REPEATED-DOSE 90-DAY ORAL TOXICITY STUDY OF PICOSITOL IN EXPERIMENTAL *WISTAR* RATS

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This study aimed to evaluate the subchronic toxicity of Picositol powder following oral administration in Wistar rats. The experiment was conducted in accordance with the guidelines of the Organization for Economic Co-operation and Development (OECD 408). Rats were administered Picositol at two doses of 0.12 sachets/kg/day and 0.36 sachets/kg/day by oral gavage for 90 consecutive days. Measurements included body weight, general observations, hematology, biochemical, gross necropsy, and histopathological examination. The results show that oral administration of both doses of Picositol to rats did not result in any clinical symptom, nor hematological, biochemical, and histological signs of variation. In conclusion, a 90-days repeated dose oral toxicity study of Picositol in Wistar rats yielded no-observed-adverse-effect at 0.12 sachets/kg/day and 0.36 sachets/kg/day.

Keywords: Picositol, repeated dose toxicity, *Wistar* rats.

I. INTRODUCTION

Polycystic Ovary Syndrome (PCOS) is the most prevalent endocrine disorder affecting women of reproductive age, with a prevalence ranging from 6% to 13%.[1] Hyperandrogenism, menstrual disorders, and polycystic ovaries on ultrasound characterize PCOS.[2] In addition, PCOS also causes many metabolic disorders, significantly reducing reproductive health and leading to negative impacts on quality of life. Therefore, PCOS is considered a public health problem requiring care.

Among current interventions, adding active ingredients with multi-dimensional mechanisms of action on metabolism and endocrine is considered a potential approach. On that basis, several products supporting the treatment of PCOS have been developed, including Picositol – a formulation that combines four ingredients, including myo-inositol, L-carnitine, folic acid, and vitamin D. The compositions have been reported to simultaneously affect multiple pathogenesis mechanisms of PCOS, improving insulin resistance, supporting ovulation, and enhancing overall reproductive health.[3-5] The ingredients in the formulation have been clinically used for a long time, either individually or in combination with other ingredients. A common prevailing belief is that medicines are harmless due to their safety profile, and the scarcity of toxicity studies. Toxicity refers to unwanted effects on biological systems. In order to evaluate biological toxicity, it is very important to choose the correct system, since no effect may otherwise be seen. Subchronic systemic toxicity is defined as adverse effects occurring after the repeated or continuous administration of a test sample for up to 90 days or not exceeding 10% of the animal's lifespan.[6]

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No matter how effective a drug may be, its clinical application is precluded in the absence of demonstrated safety. Therefore, we planned this study to investigate the toxicity of Picositol and confirm the safety of the preparation. To date, Vietnam has no report on the toxicity of Picositol. The primary objective of this study was to assess repeated dose toxicity of Picositol in experimental animals, providing more scientific evidence for its application in treating PCOS.

II. MATERIALS AND METHODS

1. Subjects

The preparation of Picositol

Picositol is manufactured by Fortex Nutraceuticals Ltd, Bulgaria, and meets GMP-WHO standards. The product is prepared in the form of powder in sachets. Each sachet contains: 2000mg myo-inositol, 100mg L-Carnitine, 400mcg folic acid, and 10mcg vitamin D (Cholecalciferol). The usual expected dosage for humans is one sachet per day, 30 - 60 minutes after meals.

Experimental animals

Adult, female Wistar rats weighing 170 – 250g were used in this study. Rats were housed in cages (ten rats/cage) under standard conditions (temperature 25°C ± 1°C; relative humidity, 80% ± 10%), with a 12-hours light/dark cycle. Standard feed was given and wate was provided ad libitum. The animals were housed for 5 – 7 days before the study was conducted at the Department of Pharmacology, Hanoi Medical University.

2. Methods

Repeated dose toxicity study

A repeated dose toxicity study was carried out according to the OECD 408 Guidance.[6]

The study was carried out in a continuous 90-day period. *Wistar* rats were divided into three groups of ten animals:

- Group 1 (control) was served as the distilled water control group. Each rat was administered 1ml of distilled water/100g b.w./day by oral route of administration.

- Group 2 was given orally Picositol at 0.12 sachet/kg/day (equivalent to the human recommended dose, conversion ratio 6).

- Group 3 was given Picositol at 0.36 sachets/kg/day (three times the dose in Group 2).

Animals were treated daily by oral route of administration once a day in the morning for 90 consecutive days and observed once daily to detect signs of toxicity.

The signs and indices were checked during the study, including:

General condition consisting of mortality and clinical signs.

Body weight changes analysis: Blood samples were analyzed by ABX Micros ES 60. The contents include: red blood cell (RBC) count, hemoglobin (Hb), hematocrit (Hct), total white blood cell (WBC) count, WBC differentials, and platelet count (PLT).

Serum biochemistry: aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, albumin, total cholesterol, and creatinine levels.

The parameters were checked at before treatment, 30 days after treatment, 60 days after treatment, and 90 days after treatment. At the end of the experiment, all animals underwent a full gross necropsy. Organs, including the heart, stomach, spleen, liver, kidneys, uterus, and ovaries, were collected from 30% of the rats in each group for macroscopic examination. The liver and kidneys from these animals were subsequently subjected to histopathological analysis. The criteria for assessing histopathological damage to the liver and kidney include: Damage scores in liver and

kidney tissue are scored as: 0 = normal, 1 = mild (1% - 30% damage), 2 = moderate (31% - 70%), and 3 = severe (> 70%), based on the % of tissue affected.[7,8]

Statistical analysis

Data were collected and processed using SPSS 20.0 and Microsoft Excel 2020 software. Differences between study groups were tested using one-way ANOVA analysis followed by the Bonferroni post-hoc test, and the Kruskal-Wallis test. Results are presented as mean ± SD. All data were considered significant at $p < 0.05$.

$^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared with the control group.

$^{\Delta}p < 0.05$, $^{\Delta\Delta}p < 0.01$, $^{\Delta\Delta\Delta}p < 0.001$ compared with the time point "before treatment".

III. RESULTS

General condition

The animals exhibited normal locomotor activity and received adequate feeding. Compared with the control group, none of the animals in all the treatment groups showed any macroscopic or gross pathological changes.

Body weight changes

Table 1 showed that after 30 days, 60 days, and 90 days of treatment, body weight in all rats increased substantially compared with body weight "before treatment". No significant difference was observed between groups treated with Picositol and the control group ($p > 0.05$).

Table 1. The effect of Picositol on body weight

Time	Body weight (g)		
	Group 1	Group 2	Group 3
Before treatment	203.00 ± 29.83	214.00 ± 34.06	222.00 ± 38.82
30 days after treatment	254.00 ± 35.02$^{\Delta\Delta\Delta}$	257.00 ± 41.11$^{\Delta\Delta\Delta}$	252.00 ± 39.10$^{\Delta\Delta\Delta}$
60 days after treatment	252.00 ± 35.84$^{\Delta\Delta}$	276.00 ± 33.73$^{\Delta\Delta\Delta}$	268.00 ± 31.90$^{\Delta\Delta\Delta}$
90 days after treatment	279.00 ± 35.73$^{\Delta\Delta\Delta}$	283.00 ± 40.57$^{\Delta\Delta}$	281.00 ± 29.61$^{\Delta\Delta\Delta}$

$^{\Delta}p < 0.05$, $^{\Delta\Delta}p < 0.01$, $^{\Delta\Delta\Delta}p < 0.001$ compared with the time point "before treatment"

Effect on hematological examination

The results of the effect on hematological indices are shown in Table 2. It showed that at all time points, there was no significant difference in RBC, Hct, Hb, MCV, PLT, WBC, lymphocytes (LYM), and neutrophils (NEU) between the groups treated with Picositol and the control group, as well as the time point before treatment ($p > 0.05$).

Effect of Picositol on biochemical parameters in the repeated toxicity study

Table 3 summarizes the effect of Picositol on the biochemical parameters of treated animals. There were no significant difference in indices, such as AST, ALT, total bilirubin, albumin, total cholesterol, and creatinine between groups treated with Picositol and the control group ($p > 0.05$).

Table 2. Effect of Picositol on hematological parameters

Parameters	Group	Before treatment	30 days after treatment	60 days after treatment	90 days after treatment
RBCs count (T/L)	Group 1	8.45 ± 0.67	8.24 ± 1.26	8.49 ± 1.13	8.42 ± 0.80
	Group 2	8.50 ± 1.48	9.05 ± 1.43	9.63 ± 1.58	8.33 ± 1.28
	Group 3	9.05 ± 1.07	8.48 ± 0.99	8.37 ± 1.50	8.62 ± 0.75
Hb (g/dL)	Group 1	11.66 ± 1.12	11.27 ± 1.33	11.26 ± 0.98	11.32 ± 0.67
	Group 2	12.07 ± 1.70	11.27 ± 1.33	11.97 ± 1.13	11.19 ± 1.91
	Group 3	11.96 ± 1.35	11.26 ± 1.32	10.82 ± 1.99	11.33 ± 0.63
Hct (%)	Group 1	45.66 ± 2.57	43.28 ± 5.88	42.23 ± 5.03	43.42 ± 2.83
	Group 2	47.37 ± 7.80	46.78 ± 7.83	45.63 ± 3.10	44.99 ± 5.70
	Group 3	47.50 ± 5.54	44.14 ± 6.40	42.12 ± 8.08	44.76 ± 4.09
MCV (fL)	Group 1	53.20 ± 3.08	51.40 ± 2.88	51.00 ± 2.31	51.80 ± 3.65
	Group 2	52.50 ± 2.59	51.60 ± 1.58	51.00 ± 1.41	50.70 ± 1.89
	Group 3	52.50 ± 2.99	51.90 ± 1.79	50.90 ± 2.33	50.30 ± 1.83
PLTs count (G/L)	Group 1	555.40 ± 107.06	562.10 ± 111.22	595.40 ± 119.77	568.00 ± 65.94
	Group 2	575.50 ± 109.13	533.10 ± 107.63	575.60 ± 112.19	530.40 ± 115.56
	Group 3	596.30 ± 91.46	542.40 ± 116.17	609.10 ± 91.48	542.50 ± 78.99
WBCs count (G/L)	Group 1	10.83 ± 1.86	9.58 ± 2.09	9.05 ± 2.17	9.58 ± 1.54
	Group 2	10.90 ± 2.24	9.93 ± 1.43	9.29 ± 1.76	9.78 ± 2.39
	Group 3	10.75 ± 1.37	9.75 ± 1.22	9.65 ± 1.58	9.53 ± 1.85
LYM (%)	Group 1	70.97 ± 6.46	67.79 ± 5.90	68.42 ± 7.14	72.94 ± 6.43
	Group 2	73.19 ± 4.68	71.22 ± 5.90	68.35 ± 6.85	69.68 ± 4.14
	Group 3	72.40 ± 7.35	70.89 ± 6.50	70.29 ± 6.60	69.20 ± 6.59
NEU (%)	Group 1	13.79 ± 3.73	16.36 ± 4.55	16.52 ± 4.53	14.66 ± 5.30
	Group 2	13.07 ± 2.51	13.22 ± 2.78	17.04 ± 4.22	15.84 ± 3.97
	Group 3	13.69± 3.34	13.62 ± 3.24	14.89 ± 3.74	16.42 ± 3.97

Table 3. Effects of Picositol on biochemical parameters

Parameters	Group	Before treatment	30 days after treatment	60 days after treatment	90 days after treatment
AST (UI/L)	Group 1	70.10 ± 13.60	70.60 ± 12.78	60.30 ± 11.73	61.10 ± 10.61
	Group 2	73.50 ± 9.20	74.50 ± 10.93	68.80 ± 7.18	68.40 ± 12.29
	Group 3	73.60 ± 10.67	73.00 ± 5.40	70.10 ± 13.01	68.30 ± 11.28
ALT (UI/L)	Group 1	41.80 ± 7.94	43.30 ± 7.29	42.10 ± 8.69	39.70 ± 7.33
	Group 2	40.20 ± 6.07	44.90 ± 7.85	43.50 ± 7.99	42.00 ± 3.02
	Group 3	44.30 ± 9.20	45.30 ± 7.09	46.30 ± 10.88	37.70 ± 7.13
Total bilirubin (µmol/L)	Group 1	7.63 ± 0.78	7.55 ± 0.62	7.53 ± 0.86	7.20 ± 0.92
	Group 2	7.66 ± 0.94	7.22 ± 0.33	7.44 ± 1.21	7.67 ± 0.70
	Group 3	7.70 ± 0.53	7.43 ± 0.77	7.94 ± 1.25	7.46 ± 1.04
Albumin (g/dL)	Group 1	2.83 ± 0.38	2.59 ± 0.62	2.89 ± 0.62	2.84 ± 0.38
	Group 2	2.79 ± 0.23	2.88 ± 0.26	2.64 ± 0.25	2.65 ± 0.48
	Group 3	2.82 ± 0.19	2.75 ± 0.28	2.64 ± 0.40	2.69 ± 0.24
Total cholesterol (mmol/L)	Group 1	36.55 ± 7.97	35.00 ± 6.08	38.42 ± 6.64	40.21 ± 5.98
	Group 2	38.62 ± 6.08	39.14 ± 3.95	36.29 ± 4.31	37.60 ± 6.82
	Group 3	38.49 ± 7.17	35.77 ± 5.83	36.66 ± 9.63	38.20 ± 6.55
Creatinine (µmol/l)	Group 1	72.00 ± 7.30	74.40 ± 7.18	74.20 ± 10.52	73.00 ± 7.42
	Group 2	70.10 ± 5.90	72.80 ± 5.09	70.50 ± 9.32	67.50 ± 9.90
	Group 3	68.90 ± 5.24	71.70 ± 5.85	71.80 ± 9.74	72.10 ± 6.98

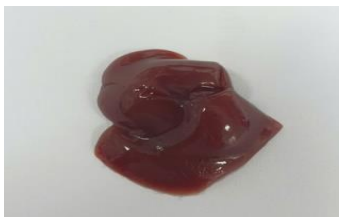
Histopathological examination

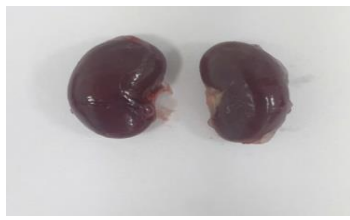
Gross pathological changes

During the repeated toxicity study, there were no gross pathological change in the liver, kidneys, spleen, stomach, heart, and reproductive organs of the control and experimental groups ($p > 0.05$) (Figure 1 - 3).

Microscopic images of liver and kidney:

In the histopathological studies, the hematoxylin and eosin stain technique was used; the sections of the kidneys and livers of treated rats showed normal general structure with no significant difference as compared to the vehicle group after 90 days of treatment ($p > 0.05$, Kruskal-Wallis test) (Table 4 and Figure 4).


Liver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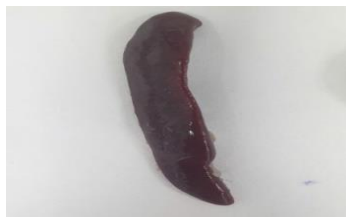
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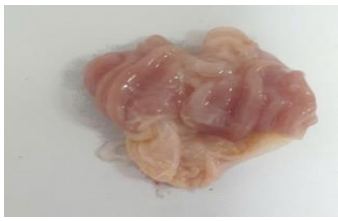
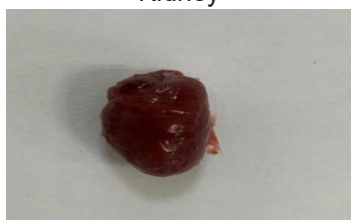

Stomach


Heart

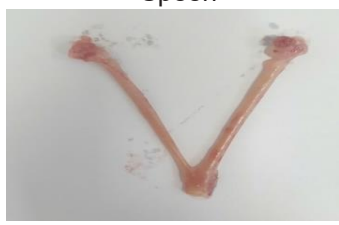
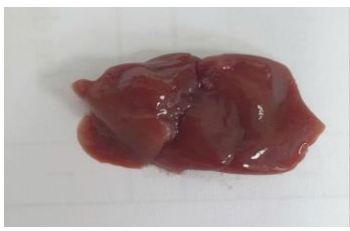
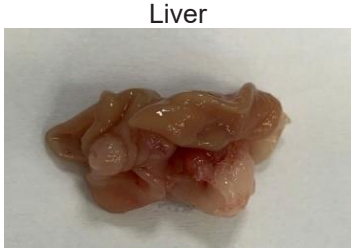

Uterus + Ovary

Figure 1. General images of group 1 organs


Liver


Kidney


Speen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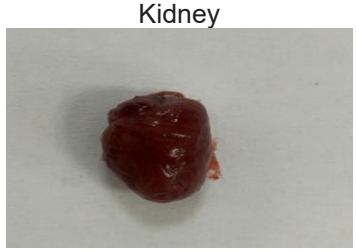

Heart

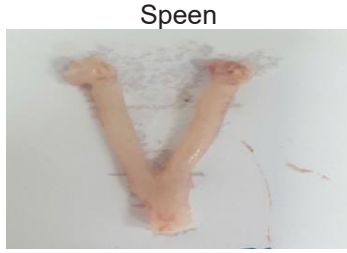
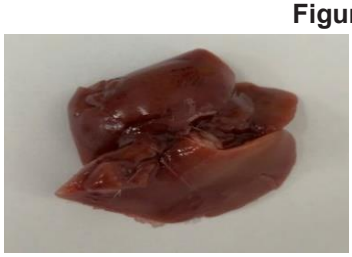
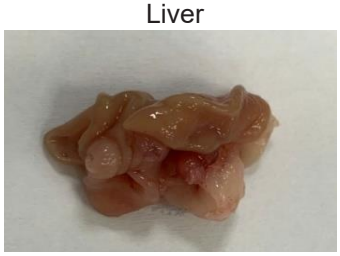

Uterus + Ovary

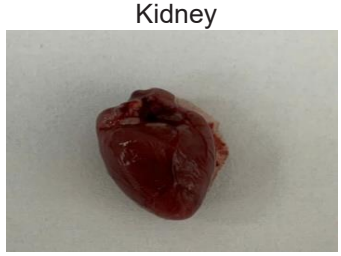
Figure 2. General images of group 2 organs


Liver


Kidney


Speen


Stomach


Heart

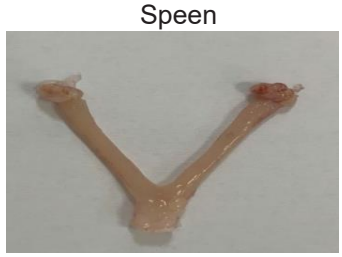

Uterus + Ovary

Figure 3. General images of group 3 organs

Table 4. Results of scoring of lesions on the liver and kidneys of rats

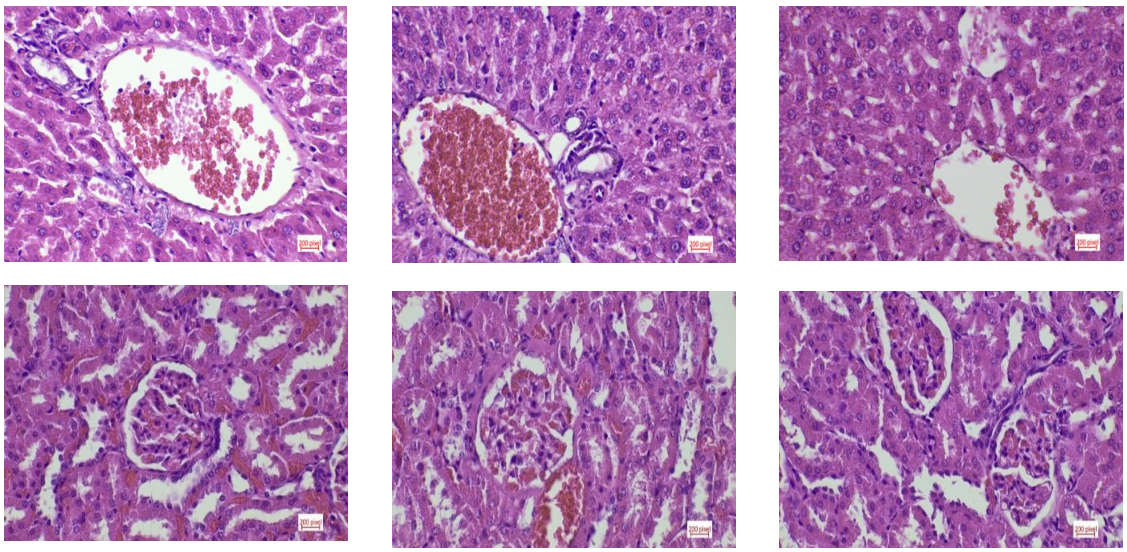
Organ	Lesions	Control	Group 1	Group 2	p (Kruskal-Wallis test)
Liver	Inflammation	0	0	0	1.00
	Periportal degeneration	0.33 ± 0.58	0.33 ± 0.58	0.67 ± 0.58	0.670
	Midzonal degeneration	0	0		1.00
	Centrilobular degeneration	0	0	1.00	1.00
	Hydropic degeneration	0	0	1.00	1.00
	Activated Kupffer cells	0	0	1.00	1.00
	Periportal necrosis	0	0	1.00	1.00
	Midzonal necrosis	0	0	1.00	1.00
	Centrilobular necrosis	0	0	1.00	1.00
Kidney	Inflammation	0.33 ± 0.58	0	0.67 ± 0.58	0.264
	Hydropic degeneration	0	0	0	1.00
	Tubular casts (hyaline)	0	0	0	1.00
	Granular casts	0	0	0	1.00
	Cellular casts	0	0	0	1.00
	Protein casts	0	0	0	1.00

IV. DISCUSSION

Toxicity is the extent to which a substance may cause harm in humans or animals. It can involve cellular injury (cytotoxicity), organ-specific effects such as kidney or liver damage, or systemic impact. To ensure human drug safety, toxicological studies are conducted in animal models to predict potential adverse effects and inform the selection of safe therapeutic doses. Subchronic toxicity studies yield data on the impact of repeated oral administration and may highlight the necessity for longer-term investigations.[6,9]

Changes in body weight are a reliable marker of the animals' overall health condition.[10] Body weights were monitored in all groups administered Picositol. The absence of significant variation compared with the distilled water control group indicates that Picositol did not disrupt the animals' normal metabolic processes.

Following 30, 60, and 90 days of treatment, no significant difference was observed in RBC, Hct, Hb, PLT, WBC, or WBC differentials between Picositol-treated animals and the control group, indicating that Picositol administration did not influence the hematological profile or the blood-forming process. There was no substantial change in AST level, ALT level, total bilirubin, total cholesterol, and albumin concentrations between the group treated with Picositol and



Group 1 Group 2 Group 3

Figure 4. Histopathology of rats during toxicity study (HE × 400)

the control group. These results indicated that Picositol had no deleterious effect on liver damage and liver function. Similarly, no significant difference was observed in blood biochemical parameters between the control group and those treated with Picositol at various dose levels ($p > 0.05$). Thus, Picositol did not affect the kidney function. Overall, the biochemical findings demonstrated that Picositol had no adverse effect on liver or kidney function. Histopathological analysis showed structural alterations of cells under light microscopy. Additional histological assessment could provide deeper insight into the potential hepatotoxicity and nephrotoxicity of Picositol. However, no notable difference was observed in the liver and kidneys between the Picositol-treated and control groups. Taken together, the results demonstrated no significant change in hematological profile, biochemical markers, or histopathological features of hepatic and renal tissues between treated and control animals.

Myo-inositol is the main ingredient of Picositol, a six-carbon cyclitol, which has been classified as an insulin-sensitizing agent, and it is commonly used in the treatment of PCOS. G Carlomagno conducted a clinical study to evaluate the safety of inositol, which showed that only the highest dose of myo-inositol (12 g/day) caused mild gastrointestinal side effects such as nausea, flatus, and diarrhea. In the comparison, each Picositol sachet only has 2g of myo-inositol, which is approximately six times lower than the dose evaluated in G Carlomagno's study.[11] Besides, the toxicological profile of L-carnitine has previously been investigated in several toxicity animal studies. In a 90-day subchronic study in rats, daily administration at doses of up to 737 mg/kg body weight did not result in significant adverse effects. According to Aouatef Bellamine, rats received diets containing up to 50,000 ppm L-carnitine L-tartrate for a period of 90 days, showed no treatment-related effects on mortality, ophthalmologic examinations, hematology, gross pathology, or histopathology.[12]

Besides, in 14-days and 90-days repeated oral dose toxicity studies of acid folic, rats given up to 1000 mg/kg body weight per day of L-5-MTHF-Na by oral gavage showed no

treatment-related adverse effect across all evaluated parameters, including clinical signs, body weight, food intake, ophthalmologic and neurological assessments, hematology, clinical chemistry, urinalysis, organ weights, and both macroscopic and microscopic examinations. A non-adverse adaptive increase in creatine kinase levels was observed in both male and female rats in the 90-days study, without any corresponding change in organ weights or histopathology.[13] Clinical trial data indicate that long-term consumption of 250 µg (10,000 IU) per day of vitamin D_3 is unlikely to cause adverse effects in nearly all individuals in the general population, thus meeting the criteria for a tolerable upper intake level.[14] The Picositol sachet contains only 10 mcg of vitamin D, which is approximately 25 times lower than the dose evaluated in the previous study.

V. CONCLUSION

Picositol at 0.12 sachets/ kg/day and 0.36 sachets/kg/day administered orally continuously for 90 days did not produce any toxic sign or evident symptom in the body weight of rodents, hematological, and biochemical parameters. Moreover, histopathological studies indicate no significant difference between control and treated animals.

Conflict of interest

The authors declare that they have no competing interests.

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