**International Journal of Environmental Sciences**
ISSN: 2229-7359
Vol. 11 No. 18s, 2025
https://www.theaspd.com/ijes.php

# The Evalution Of Acetyl-11-Keto-Beta-Boswellic Acid *(AKBA)* On Induced Autism In A Mice Model

**AL-Hasan Waleed Jawad[1], Hamid Redha Sadeghnia[2] Salman Mohammed Salman[3*]**
[1]Medicine college, Babylon University, Iraq hasanshemary@yahoo.com
[2]Medicine College, Mashhad University, Iraq hsadeghnia@gmail.com
[3]Medicine College, Babylon University, Iraq med.salman.salman@uobabylon.edu.iq

***Abstract***
***Background***: *Autism spectrum disorder (ASD), a neurodevelopmental condition, is characterized by difficulties in social interaction and communication that manifest in early childhood. The only authorized medications for the treatment of ASD are risperidone and aripiprazole.*
***Objectives***: *The research aims to demonstrate the possible therapeutic effects of acetyl-11-keto-beta-boswellic acid on an induced offspring model of autism. Additionally, to assess the influence of AKBA on interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α).*
***Materials and Methods***: *We induced the condition in mice by injecting sodium valproate (600 mg/kg) to pregnant mice. Prenatal sodium valproate-exposed mice were divided into four distinct groups: two experimental groups received AKBA (5mg/kg and 15mg/kg) and risperidone (1mg/kg), while a control group was administered normal saline. Behavioral tests, including social interaction tests, were segmented into three phases: habituation, familiarization, and testing, each lasting 10 minutes, and were performed on postnatal day 65. Additionally, assessments of anti-inflammatory markers such as TNF-α and IL-6 were done on postnatal day 66.*
***Results:*** *The study showed AKBA markedly enhanced behavioral abnormalities related to social communication and reduced neuro-inflammation in the brain. AKBA treatment significantly improved the cognitive performance of ASD mice via modulating neurogenesis, likely linked to the potent antioxidant and anti-inflammatory properties of AKBA.*
***Conclusion:*** *AKBA had significant anxiolytic and anti-inflammatory effects that enhanced behavioral activity in the mice. These data indicate that AKBA may represent a viable therapeutic option for persons with ASD.*
***Keywords***: *Autism, risperidone, AKBA , sodium valproate, mice*

## INTRODUCTION

Autism spectrum disorders (ASD) are developmental diseases characterized by deficits in communication, social interaction, and rigid behavior, often shown via limited and repetitive activities and reduced adaptation to environmental changes [1]. The disease is affected by a combination of epigenetic, environmental, and genetic variables throughout early prenatal and postnatal infancy [2].
At least 70% of individuals with Autism Spectrum Disorder exhibit co-occurring symptoms, making this occurrence expected rather than the exception.
The chemical basis suggests that many brain neurotransmitters, including dopamine (DA), serotonin (5-HT), gamma-aminobutyric acid (GABA), acetylcholine (ACh), glutamate (Glu), and histamine (HA), have a role in the development and evolution of ASD [3].
The current evidence-based therapy for Autism Spectrum Disorder in children mostly focuses on behavioral interventions to alleviate the core symptoms of the disorder. The importance of pharmacological therapies escalates with age and mostly addresses comorbid diseases linked to ASD. Risperidone and aripiprazole are the only medications approved by the Food and Drug Administration (FDA) for addressing irritability associated with Autism Spectrum Disorder (ASD) [4]. We will use the mice model owing to the significant evolutionary affinity between mice and humans,

ISSN: 2229-7359
Vol. 11 No. 18s, 2025
https://www.theaspd.com/ijes.php
along with their same genetic makeup, biological mechanisms, neurological pathways, and, to some extent, behaviors. While ASDs are exclusive to humans, extensive behavioral research on mice may clarify several fundamental deficits linked to these diseases [5].
This investigation used acetyl-11-keto-beta-boswellic acid (AKBA) as a potent neuroprotective drug, since previous studies have shown its considerable antioxidant and anti-inflammatory properties in relation to neurological disorders. Thus, AKBA demonstrated its use in our investigation.
ASD includes a range of problems classified into two primary categories:
Genetic illnesses, characterized by features linked to autism spectrum disorder including fragile X syndrome, intellectual disabilities, and tuberous sclerosis complex.
Idiopathic, indicating that the causes are unidentified. Idiopathic forms of Autism Spectrum Disorder (ASD) have been referred to by several labels throughout the years, including persistent developmental problems and Asperger syndrome. Regarding gender, there was a significant difference between males and females (autistic children) in the concentration of copper in saliva. [6].

## MATERIALS AND METHODS

### Experimental animals

This study was conducted on 60 healthy albino mice (40 female and 20 male) weighing 25-40 g and aged 12 weeks obtained from the Ministry of Science and Tikrit in salah aldeen/ Iraq. They were kept to live in the animal house of the College of the Medicine/University of Babylon and kept in typical cages made of plastic at a temperature (25±5 °C) at 60±5% humidity and 12 hr. light/dark cycle, and had free access to food pellets and water. this study was done in the college from (1/10/2024 to 1/3/2025).

### Mating and vaginal smear

After a two-week period of accommodation, split the mice into 20 groups, including one male and two females in each group for mating purposes. Inspect the female mice for the existence of a vaginal plug to verify that mating has occurred after nocturnal mating. Copulatory plugs are essential for sperm viability and ejaculate retention. Mice possess a prominent copulatory plug that adheres to the cervix and vaginal canal for 24 to 48 hours post-ejaculation [7]. Elevate the female mouse by the tail's base to momentarily elevate the hind limbs. A cotton-tipped swab may be used to detect the copulatory plug by searching for an off-white seminal coagulum next to the vaginal opening. The cotton-tipped swab cannot penetrate the vaginal canal if the copulatory plug is present.

### Preparation of animals and induction of autism

The pregnant female mice split into two groups on the $12^{th}$ day of gestation. The control groups, which consisted of ten subgroups each one had two pregnant mice were given only saline solution for injection intraperitoneally (i.p.). The experimental groups also consisted of ten subgroups each one had two pregnant mice, were received a single i.p. injection of sodium valproate at a dosage of 600 mg/kg to establish the experimental model of autism [8].

### Preparation of drugs

Sodium valproate is used as a powder for injection under the brand name Depakine® from Sanofi company at a concentration of 400 mg of Sodium Valproate per 4 ml reconstitution. The powder was dissolved in water for injection and given as a single intraperitoneal injection at a concentration of 600 mg/kg body weight after dilution with 0.9% sodium chloride solution.
The resulting powder of AKBA was dissolved in water at strength of 1mg/10mL. The powder was then delivered orally at a dosage of 5mg/kg and 15mg/kg body weight for 20 day

Risperidone is used as a solution dosage form under the name Risperdal from Janssen company at a concentration of 1mg/ml. The solution was given orally by gavage tube after dilution with distilled water at a dosage of 1mg/kg body weight during the course of twenty days.

**Experimental design**

The offspring mice were separated from the mothers on the 40$^{th}$ postnatal day and divided into 8 groups, each of them consisted of 10 animals:

Group I (control-saline group): received normal saline orally for 20 days.
Group 2 (control-AKBA group): received AKBA 5mg/kg orally for 20 days
Group 3 (control- AKBA group): received AKBA 15 mg/kg orally for 20 days.
Group 4 (control-risperidone group): received risperidone 1 mg/kg orally for 20 days (Esraa et al., 2022).
Group 5 (sodium valproate [VPA]-saline group): received normal saline orally for 20 days.
Group 6 (VPA- AKBA group): received AKBA 5mg/kg orally for 20 days (Suzan et al., 2022).
Group 7 (VPA- AKBA group): received AKBA 15 mg/kg orally for 20 days.
Group 8 (VPA-risperidone group): received risperidone 1 mg/kg orally for 20 days (Esraa et al., 2022).

**Social interaction test**

The test was performed in a three-chamber attached in rectangular shape measuring (42 cm × 19 cm × 22 cm). Each chamber was accessible through one of two apartus, each measuring 10 cm x 8 cm. The test lasted for 15 minutes and consisted of three phases each phase continued for 5minute. The starting location for each phase is the central chamber.

**In phase I (adaptation)**, the test mouse was placed in the central chamber of the apparatus, with the other two compartments closed, and given five minutes to investigate their surroundings.

**In phase II (familiarization)**, The test mouse was positioned in the center place of the apparatus, and the doors to the lateral chamber were opened, with no wire cage or stimulus mouse present during this phase. A test mouse was let pass through the three-chambered apparatus freely for 5 minutes, during which the frequency of its movements between locations was recorded to provide a baseline for the animal's behavior **(figure 1).**

**Figure 2:** The movement of the mouse from chamber to chamber in social interaction test

**Phase III (Sociability)**, At the midpoint of each lateral chamber, which has a wire cup with a diameter of 10 cm, an unknown mouse was placed in the wire cup of the right chamber, while the wire cup of the left chamber remained vacant. The testing mouse was placed in the central chamber and allowed unrestricted access to investigate all three chambers. The experiment seeks to determine if the testing mouse favors a social

setting over a non-social one by comparing the duration spent alone in a wire cup chamber to the duration spent with an unknown mouse (**figure 2**).

**Figure 3:** Experimental mouse spending time with strange mouse in social interaction test

**Decapitation of mice and isolation of brain**

After departing 24 hours following the last treatment dose and completion of a series of behavioral tests, mice were decapitated. Surgical shears were used to cut off the head from the body. The skull was revealed by making a midsagittal scalp incision, pulling back the skin, and then using sharp scissors to open the skull's back. Using forceps, the skull was peeled back to reveal the mouse's brain. The brain was carefully removed from the skull and then the olfactory bulb and cerebellum were severed taking only two brain's hemispheres. Two hemispheres were washed by using phosphate-buffered saline PBS 10% (w/v). The brain was then promptly cooled by placing them in eppendorf tube on ice. After which placed in the refrigerator and frozen at -20°C.

**Anti-inflammatory assessment**

Using an enzyme-linked immunosorbent assay kit, the IL-6 and TNF-α concentrations in the tissue were determined.

**Ethical approval**

The committee of publication ethics in the College of Medicine / University of Babylon in Iraq approved this study. According to document no, a local ethics committee agreed on the study protocol, on 1/9/ 2024.

**Statistical analysis**

The 26$^{th}$ edition of the Statistical Package for the Social Sciences (SPSS) was used for data processing, data entry, and analysis. The study's results were examined using a one-way analysis of variance (ANOVA) and post hoc least significant difference testing. Independent samples t-test was used to compare the mean length of the tail between VPA and control groups. The results were shown as (Means ± SEM). The mean rates of tail abnormalities were compared between groups using a chi-squared test and the results were shown as percentages. P-values of ($p \leq 0.05$) and ($p \leq 0.001$) were used to classify differences as statistically significant and highly significant respectively.

## RESULTS

**Social interaction test**

**A-Number of transitions between chambers**

**International Journal of Environmental Sciences**
ISSN: 2229-7359
Vol. 11 No. 18s, 2025
https://www.theaspd.com/ijes.php

The number of transitions between chambers was considerably decreased (p≤ 0.001) in the VPA-saline group relative to other groups, whereas it was significantly increased (p≤ 0.001) in both low and high-dose VPA-AKBA and VPA-risperidone groups compared to the VPA-saline group.
The number of transitions between chambers was markedly increased (p= 0.039), (p= 0.044), and (p= 0.01) in the high-dose control-AKBA group compared to the control-saline, low-dose control-AKBA, and control-risperidone groups, respectively.
**(figure 4)**.

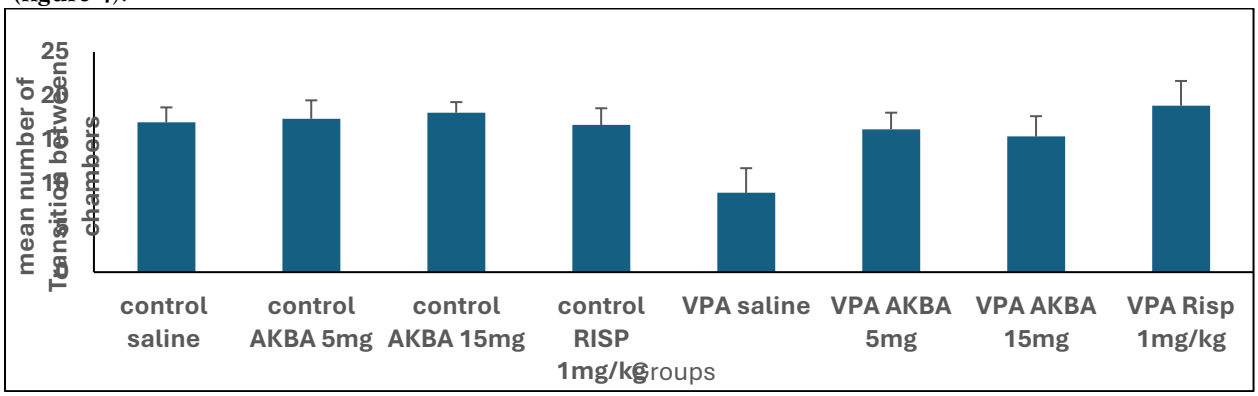


**Figure 4:** Effect of *acetyl-11-keto-beta-boswellic acid* and risperidone on the number of transitions between chambers in social interaction test. The results expressed as (Means ± SEM). No. of mice = 10 for each group

**B-Time spent with strange mice**
The time spent interaction with strange mice was considerably increased (p≤ 0.001) in both low and high-dose VPA-AKBA and VPA-risperidone groups compared to the VPA-saline group, whereas it was significantly reduced (p≤ 0.001) in the VPA-saline group relative to the other groups.
The time spent with the strange mice was markedly increased (p= 0.04) in the high-dose VPA-AKBA group relative to the VPA-risperidone group, and significantly increased (P= 0.006) and (p=0.022) in the high-dose control-AKBA group compared to the control-saline and control-risperidone groups, respectively.
**(figure 5)**.

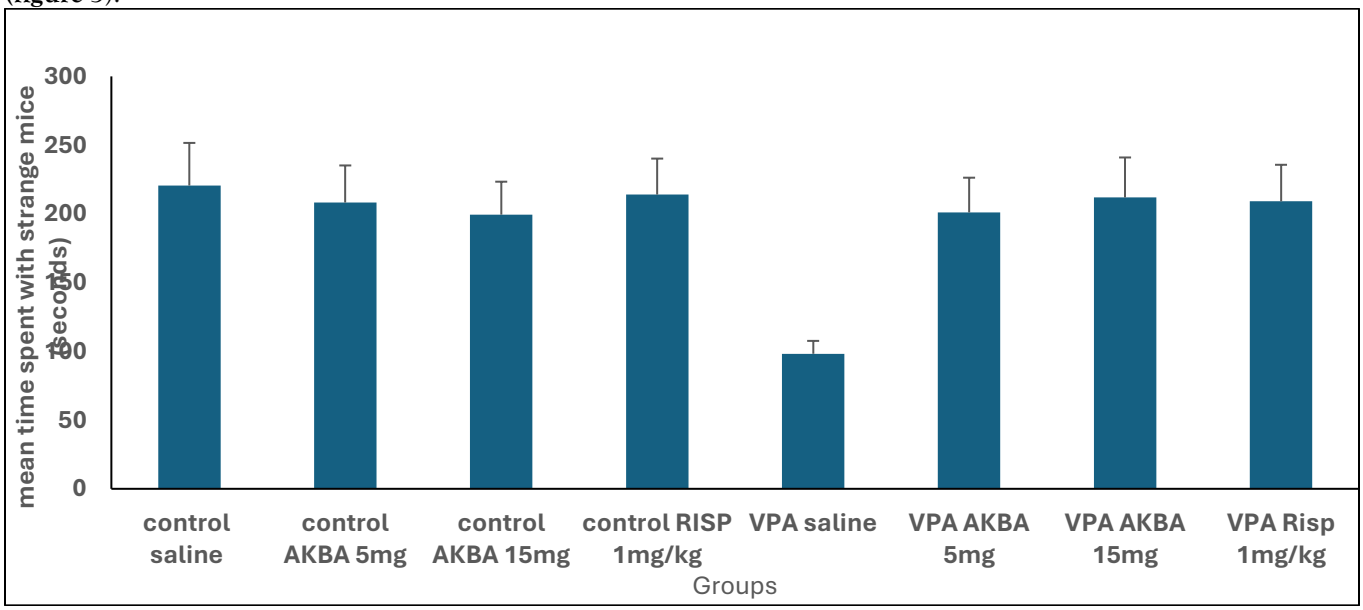

**Figure 5:** Effect of *AKBA* and risperidone on the time spent with strange mice in social interaction test. The results expressed as (Means ± SEM). No. of mice = 10 for each group.

**Anti-inflammatory tests**

**A-Tumor necrosis factor alpha (TNF-α)**

The concentration of TNF-α was highly significantly raised (p≤ 0.001) in the VPA-saline group as compared with other groups and the concentration of TNF-α was highly significantly reduced (p≤ 0.001) in both low and high-dose VPA-*AKBA* and VPA-risperidone groups as compared with the VPA-saline group.

The concentration of TNF-α significantly declined (p= 0.028) and (p= 0.014) in both low and high-dose control-*AKBA* groups respectively as opposed to the control-saline group (**figure 6**).

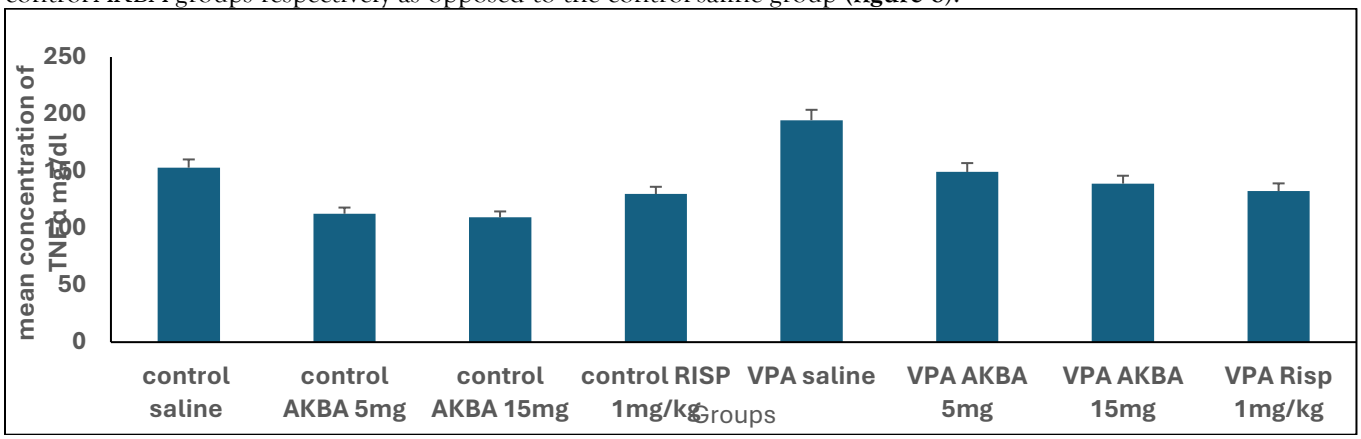


**Figure 6:** Effect of *AKBA* and risperidone on the concentration of TNF-α. The results expressed as (Means ± SEM). No. of mice = 10 for each group.

**B-Interleukin-6 (IL-6)**

The level of IL-6 was highly significantly reduced (p≤ 0.001) in high-dose control-*AKBA* group in contrast to the control-saline group, while the level of IL-6 was significantly decreased (p= 0.024) and (p= 0.018) in low-dose control-*AKBA* and control-risperidone groups respectively as compared with the control-saline group.

The concentration of IL-6 was highly significantly increased (p≤ 0.001) in the VPA-saline group as opposed to other groups and the concentration of IL-6 was highly significantly declined (p≤ 0.001) in VPA-risperidone and both high and low dose VPA-*AKBA* groups as compared with the VPA-saline group (**figure 7**).

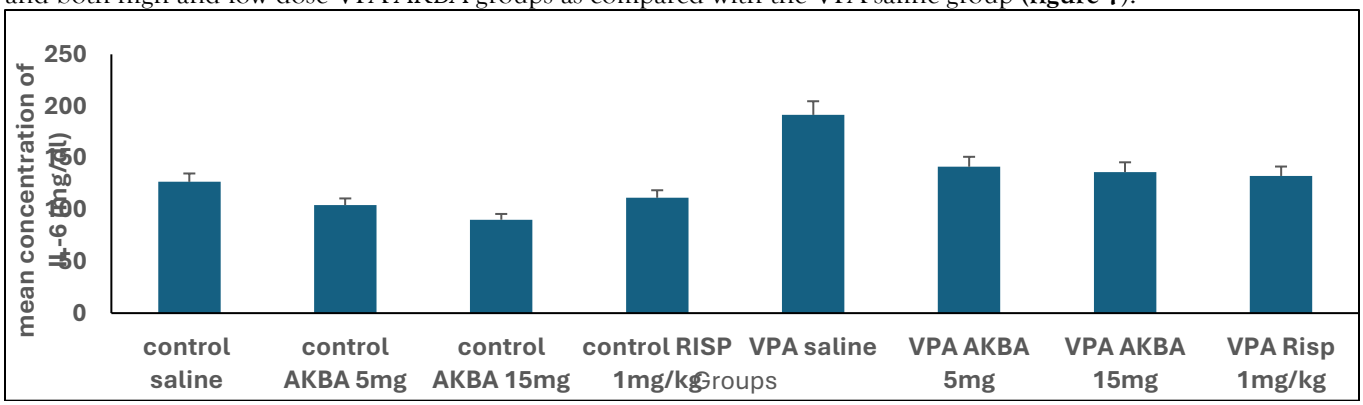


**Figure 7:** Effect of *AKBA* and risperidone on the concentration of IL-6. The results expressed as (Means ± SEM). No. of mice = 10 for each group.

**International Journal of Environmental Sciences**
ISSN: 2229-7359
Vol. 11 No. 18s, 2025
https://www.theaspd.com/ijes.php

## DISCUSSION

The present investigation is the first to investigate if AKBA has an impact on ASD. By injecting sodium valproate into the peritoneal cavity of pregnant mice on gestational day 12, the researchers seek to create an animal model of autism. Mice given sodium valproate during prenatal development showed the following behavioral changes: (i) decreased sociability and social creativity; (i) increased locomotor activity, including higher anxiety scores and decreased explorative action; (ii) increased oxidative stress; and (iv) increased release of inflammatory cytokines. In contrast, AKBA and risperidone treatment increased the offspring's behavioral difficulties while decreasing oxidative stress and neuroinflammation.

**Effects of *AKBA* and Risperidone on the Social Interaction Test Results:**

Autism spectrum disorder is a group of neurodevelopmental diseases characterized by social and communication issues [9] . The findings of this investigation demonstrate that mice prenatally exposed to VPA had a lower preference for social engagement (number of transitions between chambers and time spent with stranger animals) compared to controls (figures 3.7, 3.8), which is consistent with prior research. Social dysfunction may result from a variety of circumstances, including cognitive deficits necessary for identifying and processing socially significant signals, as well as an inability to adjust to changing social contexts . Meanwhile, therapy with AKBA and risperidone improved social behavior in VPA prenatally exposed mice. These findings are consistent with previous research.

This study found that AKBA at doses of 5mg/kg and 15mg/kg increased both the duration of time spent with unfamiliar mice and the number of transitions between chambers in the ASD mice model, and that the high dose 15mg/kg VPA-AKBA increased the duration spent with stranger mice compared to the VPA-risperidone group.

In the control groups, the high dose of 15mg/kg control-AKBA resulted in a significant increase in the number of transitions between chamber tests compared to the control-saline and control-risperidone groups (figure 3.7). These findings suggest that at a dose of 15mg/kg, AKBA has a greater positive effect on social behavior than risperidone. Treatment with AKBA plus risperidone improved the poor social interactions of VPA-treated mice.

**Anti-inflammatory activity of *AKBA* and risperidone assessed by TNF-α and IL-6 levels:**

Neuropathology is described by the generation of inflammatory cytokines, such as IL-6 and TNF-a, among other inflammatory cytokines. When the values of the VPA-saline group were compared to the values of the control group, the results showed that there was a significant rise in the TNF-a and IL-6 levels in the VPA-saline group's supernatants **(figure 3.15) (figure 3.16)**. This is consistent with the findings of the earlier research [10].

By the activation of a cascade of events involving chemokines, cytokines, and growth factors, TNF-a is able to cause an inflammatory response. Inflammatory reactions can be caused by IL-6 acting as a trigger in the nuclear factor kappa B (NF-kB) signal transduction pathway, which stimulates the transcription and releasing of subsequent inflammatory mediators. It is possible for pro-inflammatory cytokines to trigger the production of reactive oxygen species (ROS), which in turn may activate the transcription factor NF-kB and result in an inflammatory response. NF-kB is then moved into the nucleus, where it activates a number of genes involved in the inflammatory response [11].

The treatment of mice that had been prenatally exposed to VPA with AKBA and risperidone resulted in a reduction in the levels of the inflammatory cytokines TNF-a and II-6. The levels of TNF-a and IL-6 were reduced in the VPA-risperidone group when compared with the VPA-AKBA groups, and these amounts were also significantly decreased in the 15mg/kg AKBA group in comparison with the 5mg/kg AKBA group[12]. The level of IL-6 significantly declined in the low-dose control-*AKBA* and control-risperidone groups and

highly significantly declined in the high-dose control-*AKBA* as opposed to a control-saline group **(figures 3.15, 3.16)**. These results indicate that both *AKBA* and risperidone had potent anti-inflammatory activity.

## CONCLUSION

The following can be concluded based on the findings of the current investigation.

1- The data from the results shows increased in social activity with stranger mouse and number of Transition between chambers after treatment with AKBA for 20 days in social interaction test.

2- AKBA can ameliorate ASD-like symptoms (hyperactivity, anxiety and fearing), so it has potent anxiolytic activity in open field test.

3- According to the results, it could be concluded that AKBA had strong antioxidant and free radical scavenger activities as compared with risperidone. At the dose 5mg/kg, AKBA had stronger effect on lipid peroxidation (MDA) than risperidone.

4- AKBA had potent anti-inflammatory effect by decreasing IL-6 and TNF-a in ASD mice, but this effect is less as compared with risperidone.

**Financial support and sponsorship**

Nil.

**Conflicts of interest**

There are no conflicts of interest.

**REFERENCES**

**1-**Sanchez Matos, J., Barberino, A.T.M.S., de Araujo, L.P. *et al.* Potentials and Limitations of the Bioconversion of Animal Manure Using Fly Larvae. *Waste Biomass Valor* 12, 3497–3520 (2021)

**2-** Thabault M, Turpin V, Alexandre M, Mohamed J, Matthieu E, Laurie G. Cerebellar and striatal implications in autism spectrum disorders: From clinical observations to animal models. Int J Mol Sci 2022;23:2294

**3-** Eissa N, Al-Houqani M, Sadeq A, Ojha SK, Sasse A, Sadek B. Current Enlightenment About Etiology and Pharmacological Treatment of Autism Spectrum Disorder. Front Neurosci. 2018 May 16;12:304. doi: 10.3389/fnins.2018.00304. PMID: 29867317; PMCID: PMC5964170.

**4-** Aishworiya R, Valica T, Hagerman R, Restrepo B. An Update on Psychopharmacological Treatment of Autism Spectrum Disorder. Neurotherapeutics. 2022 Jan;19(1):248-262. doi: 10.1007/s13311-022-01183-1. Epub 2022 Jan 14. PMID: 35029811; PMCID: PMC9130393.

**5-** Lazaro, J & Kitzmann, Magali & Poul, Marie-Alix & Vandromme, Marie & Lamb, Ned & Fernandez, Anne. (1997). Lazaro, J. B., Kitzmann, M., Poul, M. A., Vandromme, M., Lamb, N. J. & Fernandez, A. Cyclin dependent kinase 5, cdk5, is a positive regulator of myogenesis in mouse C2 cells. J. Cell Sci. 110 (Pt 10), 1251-1260. Journal of cell science. 110 ( Pt 10). 1251-60.

**6-** Abdulla, Wasan Lafta; Abd, Suha Talal; Alkhayoun, Juman Dhia1; Ghathwan, Khalid H.; Ali, Alaa L.. The Effect of Gender on the Level of Some Salivary Elements in Children with Autism Spectrum Disorder. Medical Journal of Babylon 22(1):p 140-145, January-March 2025.

**7-** Michael Lough-Stevens, Caleb R Ghione, Matthew Urness, Adelaide Hobbs, Colleen M Sweeney, Matthew D Dean, Male-derived copulatory plugs enhance implantation success in female *Mus musculus*, *Biology of Reproduction*, Volume 104, Issue 3, March 2021, Pages 684–694.

**8-** Bon-Kwon Koo, M.D., Xinyang Hu, M.D., Jeehoon Kang, M.D., Jinlong Zhang, M.D. 6-5087, Jun Jiang, M.D., Joo-Yong Hahn, M.D. Chang-Wook Nam, M.D. for the FLAVOUR Investigators Published August 31, 2022 N Engl J Med 2022;387:779-789 DOI:

**9-** Liu, H., Yang, A., Song, J., et al. (2021) Ultrafast, Sensitive, and Portable Detection of COVID-19 IgG Using Flexible Organic Electrochemical Transistors. Science Advances, 7, eabg8387.

**10-** Spampinato, Simona Federica, Grazia Ilaria Caruso, Rocco De Pasquale, Maria Angela Sortino, and Sara Merlo. 2020. "The Treatment of Impaired Wound Healing in Diabetes: Looking among Old Drugs" *Pharmaceuticals* 13, no. 4: 60

**11-** Li, B., Deng, A., Li, K. *et al.* Viral infection and transmission in a large, well-traced outbreak caused by the SARS-CoV-2 Delta variant. *Nat Commun* 13, 460 (2022).

**12-** Gilbert, Marius et al. The Lancet, Volume 395, Issue 10227, 871 – 877