MUDANÇA NA QUÍMICA SANGUÍNEA, CITOCINAS PRO-INFLAMATÓRIAS E GENES APOPTÓTICOS APÓS O USO DE METAFETAMINA EM RATOS EXPERIMENTAIS

CHANGE IN BLOOD CHEMISTRY, PRO-INFLAMMATORY CYTOKINES, AND APOPTOTIC GENES FOLLOWING METHAMPHETAMINE USE IN EXPERIMENTAL RATS

صحرایی موشهای در متامفتامین از استفاده دنبال به که آپوپتوتیک ژنهای و التهابی پیش سینوکینه های ، خون شیمی در تغییر شوند می استفاده آزمایشی

GOUDARZI, Zahra¹; HOSEINI Seyed Ebrahim^{1*}; MEHRABANI, Davood^{2,3,4,5}; HASHEMI, Seyedeh Sara³

¹ Department of Biology, Shiraz Branch, Islamic Azad University, Shiraz, Iran

² Stem Cell Technology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

³ Burn and Wound Healing Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

⁴ Center of Comparative and Experimental Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

⁵ Department of Oncology, University of Alberta, Edmonton, Alberta, Canada

* Corresponding author
e-mail: mehrabad@outlook.com

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RESUMO

A metanfetamina (METH) é uma substância ilícita abusada globalmente com níveis epidêmicos em todo o mundo. Este estudo teve como objetivo investigar as alterações na química do sangue e citocinas pró-inflamatórias após o uso de metanfetamina em ratos experimentais. Um total de quarenta e cinco ratos fêmeas foram aleatoriamente dedicados a três grupos iguais de experimental recebendo METH por via subcutânea (0,4 mg / kg, em 0,6 mL de volume) por 21 dias, o sham recebeu 0,6 mL de solução salina normal e o controle recebeu 0,6 mL destilado água, de forma idêntica. O teste do labirinto em cruz elevado foi usado para confirmar mudanças cognitivas e de ansiedade após o uso de METH até três semanas. Química do sangue e citocinas inflamatórias foram avaliadas após o uso de METH até 21 dias. Os resultados mostraram um aumento da ansiedade. Os níveis séricos de fator de crescimento transformador beta (TGF-β), interleucinas de IL-15, IL-17 e níveis de adenosina desaminase xantina oxidase foram anotados. No entanto, a contagem de leucócitos (leucócitos) demonstrou uma tendência decrescente. Não houve outras alterações na química do sangue após o uso de METH. Pôde-se observar, entretanto, que a metanfetamina aumenta a ansiedade e faz algumas alterações na química do sangue e nas citocinas pró-inflamatórias. Este estudo pode ajudar a tomar melhores decisões sobre a prevenção e até mesmo o tratamento de pessoas que tomam metanfetamina.

Palavras-chave: metanfetamina, ansiedade, química do sangue, apoptose, citocinas inflamatórias.

ABSTRACT:

Methamphetamine (METH) is a globally heavily abused illicit substance with epidemic levels worldwide. This study aimed to investigate changes in blood chemistry and pro-inflammatory cytokines following methamphetamine use in experimental rats. A total of forty-five female rats were randomly devoted to three equal groups of experimental receiving METH subcutaneously (0.4 mg/kg, in 0.6 mL volume) for 21 days, sham received similarly 0.6 mL normal saline, and the control received 0.6 mL distilled water, identically. The elevated plus-maze test was used to confirm cognitive and anxiety changes following METH use until three weeks. Blood chemistry and inflammatory cytokines were evaluated after METH use until 21 days. The results showed an increase in anxiety. The serum levels of transforming growth factor-beta (TGF-β), interleukins of IL-15, IL-17, and adenosine deaminase xanthine oxidase levels were noted. However, white blood cell (WBC) count demonstrated a decreasing trend. There were no other changes in blood chemistry after METH use. It could be observed, however, that methamphetamine increases anxiety and makes some changes in blood chemistry and pro-

inflammatory cytokines. This study can help make better decisions about the prevention and even treatment of people taking methamphetamine.

Keywords: *methamphetamine, anxiety, blood chemistry, apoptosis, inflammatory cytokines*

چکیده:

مت آمفتامین (METH) یک ماده غیرقانونی است که در سطح جهانی به شدت مورد سوء استفاده قرار گرفته و دارای سطح اپیدمی در سراسر جهان است. این مطالعه با هدف بررسی تغییرات شیمیایی خون و سیتوکین های پیش التهابی به دنبال استفاده از مت آمفتامین در موش های آزمایشگاهی انجام شد. در مجموع چهل و پنج موش ماده به طور تصادفی به سه گروه مساوی از آزمایش تجویز زیر جلدی (0.4 میلی گرم در کیلوگرم ، در حجم 0.6 میلی لیتر) به مدت 21 روز اختصاص داده شد ، شش به طور مشابه 0.6 میلی لیتر نرمال سالین دریافت کرد و شاهد 0.6 میلی لیتر مقطر دریافت کرد. آب ، به طور یکسان. برای افزایش تغییرات شناختی و اضطرابی بدنبال استفاده از METH تا سه هفته ، از آزمایش ماز بعلاوه پیچ و خم استفاده شد. شیمی خون و سیتوکین های التهابی پس از استفاده از METH تا 21 روز مورد بررسی قرار گرفت. نتایج افزایش اضطراب را نشان داد. سطح سرمی تبدیل فاکتور رشد بتا (TGF- β)، اینترلوکینهای IL-15 ، IL-17 و آدنوزین دی آمیناز گرانانتین اکسیداز مشخص شد. با این حال ، تعداد گلبول های سفید (WBC) روند کاهشی را نشان می دهد. پس از استفاده از METH هیچ تغییر دیگری در شیمی خون مشاهده نشد. با این وجود می توان مشاهده کرد که مت آمفتامین اضطراب را افزایش می دهد و برخی تغییرات را در شیمی خون و سایتوکاین های پیش التهاب ایجاد می کند. این مطالعه می تواند به تصمیم گیری بهتر در مورد پیشگیری و حتی درمان افرادی که مت آمفتامین مصرف می کنند ، کمک کند.

واژه های کلیدی: مت آمفتامین ، اضطراب ، شیمی خون ، آپوپتوز ، سیتوکین های التهابی

1. INTRODUCTION:

Amphetamine type stimulants include amphetamine, methamphetamine (METH), and 3,4-methylenedioxymethamphetamine (MDMA, ecstasy). METH is easily manufactured in clandestine drug laboratories and is available as a ground, whitish powder; pills; sticky, waxy base; and crystalline shards (Ice) (McKetin *et al.*, 2005). It was first synthesized in 1893 in Japan (Suwaki, 1991). METH can be used orally, nasally, or intravenously to produce a euphoric high for the consumer and causes inhibition of fatigue, enhancement of mental acuity, mood, social and sexual function, and a reduction in appetite (Shin *et al.*, 2017). Prolonged use at high levels results in independence. METH is a spinoff of amphetamine, which used to be widely prescribed in the 1950s and 1960s as a medicine for depression and obesity, achieving a height of 31 million prescriptions in the United States in 1967. Until the late 1980 ser (Anglin *et al.*, 2000). Methamphetamine-associated psychosis (MAP) represents an intellectual sickness precipitated with the aid of chronic methamphetamine use in a subset of users. The prevalence of the ailment has multiplied in quite a few countries in Europe and Asia, the place methamphetamine use has increased. MAP stays challenging to distinguish from main psychiatric disorders, mainly schizophrenia, growing issues in prescribing remedy plans to sufferers (Chiang *et al.*, 2019). Nonetheless, the prevalence of METH use has increased in both Europe and Asia, including Iran, too (Wang *et al.*, 2016). It was robustly illustrated to produce psychotic symptoms as a condition known as METH-associated psychosis (MAP) (Glasner-Edwards and Mooney, 2014;

Schwarzbach *et al.*, 2020).

There is no pharmacotherapy for METH use disorder to be approved by the Food and Drug Administration (Chan *et al.*, 2019). Accurate identification of exposure to METH can have important implications for the care of the abuser. Sampling blood is one of the first types of biological material analyzed in METH abusers to detect drug complications (Plotka *et al.*, 2014). METH abuse is related to activation of the innate immune response, the concerning cellular and chemical elements, and inflammatory cytokines. The literature collectively displays an association of METH use with innate immune response activation in CNS with neurotoxicity (Clark *et al.*, 2013).

The innate immune response activation is an early stage of neuroinflammatory reaction to insult that can cause migration of inflammatory cells through the blood-brain barrier (BBB) into the CNS parenchyma (Clark *et al.*, 2013). Microglial activation is linked to the production of several pro-inflammatory molecules, including chemokines, cytokines, and nitric oxide (Minagar *et al.*, 2002). Although the negative effects of METH abuse are considered significant problems, few studies have been conducted on changes in blood cells and blood chemistry and pro-inflammatory cytokines.

This study aimed to assess the anxiety and changes in blood chemistry and pro-inflammatory cytokines following methamphetamine use in experimental rats.

2. MATERIALS AND METHODS

2.1. Ethics

The Ethics Committee for Animal Use of Islamic Azad University, Shiraz Branch, approved the study protocol on September 30, 2018 (7-E-IR-MIAU.REC.80-B-2018), and all experiments were undertaken according to Iran Veterinary Organization guidelines.

2.2 Chemicals and Reagents

Except where otherwise stated, all chemicals were obtained from Sigma (Sigma Chemical Co., St. Louis, MO, USA).

2.3. Preparation of METH

METH (Sigma-Aldrich, USA) was a donation by the Shiraz Branch of Islamic Azad University in Shiraz, Iran, with written permission for research purposes. Before use in experiments, Methamphetamine solution in normal saline was prepared with a concentration of 66.6%. To make METH, we dissolved 66.6 mg of METH in 100 cc of normal saline.

2.4 Animals and grouping

A total of forty-five females, with 8 weeks old Wistar rats, weighing from 200 to 220 g were purchased from the Comparative and Experimental Medicine Center of Shiraz University of Medical Sciences, Shiraz, Iran. The animals were kept under controlled environmental conditions of 22 °C, 70 % humidity, a 12-h light and dark cycle (lights on at 8:00 a.m.), and free access to a standard diet and tap water. Before the experiments, the accommodation to their condition was carried out.

The rats were randomly divided into three equal groups (Table 1) of experiments receiving 0.4 mg/kg of METH subcutaneously in 0.6 mL volume for 21 days. The sham group received subcutaneously 0.6 mL normal saline for 21 days, and the control group received 0.6 mL distilled water identically. Daily interventions were performed from 08:00 a.m. to 12:00 a.m.

2.5. Confirming METH addiction by anxiety assessment

A plus-maze with two 50×10 cm opposite open arms confined by 40 cm height walls was

applied, while the arms were connected by a 10×10 cm central square, shaped like a “plus”, and elevated 50 cm from the floor was lit by a dim light. The interventions were video-recorded, while the camera was at a 50° angle above the maze, and the anxiety scores were recorded in an adjacent room. The number of entries, together with the percent time spent in open arm (OAT), closed arm (CAT), and central parts of the maze, were collected. Head dipping for the exploratory movement of the head/shoulders of the rats over the side of the maze was also investigated (Holmes *et al.*, 2000).

When all four paws were inside the arm, an arm entry was registered; and exit from an arm was when the forepaws were outside that arm. On test day before starting experiments, the animals were transferred undisturbed to a dimly illuminated room for 1 h. As rats are usually reactive to man's direct handling, a cylindrical cardboard tube was used for individual transportation of the animals from the cage to the plus-maze. Scoring was conducted for 5 min by an observer who was blind to the protocol, and the data were directly entered into a PC computer. All tests were carried out between 8.00 a.m. and 12.00 a.m., and the maze was thoroughly cleaned (wet and dry cloths) between successive experiments.

2.6. Hematological assessment

A capillary glass tube was inserted into the medial canthus of the right eye above the lacrimal duct under deep anesthesia, and 2.5 ml of blood was collected from the orbital sinus. Aliquots were prepared and dispensed in tubes with EDTA or sodium citrate or without any anticoagulant for hematological assessment of transforming growth factor-beta (TGF-β), interleukins of IL-15, and IL-17, adenosine deaminase (ADA), xanthine oxidase (XO), white blood cells (WBC), red blood cells (RBC), hemoglobin (Hb), hematocrit (HCT), platelet (PLT), triglycerides (TG), cholesterol, high-density lipoprotein cholesterol (HDL), blood urea nitrogen (BUN), total bilirubin, creatinine, uric acid, albumin, total protein, alkaline phosphatase (ALK), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels.

Blood urea nitrogen (BUN) levels, creatinine, uric acid, triglycerides (TG), cholesterol, high-density lipoprotein cholesterol (HDL), Total bilirubin, albumin, total protein, alkaline phosphatase (ALK), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were measured biochemically by an autoanalyzer (Permium24i,

Japan) and Bionic assay kit.

By using an electronic cell counter (Baker 9000 Automated Cell Counter, Biochem Immunosystems, Inc., Allentown, PA), a complete blood count was done. A Coulter Dacos Chemistry Analyzer (Coulter, Miami, FL) was employed to evaluate uric acid levels. RBC count was carried out with a hemocytometer using a solution of 3.13% trisodium citrate as diluents applying standard hemocytometer protocol was used to count the RBC.that integrated the number of RBC counted, dilution factor, as well as area and depth of the chamber. A hemocytometer conducted the platelet count after 1 in 20 dilutions of the sample in 1% ammonium oxalate. WBC count was undertaken identically. Hemoglobin concentration was detected using 1 in 20 dilutions and at a wavelength of 540 nm (Baker *et al.*, 2000).

The adenosine deaminase (ADA, ZellBio GmbH, Germany) assay kit quantitatively assessed rat ADA in serum based on the biotin double antibody sandwich technology. Xanthine oxidase activity (XOX, ZellBio GmbH, Germany) assay kit evaluated XOX activity in serum samples. All tests except leukocyte differential counts were done on the day of blood collection, while all biochemical and hematological experiments were duplicated. TGF- β , IL-15, IL-17, ADA, and XO levels were assessed by ELISA (Stat Fax® 4200 - Awareness Technology, USA – Florida)

2.7. Statistical analysis

The effect of METH on the number of entries and the percent time spent in OAT, CAT, central parts of the maze, and head dipping were investigated between the three groups at the end of the first, second, and third week. The obtained data were exhibited as mean $\pm$ SEM and statistically analyzed using SPSS software (Version 21, Chicago, IL, USA) by one-way ANOVA, Post-hoc Tukey's, and independent Student t-tests. P values $\leq$ 0.05 were defined as statistically significant.

3. RESULTS AND DISCUSSION:

3.1 METH addiction

METH could significantly increase the anxiety level after METH use ($p < 0.05$) based on OAT findings denoting a significant difference between the experimental and the control group (Figure 1). Regarding CAT after METH use, there

was a significant difference between the experimental and the control group ($p < 0.0005$), revealing that METH could significantly increase the anxiety level in experimental rats (Figure 2). Considering the effect of METH on percent time spent in central parts of the maze after METH consumption, there was a significant difference between the experimental and the control group, demonstrating that the METH use could significantly increase the amount of anxiety in the experimental group ($p < 0.05$, $p < 0.0005$) (Figure 3). Evaluation of METH effect on head dipping of the animals revealed a significant difference between the experimental and the control group of rats treated with METH ($p < 0.001$, $p < 0.0005$, $p < 0.0005$) (Figure 4).

3.2. Hematological assessment

The effects of methamphetamine on hematological factors including white blood cells, red blood cells, hemoglobin, hematocrit, platelets, triglycerides, HDL and cholesterol in female rats are shown in Table 2. The results of the effect of methamphetamine on the white blood cell count of female rats indicate that the white blood cell count in the first and second treatments and the Sham group were significantly different from the control group ($P < 0.05$). But there was no significant difference between the number of white blood cells in the third treatment and the control group ($P > 0.05$). For the other factors studied in this study, including red blood cells, hemoglobin, hematocrit, and platelets, no significant differences were observed between the treatment and control groups ($P > 0.05$).

The results related to the effect of methamphetamine on TG level showed a significant difference between the Sham group with the control group and the treatment group in the first week with the control group ($P < 0.05$). The amount of TG in different periods of treatment with methamphetamine and control group and Sham numerically shows an irregular increase trend. The highest rate is related to Sham group and the lowest is related to treatment in the first week. The effect of methamphetamine on Chol and HDL did not show a significant difference ($P > 0.05$). Regarding TGF- β , IL-15, IL-17, ADA, and XO levels, a significant increase was noted following METH use ($p < 0.001$) (Table 4). For WBC count, there was a decrease ($p = 0.009$). However, regarding RBC count, Hb, HCT, PLT, BUN, creatinine, uric acid, TG, cholesterol, HDL, total bilirubin, albumin, total protein, and ALK, no changes in the values were noticed following METH administration. Furthermore, the levels of

AST and ALT were increased in the METH groups in comparison with Control and Sham groups Table 3.

3.3. Discussion

During the past two decades, there has been a tremendous expansion of knowledge regarding how the central nervous system responds to noxious stimuli such as psychostimulants of abuse (methamphetamine, cocaine, ecstasy) by immune response including inflammatory cytokines (IL-1b, IL-6, TNF- α), blood chemistry and gene expression. It was shown that the pro-inflammatory cytokines active in the innate immune process are keys to injury response, extended or amplified production (Clark *et al.*, 2013). Substance use, such as METH, can cause behavioral modifications such as anxiety (Kamali-Sarvestani *et al.*, 2020; Sadeghi Bimorgh *et al.*, 2020). In this study, similarly, an increasing trend for anxiety level was noted following METH use.

Pro-inflammatory cytokines are involved in the maintenance and homeostasis of the immune system, inflammation, and host defense (Li *et al.*, 2020). METH abuse results in severe dysregulation in the peripheral immune response and causes an imbalanced expression in chemokines, cytokines, and other molecular factors due to neuronal injury (Tran *et al.*, 2018). In this study, pro-inflammatory cytokines of IL-15, IL-17, and TGF- β demonstrated a significant increase following METH use.

There has been markedly increased expression of TNF- α (Mahajan *et al.*, 2006) and ultimately induced apoptosis (Shen *et al.*, 2020), leading to an elevated expression of IL-1 β resulting in neurotoxic activity (Du *et al.*, 2017), and exacerbated IL-2 secretion (Bortell *et al.*, 2015), and up-regulated IL-6 level (Wang *et al.*, 2017), a rise in IL-8 to be a significant marker of anxiety and depression (Huckans *et al.* 2015), a considerable enhancement of IL-10 (Burns and Ciborowski, 2016), a significantly increased expression of IL-12 (Peerzada *et al.*, 2013) and a greatly enhanced expression of IFN- α (Li *et al.*, 2020).

There were no data available in the literature regarding changes in IL-15, IL-17, and TGF- β after METH use. However, an increased IL-15, IL-17, and TGF- β expression were shown in patients with inflammatory diseases (Chen *et al.*, 2018). Several studies revealed that TGF- β is the quintessential cytokine of Th17 cell differentiation. It was found that in the presence of TGF- β , polarization to the Th17 cells happens and make

these cells secrete IL-17A, IL-17F, IL-21, and IL-22, that leads to inflammation, tissue remodeling, metaplasia, and hyperplasia modifications in the tissue (Chen *et al.*, 2018), that can explain the rise in TGF- β and as a consequence the increase in IL-17.

Up-regulation of pro-inflammatory cytokines, including IL-17, has been previously reported during allergic inflammation (Vanvalkenburgh *et al.*, 2011), which is in line with our results following METH use. A high expression level of TGF- β , IL-15, and IL-17 was detected in mice with chronic inflammatory diseases (Salehi *et al.*, 2016; Wang *et al.*, 2018) in agreement with our findings METH consumption too. The study provides in vivo evidence for the involvement of the IL-15, IL-17, and TGF- β after METH use with significantly up-regulated IL-15, IL-17, and TGF- β .

These findings denoted an increase in the ADA level following METH use, which was time-dependent. The extracellular adenosine level is regulated by uptake into cells and by metabolism undertaken by adenosine kinase (AKA) and ADA (Meghji, 1991). Adenosine as a neuromodulator is released from metabolically active or stressed cells, and adenosine analogs were demonstrated to exert a neuroprotective effect in METH neurotoxicity in mice (Delle Donne and Sonsalla, 1994; Gołembiowska and Zylewska, 2000). These findings are in agreement with the results of this study.

XO, as the enzyme responsible for the catabolism of purines and their conversion into uric acid, was illustrated can be overexpressed after stress, which eventually can lead to inflammation in the tissue (Maimaiti *et al.*, 2019). It was shown that multiple doses of METH could dramatically raise extracellular concentrations of dopamine and serotonin, with significant behavioral and neurochemical consequences. These neurotransmitters can, in turn, increase cytotoxic quinones and reactive oxygen species (Kokoshka *et al.*, 1998). METH also creates an environment disturbing the balance between oxidative stress and antioxidant defense (McDonnell-Dowling, 2017). Therefore, the increase in xanthine oxidase in our study can be explained by the inflammatory effect of METH administered in rats too. As METH has significant effects on both the innate and adaptive immune responses (Peerzada *et al.*, 2013; Sriram *et al.*, 2015), it can lead to a reduction in the numbers of leukocytes rendering individuals susceptible to certain diseases and infections (Mahajan *et al.*, 2006). This decline in WBC count was also noted in this study in line with the METH effect.

4. CONCLUSIONS:

It was concluded that there was an increase in anxiety following methamphetamine use in animal model. Also, it could be observed that the use of methamphetamine led to an increase in inflammation, ALT, AST, ADA and xanthine oxidase after consumption. The results of the present study also showed that methamphetamine has an effect on the white blood cell and TG where the lowest rate of TG is related to treatment in the first week. Further research is required to confirm these findings and determine involved mechanisms in other animals, and humans.

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Table 1. Groups of study

Group	Description	Number of animals
METH	0.4 mg/kg of METH subcutaneously in 0.6 mL volume for 21 days	15
Sham	subcutaneously 0.6 mL normal saline for 21 days	15
Control	0.6 mL distilled water for 21 days	15
Total		45

Table 2: Blood chemistry profile after METH use (Mean± Standard deviation)

Variable		Group
		WBC
		7.63±1.81
		7.63±0.55
		P-v ¹
		N/A
		RBC
		6.97±0.63
		7.20±0.45
		P-v
		N/A
		HB
		14.00±0.84
		15.43±0.57
		P-v
		N/A
		HCT
		40.78±2.97
		48.57±0.92
		P-v
		N/A
		PLT
		717.75±101.3
		712.67±31.18
		P-v
		N/A
		TG
		95.75±22.42
		85.00±4.35
		P-v
		N/A
		CHOL
		69.00±10.98
		71.33±14.01
		P-v
		N/A
		HDL
		39.00±5.35
		34.67±0.57
		P-v
		N/A

1- P-value

Table 3: Liver and renal function tests after METH use (Mean± Standard deviation).

METH weeks	3 rd	METH weeks	METH 2 nd weeks	METH 1 st week	Sham	Control	Variable
27.50±4.50		24.00±3.91		25.67±2.30	29.00±6.05	27.00±1.00	BUN
1.00		0.87		0.99	0.54	N/A	P-V ¹
0.48±0.09		0.53±0.50		0.633±0.057	0.50±0.00	0.56±0.05	CRT ²
0.33		0.894		0.67	0.03	N/A	P-V
6.50±1.02		4.90±0.49		5.90±0.52	7.03±0.095	6.6±0.11	UA ³
0.99		0.01		0.56	0.002	N/A	P-V
6.51±0.26		6.38±0.37		6.83±0.057	6.40±0.133	6.46±0.14	T-PRO ⁴
0.99		0.99		0.348	0.163	N/A	P-V
166.50±019.22		138.75±20.41		107.00±22.60	107.50±11.09	112.33±2.51	AST
0.008		0.304		0.995	0.001	N/A	P-V
61.00± 24.38		5275±7.63		46.00±4.58	50.75±7.63	51.67±2.08	ALT
0.87		1.00		0.98	0.64	N/A	P-V
329.75±63.17		286.00±74.97		362.00±153.189	394.00±8.04	374.67±14.29	ALK
0.93		0.57		0.84	0.36	N/A	P-V

¹- P- value

²- CREATININE

³- URIC ACID

⁴- TOTAL PROTEIN

Table 4: Pro-inflammatory cytokines following METH use (Mean± Standard deviation).

METH 3 rd weeks	METH 2 nd weeks	METH 1 st week	Sham	Control	Variable	
					Group	
514.50±13.89	672.25±23.51	764.66±25.54	153.50±18.41	262.00±208.71	IL-15	
0.012	0.0001	0.0001	0.0001	N/A	P-v ¹	
504.00±8.36	654.25±22.76	763.66±14.04	193.00±9.12	179.66±9.01	IL-17	
0.0001	0.0001	0.0001	0.0001	N/A	P-v	
844.50±19.22	1001.75±56.59	1079.33±58.79	210.00±7.07	209.66±2.30	TGF-β	
0.0001	0.0001	0.0001	0.0001	N/A	P-v	
5651.00±80.51	4420.75±48.35	257.33±58.22	199.25±4.64	196.33±6.42	ADA	
0.0001	0.0001	0.0001	0.0001	N/A	P-v	
135.25±3.86	112.50±1091	72.00±3.60	5.25±0.50	5.33±1.52	XO	
0.0001	0.0001	0.0001	0.0001	N/A	P-v	

¹- P-value

Figure 1. The effect of METH at the end of the first, second, and third week on the mean percentage of open arm time (OAT) between the three groups (* $p < 0.05$).

Figure 2. The effect of METH on the mean percentages of time spent in the closed arm time (CAT) at the end of the 1st, 2nd, and 3rd week between the three groups (* $p < 0.0005$).

Figure 3. The effect of METH on the mean number of times rats passing through the edge of center cross at the end of the first (* $p < 0.05$), second (* $p < 0.0005$), and third week between the three groups.

Figure 4. The effect of METH on the mean number of head dipping at the end of the first (* $p < 0.001$), second (** $p < 0.0005$), and third week (** $p < 0.0005$) between the three groups.