicity assessment

General condition

Picositol orally administered at 5000 mg/kg in *Wistar* rats did not induce any death or toxic symptoms in treated animals. All animals displayed normal behavior throughout the study and survived until the end of the 14-day experiment period. During the entire observation period, they did not present any significant clinical alteration. Furthermore, after 14 days, a similar increase in body weight was observed in the control and Picositol (5000 mg/kg) groups, indicating normal growth and no adverse effect of Picositol on body weight between these groups and the controls (Table 1 & Table 2). As a result, the LD_{50} of Picositol was greater than 5000 mg/kg of body weight.

Across all experimental rats (both the control and toxicity test groups), no organ weight change, pathological change, or gross morphological change were observed in the brain, heart, lungs, liver, spleen, pancreas, kidneys, or the entire digestive system of the animals.

Table 1. Body weight of rats treated with Picositol

Treatment duration (days)	Weight (gram)	
	Normal control	Picositol 5000 mg/kg
Before treatment	173.33 ± 15.28	163.33 ± 30.55
After 14 days of treatment	200.00 ± 10.00 $^{\Delta}$	180.00 ± 30.00 $^{\Delta}$

$^{\Delta}$ $p < 0.05$. $^{\Delta\Delta}$ $p < 0.01$. $^{\Delta\Delta\Delta}$ $p < 0.001$ value was obtained by Student's T-test. compared with the time point "before treatment."

Table 2. Effects of Picositol on the weight of selected organs

Weight (g/100 g)	Normal control	Picositol 5000 mg/kg
Liver	3.55 ± 0.38	3.54 ± 0.28
Kidneys	0.70 ± 0.21	0.61 ± 0.08
Speen	0.34 ± 0.07	0.42 ± 0.05
Heart	0.31 ± 0.02	0.34 ± 0.08
Lungs	0.86 ± 0.11	0.68 ± 0.10

**p<0.05; **p<0.01; ***p<0.001 compared with the normal control*

2. Letrozole induced PCOS study

Serum hormonal assay

Table 4 shows that serum testosterone was significantly increased, whereas progesterone and estradiol were decreased markedly in the animals after administration of letrozole for 21 days, as compared to the normal control group. Treatment with standard drug causes a significant decrease in testosterone, and the levels of estradiol and progesterone were improved as compared to the disease control group. Picositol treatment at both doses also improved the hormonal profile, with a reduction in testosterone and partial normalization of estradiol and progesterone levels.

Table 3. Effect of Picositol administration on hormone concentrations in letrozole induced PCOS rats

Group	Testosterone (nmol/L)	Estradiol (pg/mL)	Progesterone (ng/mL)
Normal control	0.153 ± 0.05	22.95 ± 4.55	13.65 ± 3.82
Model (PCOS)	1.211 ± 0.42***	17.88 ± 5.40*	7.45 ± 1.95***
Metformin 70 mg/kg/day	0.712 ± 0.19[b]	24.64 ± 6.19[a]	8.21 ± 1.78
Clomiphene 1 mg/kg/day	0.203 ± 0.06[c]	23.12 ± 5.50[a]	11.92 ± 2.75[c]
Picositol 0.12 sachets/kg/day	0.871 ± 0.23[a]	17.860 ± 4.85	10.380 ± 2.70[a]
Picositol 0.24 sachets/kg/day	0.76 ± 0.24[b]	18.86 ± 3.93	8.22 ± 2.37

*Values are expressed as Mean ± SD (n = 10). *p<0.05; **p<0.01; ***p<0.001 compared to the normal control group. [a]p<0.05; [b]p<0.01; [c]p<0.001 compared to the model group (Student's t-test)*

Effect of Picositol preparation on the relative weight of organs

Table 4 shows that, in the model group, the relative weights of the periovarian fat and ovaries were significantly increased, while the relative uterine weight was significantly decreased compared with the normal group ($p < 0.001$). Treatment with clomiphene and Picositol reduced both periovarian fat and ovarian weights and tended to restore uterine weight toward normal values. Notably, the effects of Picositol were comparable to those of the positive control drugs, with no significant difference observed among the treated groups for the evaluated parameters.

Table 4. Effects of Picositol on relative and absolute organ weights

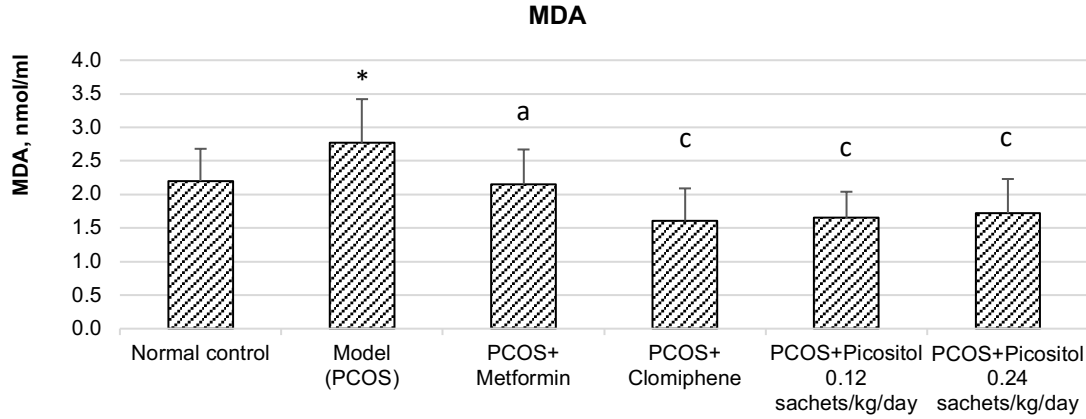
Group	Weight (g/100g)		
	Fat	Uterus	Ovary
Normal control	2.071 ± 0.57	0.102 ± 0.03	0.038 ± 0.01
Model (PCOS)	3.101 ± 0.84**	0.063 ± 0.018**	0.053 ± 0.013**
Metformin 70 mg/kg/day	2.481 ± 0.37[a]	0.078 ± 0.03	0.050 ± 0.01
Clomiphene 1 mg/kg/day	2.307 ± 0.73[a]	0.081 ± 0.01[a]	0.035 ± 0.01[b]
Picositol 0.12 sachets/kg/day	2.127 ± 0.44[b]	0.076 ± 0.02	0.042 ± 0.01[a]
Picositol 0.24 sachets/kg/day	1.845 ± 0.48[b]	0.077 ± 0.02	0.040 ± 0.01[a]

**p < 0.05. **p < 0.01. ***p < 0.001 compared with the normal control group*

[a]p<0.05; [b]p<0.01; [c]p<0.001 compared with the model group (Student's t-test)

Antioxidant activity of Picositol via modulation of serum MDA and GSH levels

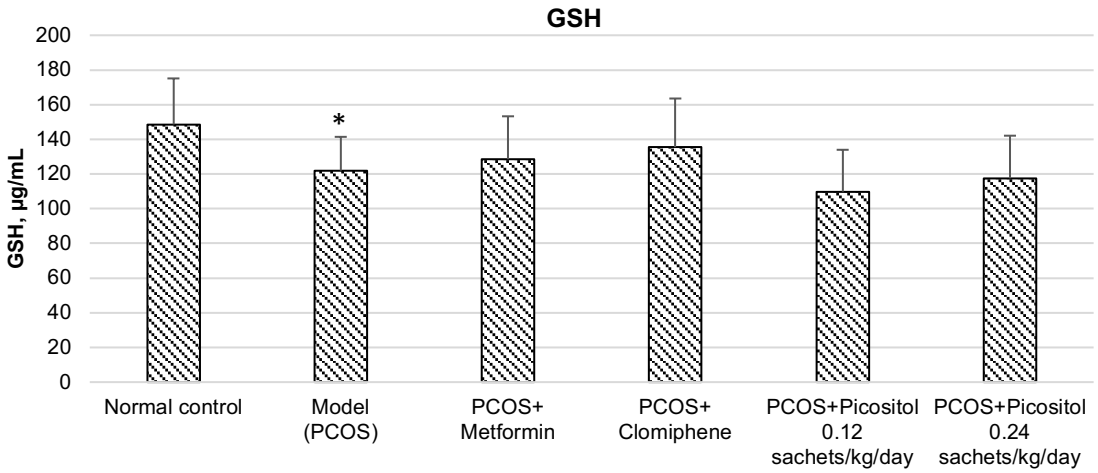
Figures 1 and 2 show the changes in serum MDA and GSH levels in the study groups. The model group showed an increase in MDA content and a decrease in GSH content compared to the normal group. Furthermore, MDA content in the groups treated with Picositol was increased compared to the model group, a statistically significant difference (p < 0.001). However, there was no significant difference in GSH content when comparing the Picositol-treated groups and the model group.



**p<0.05 compared with the normal control group*

[a]p<0.05; [b]p<0.01; [c]p<0.001 compared with the group (Student's t-test)

Figure 1. Effect of Picositol preparation on serum MDA levels



**p<0.05 compared with the normal control group*

[a]p<0.05; [b]p<0.01; [c]p<0.001 compared with the group (Student's t-test)

Figure 2. Effect of Picositol on serum GSH levels

IV. DISCUSSION

The study results show that Picositol does not cause any signs of acute toxicity when administered orally at a dose of 5000 mg/kg in accordance with OECD 423 guidelines. This safety profile may be explained by the characteristics of its two main components, myo-inositol and L-arginine. Both components have been widely demonstrated to be safe in numerous toxicity studies and risk assessments. Myo-inositol is a water-soluble compound naturally present in the cells of all living organisms. Available toxicity data indicate that myo-inositol has a very high safety margin: the oral LD_{50} in mice is approximately 10 g/kg body weight and QSAR analysis predicts an oral LD_{50} in rats of up to 19.5 g/kg.[10] These values are many times higher than the 5000 mg/kg test dose used in this study, indicating that myo-inositol is unlikely to cause acute toxicity even at very large doses. Similarly, L-arginine has also been reported to have a high safety profile. This amino acid is non-essential for most adult mammals and is considered safe for consumption at high levels in foods. A risk assessment by Shao and Hathcock (2008) concluded that arginine doses up to 20 g/day are safe for healthy adults.[11] Moreover. EFSA (2018) also confirmed that L-arginine produced by microbial fermentation is safe for all animal species when used as a feed additive. These data further reinforce the very wide safety margin of L-arginine.[12] Since both myo-inositol and L-arginine have excellent safety profiles, with very high oral LD_{50} values and no toxicity at large doses, the absence of acute toxicity of Picositol, a formulation containing these two components at a dose of 5000 mg/kg, is fully consistent with the existing toxicity evidence.

Although there are numerous animal models for studying human PCOS, a fully convincing model has not yet been established.[13] In this study, the oral letrozole-induced PCOS model was administered continuously for 21 days, which was considered to produce a phenotype most similar to human PCOS. Previous reports have indicated that letrozole is a non-steroidal

aromatase inhibitor that blocks the conversion of testosterone to estradiol. This results in increased androgen levels along with decreased estradiol and progesterone, manifesting as an anovulatory phenotype, consistent with findings from previous studies.[14,15] However, in this study, we did not assess plasma LH and FSH levels, which is considered a limitation of the research.

Several studies have reported that oxidative stress is one of the pathological factors contributing to PCOS.[16,17] PCOS is associated with oxidative stress, leading to increased androgen production. In the present study, we observed that PCOS animals exhibited elevated MDA levels, indicating tissue damage caused by free radicals, and decreased GSH levels, reflecting impaired antioxidant defense. The inverse correlation between increased MDA and decreased GSH serves as an important indicator of ovarian dysfunction in PCOS.

In humans, PCOS is often associated with obesity, increased visceral fat, and metabolic disturbances. Visceral fat surrounding the abdomen and ovaries may play a critical role in the pathophysiology and clinical manifestations of PCOS, including insulin resistance, hyperandrogenism, and hormonal imbalances.[1] The letrozole-induced model in this study significantly decreased relative uterine weight and significantly increased relative fat weight as well as relative ovarian weight. This differs slightly from the previous findings of Hasan Kafali and Mamata Jadhav, which reported no significant change in ovarian weight, although uterine weight was reduced following letrozole treatment.[14,18] Treatment with Picositol significantly reduced relative ovarian weight and relative fat weight.

Picositol's beneficial effects on fertility are linked to its phytochemical constituents, such as myo-inositol, antioxidant activity, and L-carnitine, as well as vitamin D and folic acid. These compounds provideto hormonal balance, and promote the maturation of oocytes. Myo-inositol modulates multiple molecular pathways that are directly involved in reproductive function, thereby enhancing oocyte and embryo quality in assisted reproductive techniques. Several studies have shown that myo-inositol promotes oocyte maturation and may represent a promising therapeutic approach for restoring spontaneous ovulation.[4]

L-carnitine plays a pivotal role in cellular energy metabolism and possesses strong antioxidant capacity, protecting cells from oxidative stress-induced damage. Moreover, L-carnitine may influence central nervous system activity, thereby modulating the hypothalamic-pituitary-gonadal axis and supporting reproductive regulation. Recent evidence has highlighted its positive effects on female fertility, including increased gonadotropin and hormone levels, improved oocyte quality, and higher pregnancy rates.[7]

The obtained results will contribute to providing scientific evidence on the safety and effects of Picositol in an *in vivo* PCOS model. This will serve as a foundation for the development and application of Picositol as an adjunct therapy for PCOS.

V. CONCLUSION

Picositol is safe at a dose of 5000 mg/kg according to OECD 423 and improves multiple abnormalities in the PCOS model. The preparation markedly reduces MDA levels, lowers testosterone, slightly increases progesterone, and decreases fat and ovarian weights with estradiol showing a trend toward improvement. Overall, Picositol demonstrates beneficial effects on oxidative, hormonal, and

morphological parameters related to PCOS, although further studies are needed to clarify its long-term efficacy.

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