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Evaluation of acute oral toxicity of a herbal semen quality enhancer

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ABSTRACT

The objective of this study was to assess the acute oral toxicity of AV/SGB/27 (M/s Ayurvet Limited) in accordance with OECD-423 guideline. AV/SGB/27 is a herbal formulation for enhancing semen quality of livestock. Nine healthy and adult nulliparous nonpregnant Swiss albino female mice, weighing 24-28 g, were used in this study. After being given test material orally, the mice were examined for toxic effects and mortality. Changes in body weights, symptoms of toxicity, histological appearances of liver, kidney, and lungs, and biochemical parameters were used to assess the toxicity. No toxic effects or mortalities were noticed until the trial was completed, indicating that AV/SGB/27 is safe for oral consumption.

Keywords: Acute oral toxicity, AV/SGB/27, OECD 423, Semen quality, Herbal.

INTRODUCTION

Profitability in any commercial livestock-breeding unit is closely related to the reproductive efficiency of animals. Fertility of the breeding males is important to reduce production losses and to achieve better economy of the farm. Many herbs have the ability to enhance vigor and fertility in order to improve reproduction. Low testosterone levels, toxicity causing low sperm count, and poor sperm motility can all be addressed with herbal therapies. Although the causes of infertility are various and undetermined, oligozoospermia (low sperm concentration) and asthenozoospermia (low sperm motility) are the most common causes of male infertility [1, 2]. Hormonal imbalances, mineral deficiencies, rearing stresses, and radiations are notable factors that lower reproductive performance. So, better semen quality is important for improving fertility, conception rate and farm economy.

AV/SGB/27 (M/s Ayurvet Limited, Baddi, India) is a scientific blend of effective medicinal herbs and minerals for improving semen quality, fertility, and vigor of the breeding males. In various studies, its key ingredients such as *Withania somnifera*, *Mucuna pruriens*, *Asparagus racemosus*, and *Tribulus terrestris*, have been used as natural remedies for thousands of years in Indian and Chinese medicine, to improve sperm quality and erectile functions, promote sexual behaviors and increase hormonal levels [3, 4, 5].

These herbal ingredients promote spermatogenesis by improving the testicular, seminal vesicular and epididymal functions. They stimulate testicles to produce more and high-quality sperm. They also improve the sperm count and the quality by boosting the number of LH-FSH-producing basophil cells in the pituitary. AV/SGB/27 is hormone-free, leaves no residue, and is environment and animal-friendly. The aim of this study was to determine the acute oral toxicity of AV/SGB/27.

MATERIALS AND METHODS

The present research was performed at the Dept. of Vet. Pharmacology and Toxicology at the Post Graduate Institute of Veterinary and Animal Sciences (PGIVAS), Akola, Maharashtra. Institutional Animal Ethics Committee of PGIVAS, Akola, approved the research protocol with approval number 312/4/14/2000/20; dated 06-03-2020.

Nine healthy and adult nulliparous nonpregnant Swiss albino female mice, weighing 24-28 g, were used in this study. The animals were obtained from the lab animal resource unit, Dept. of Pharmacology, PGIVAS, Akola, Maharashtra. Animals were cared for in accordance with IAEC SOPs and CPCSEA guidelines. Picric acid staining was used to identify the animals. For easy monitoring, the number of animals in single cage was kept at three with good housing conditions. The animals were subjected to 12-hour light and 12-hour dark cycle with maintained 25 ± 20 °C temperature and 70% relative humidity. All mice were fed a standard pelleted feed and had unlimited access to water [6].

For acclimatization to laboratory environment, all mice were maintained in the cages for five days. Thereafter, all mice were fasted for the night; food but not water was withheld for 3-4 hrs. All mice were weighed after the fasting period and the test material was given to them per os. The test sample was given to 3 mice in Group-I with dose rate of 300 mg/kg of body weight (b.wt.). If signs of toxicity not appeared in Group-I, then other 6 mice in Group-II were administered the limit dose of the test materials *i.e.* at 2000 mg/Kg of b.wt. Following administration of the test material, no mice were fed for 1-2 hrs in both groups.

The animals were observed for toxic effects and fatalities, as well as the LD_{50} value, at least once for the first 30 minutes and frequently during first 24 hrs, and further for 14 days. Changes in the eyes, skin and coat as well as changes in the respiratory, circulatory, and central nervous systems, as well as autonomic and somatic activity were noted.

Clinical signs like muscular tremors, lethargy, sleep, diarrhea, salivation, and convulsions, if seen, were noted. Animals were euthanized after 14 days of examination, and necropsy, along with the histopathological study of the liver, heart, lungs, and kidneys, were performed. Biochemical characteristics such as alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and creatinine were analyzed in the blood samples.

The data of biochemical parameters were analyzed statistically using one way ANOVA followed by complete randomized design.

RESULTS AND DISCUSSION

The b.wt. of mice were measured individually during 0, 7, and 14 days of the study. The b.wt. of Group-I and Group-II continued to increase during the study period (Table 1). No mortality and abnormal sign was seen after oral administration of AV/SGB/27 at 300 mg/Kg b.wt. and 2000 mg/Kg b.wt. to G-I and G-II mice, respectively. The LD_{50} of AV/SGB/27 was more than 2000 mg/Kg as no mortality occurred at this limit dose *i.e.* the limit dose, which can be given by orally.

Necropsy after 14 days did not showed any significant changes in the gross appearance of liver, heart, lungs, and kidneys. Similarly, no changes were seen in the histopathological appearances of liver, kidneys, heart, and lungs in any of the animals (Figure 1). While blood biochemical parameters differed significantly in the values of AST, ALT, and creatinine between G-I and G-II (Table 2), the values were well within their normal ranges in both the groups, indicating no liver or kidney damage.

AV/SGB/27 is prepared from parts of plants like *Withania somnifera*, *Mucuna pruriens*, *Asparagus racemosus*, and *Tribulus terrestris*, which belong to the Generally Regarded as Safe (GRAS) category. The extracts of *T. terrestris, W. somnifera* and *M. pruriens* regulating Nrf2/HO–1 and NF-κB pathways and enhance sexual activity and behavior by increasing the androgen levels and simultaneous lower the ROS levels [5]. *W. somnifera*, having active component like sitoindosides and withaferin A [7, 8], reduce lipid peroxidation by elevation of superoxide dismutase, free radical scavenging enzymes, catalase, and glutathione peroxidase levels [7]. The active component in *T. terrestris* is protodioscin, which improves semen quality by increasing androgen level and enhances ejaculation [9, 10]. *A. racemosus* and *M. pruriens* contain active ingredients like shatavarin I-IV and mucunine, which have anti-oxidant and immunomodulatory activity [11, 12]. *M. pruriens* significantly increases sperm motility and sperm concentration. Further, it decreases major anomalies of the sperm [5, 13]. Moreover, *M. pruriens* increases erectile function, sexual desire and overall satisfaction and orgasmic function as well as increases semen volume, total sperm count, and rapid linear progressive sperm, and decreases non-progressive and immotile sperm count [14]. Similarly, *A. racemosus* is useful for infertility and decreased libido [3]. Thus, AV/SGB/27 can be used to improve semen quality and infertility problem in male animals without exerting any toxic effects.

Table 1: Individual weekly body weights and mortality of mice during experimental period

Dose	Animal number	Body weight (g) on day			Mortality
		0	7	14	
300 mg/Kg b.wt. orally (G-I)	1	24	26	26	No
	2	27	29	29	-
	3	28	28	30	-
	Mean ± SD	26.33 ± 1.20	27.67 ± 0.88	28.33 ± 1.20	
2000 mg/Kg b.wt. orally (G-II)	1	25	26	27	No
	2	26	27	28	-
	3	26	27	29	-
	4	27	28	28	-
	5	27	27	29	-
	6	28	29	30	-
	Mean ± SD	26.50 ± 0.43	27.33 ± 0.42	28.33 ± 0.43	

Table 2: Mean ± SD ALT, AST, ALP and creatinine values of experimental animals

Dose	ALT (U/L)	AST (U/L)	ALP (U/L)	Creatinine (mg/dL)
300 mg/Kg b.wt (G-I)	42.34±0.59[b]	49.71±0.84[b]	119.01±2.63	0.45±0.012[b]
2000 mg/Kg b.wt (G-II)	45.72±0.85[a]	55.18±0.58[a]	125.59±2.09	0.55±0.012[a]

[a-b] Mean ± SD bearing different superscripts differ significantly ($p<0.05$) within columns

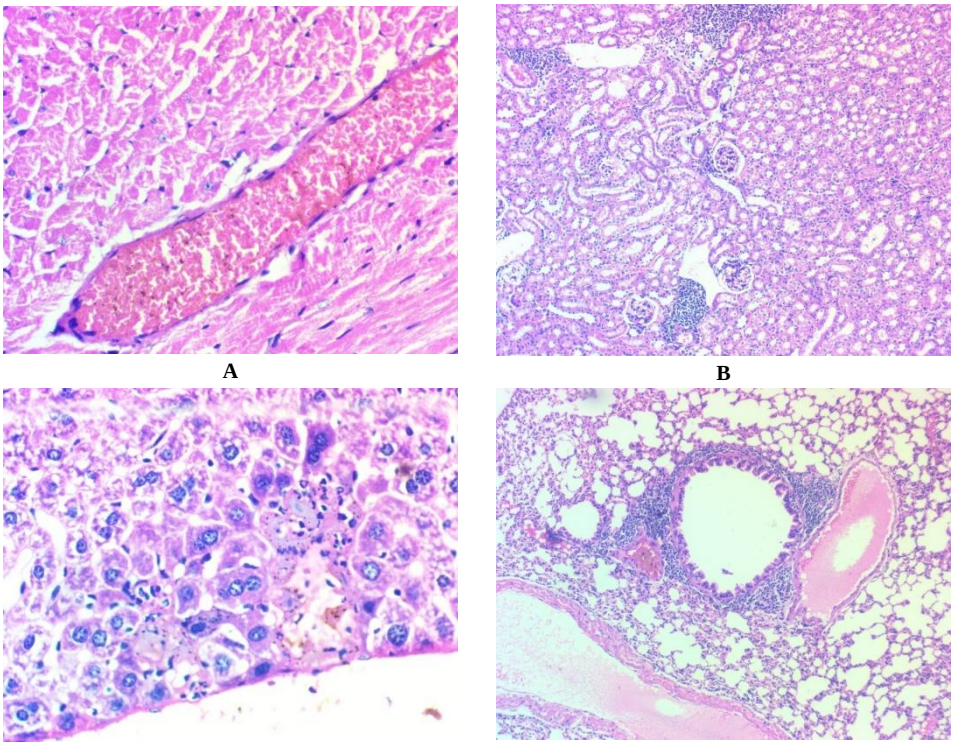

Figure 1: Histopathological appearances of A-heart, B-kidneys, C-liver and D-lungs of mice receiving AV/SGB/27 (2000 mg/Kg b.wt.)

CONCLUSION

AV/SGB/27 produced no acute oral toxicity, as indicated by the absence of mortality, the absence of clinical signs, and the absence of gross or histopathological changes, even when given upto the threshold dose in mice (2000 mg/Kg b.wt.). Based on the results, the herbal medicine is safe to take orally.

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Conflict of Interest

None declared.

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None declared.

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