



***IN VIVO* EVALUATION OF THE EFFECT OF HERBAL SUPPLEMENTS ON THE NERVOUS SYSTEM BASED ON KATALASE, SOD, MDA, AND DC BIOMARKERS**

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**ANNOTATION:** In this study, the effects of the plant-based biologically active supplements “As-Sabr” and “As-Sedan” on the nervous system were evaluated *in vivo* based on catalase, superoxide dismutase (SOD), malondialdehyde (MDA), and diene conjugate (DC) biomarkers in an  $AlCl_3$ -induced neurotoxic model. Experiments conducted on 30 laboratory rats demonstrated that the neurotoxic model caused a decrease in antioxidant enzyme activity and an increase in lipid peroxidation markers. In the groups receiving the supplements, catalase and SOD activity increased, while MDA and DC levels decreased, and protein metabolism was restored. The obtained results confirmed the neuroprotective and antioxidant properties of these supplements.

**Keywords.** nervous system; catalase; superoxide dismutase (SOD); malondialdehyde (MDA); diene conjugates (DC); lipid peroxidation; antioxidant defense; “As-Sabr”; “As-Sedan”; *in vivo* model

## **INTRODUCTION**

At present, one of the key pathogenic factors in nervous system disorders is the increase in oxidative stress and lipid peroxidation. In neurodegenerative syndromes, vegetative-vascular dystonia (VVD) and neurocirculatory dystonia (NCD), reactive oxygen species (ROS) damage cell membranes, reduce antioxidant enzyme activity, and disrupt neurotransmitter metabolism. Therefore, *in vivo* evaluation of biochemical markers such as catalase, superoxide dismutase (SOD), malondialdehyde (MDA), and diene conjugates (DC) serves as an important diagnostic criterion in assessing the functional state of the nervous system. These markers reflect the initiation of lipid peroxidation (DC), its progression and final products (MDA), as well as the activity of the antioxidant defense system (catalase and SOD). Plant-based biologically active supplements “As-Sabr” and “As-Sedan” contain flavonoids, phenolic acids, B-group vitamins, macro- and microelements, and essential oil components, providing the ability to reduce oxidative stress, activate antioxidant enzymes, and restore membrane stability. For this reason, the aim of this study was to evaluate the effects of “As-Sabr” and “As-Sedan” on the nervous system in an  $AlCl_3$ -induced neurotoxic model based on catalase, SOD, MDA, and DC biomarkers under *in vivo* conditions.

## **MATERIALS AND METHODS**

Thirty male laboratory rats (*Rattus norvegicus*) weighing 150–200 g were used in the study. The animals were acclimatized for 2 weeks under 22–24 °C temperature, 40–60% relative humidity, with free access to food and water. The diet included wheat, pistachio, dairy products, beans, egg products, meat, and pasta.

**Induction of the neurotoxic model.** To induce neurotoxic conditions, rats received intraperitoneal injections of a 10% aluminum chloride ( $AlCl_3$ ) solution for 3 consecutive days. This neurotoxin disrupts impulse transmission in the central nervous system, increases oxidative stress, and impairs cognitive functions.



Experimental groups. Animals were divided into 3 groups:

Control- healthy rats. Neurotoxic model- AlCl<sub>3</sub>-treated rats without supplements.

Experimental- AlCl<sub>3</sub>-treated rats receiving “As-Sabr” or “As-Sedan” supplements for 1 month.

Determination of biomarkers. Malondialdehyde (MDA): determined by the thiobarbituric acid reactive substances (TBARS) method. To 2.5 ml of citrated blood, 2.5 ml of 10% trichloroacetic acid was added and centrifuged at 3000 rpm for 15 minutes. To the supernatant, 1.5 ml of 0.8% thiobarbituric acid solution was added and heated in a boiling water bath for 15 minutes. After cooling and centrifugation, the optical density was measured at 532 nm. Diene conjugates (DC): determined by the method of Khishiktuyev et al. (1996). A heptane–isopropanol (1:1) mixture was added to plasma, shaken for 15 minutes, and centrifuged at 6000 rpm for 10 minutes. The lipid fraction was separated and treated with 0.01 N hydrochloric acid. Absorbance was measured at 232 nm. Catalase: activity calculated by measuring the rate of H<sub>2</sub>O<sub>2</sub> decomposition spectrophotometrically (410 nm) with ammonium molybdate used to stop the reaction. Results were expressed in mkat/L. Superoxide dismutase (SOD): determined by the method of Misra and Fridovich (1972). Superoxide anion is generated in the PMS/NADH system and converts NBT (nitrotriazolium blue) into colored formazan. In the presence of SOD, color formation decreases. Activity was calculated at the 50% inhibition point (per gram of protein). Protein content: determined by the Biuret method (540–560 nm) to normalize biomarker concentrations.

Equipment and reagents. Spectrophotometer K-7000 (Andru Instruments, China)- for MDA, DC, catalase, and SOD assays. Centrifuge Mini-7 (BIOBASE, China). Ultrapure water system, analytical balance OHAUS. Thiobarbituric acid, trichloroacetic acid, heptane, isopropanol, ammonium molybdate, PMS, NADH, and NBT- all reagents were of analytical grade.

Statistical analysis. Data were expressed as ±SD. Significance between the groups was assessed using Student’s t-test at p < 0.05.

Results. The results obtained during the experiment demonstrated that in the aluminum chloride (AlCl<sub>3</sub>)-induced neurotoxic model, all major biomarkers of oxidative stress underwent significant alterations. The level of MDA (a terminal product of lipid peroxidation) increased 1.5–2 times in the neurotoxic model compared to the control group (p < 0.01). In the groups treated with “As-Sabr” and “As-Sedan,” MDA levels significantly decreased and approached control values.

**Table 1. *In vivo* changes in catalase, SOD, MDA, diene conjugates, and protein biomarkers in rats treated with “As-Sabr” and “As-Sedan.”**

Indicator	Control group (mg/dl or mkat/l)	Neurotoxic model (AlCl <sub>3</sub> )	“As-Sabr” AlCl <sub>3</sub>	+	“As-Sedan” + AlCl <sub>3</sub>
Catalase (mkat/l)	32.25	23.25	34.67		33.90
SOD (mkat/l)	4.67	5.53	5.96		5.80
MDA (nmol/ml)	2.78	3.64	2.50		2.55
Diene conjugates (absorbance at 232 nm)	1.23	1.91	1.12		1.15
Protein (mg/dl)	16.95	24.76	31.00		30.80

The amount of diene conjugates (DC) was also elevated in the AlCl<sub>3</sub> group; in the animals receiving the supplements, DC levels decreased, indicating reduced membrane lipid peroxidation.



Catalase activity markedly decreased in the neurotoxic group compared to the control. Administration of “As-Sabr” and “As-Sedan” restored catalase activity and, in some cases, increased it beyond control values (an indication of enhanced antioxidant defense). SOD activity also decreased in the  $AlCl_3$  group. Supplementation increased SOD activity, restoring the ability to neutralize superoxide radicals.

Protein levels in the experimental group were higher than in the control group, showing restoration of metabolic processes and improvement of the trophic state of the examined tissues. Overall, extracts of “As-Sabr” and “As-Sedan” reduced oxidative stress in the neurotoxic model, increased the activity of antioxidant defense enzymes, and decreased lipid peroxidation markers.

### **CONCLUSION**

In vivo studies conducted on the  $AlCl_3$ -induced neurotoxic model showed that increased MDA and DC levels and decreased catalase and SOD activity lead to pronounced oxidative stress in the central nervous system. The dietary supplements “As-Sabr” and “As-Sedan” corrected these pathological changes: they significantly reduced MDA and DC levels, increased catalase and SOD activity, and normalized protein concentration. These preparations possess neuroprotective and antioxidant properties, counteracting oxidative stress induced by neurotoxins such as  $AlCl_3$  and ensuring metabolic stability of nervous tissue cells.

Thus, the dietary supplements “As-Sabr” and “As-Sedan” may be regarded as promising natural preparations for the prevention and treatment of neurodegenerative processes.

### **References**

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