Research Journal of Chemical and Environmental Sciences
Res J. Chem. Environ. Sci. Vol 8 [4] August 2020: 15-17
Online ISSN 2321-1040
CODEN: RJCEA2 [USA]

Website: www.aelsindia.com/rjces.htm

RJCES

ORIGINAL ARTICLE

Evaluation of acute oral toxicity of a polyherbal liver stimulant

Vikas Vasant Karande[1], Vaishnavi Sanjay Gagare[1], Sunidhi[2]*, Ravikanth Kotagiri[2], Bhaskar Ganguly[2]

[1]Department of Pharmacology and Toxicology, Krantisinh Nana Patil College of Veterinary Science, Shirwal, 412801. India.

[2]Department of Clinical Research, Research and Development Unit, Ayurvet Limited, Baddi, 173205. India.

*Corresponding Author: clinical@ayurvet.in

ABSTRACT

A study was undertaken to evaluate the potential of Superliv® DS (M/s Ayurvet Limited, India) for acute oral toxicity according to OECD 423 guidelines. Superliv® DS is a liver formula for better growth and production in poultry, fish, prawns and shrimps. Six (3 male and 3 female) Swiss albino mice were used for the study, where each animal served as its own control. The animals were observed for the manifestation of toxic effects and mortality following the oral administration of the limit dose of test substance @ 2000 mg/Kg body weight. No toxic effects or mortalities were observed till 14 days and Superliv® DS was found to be safe for oral use.

Keywords*: acute oral toxicity; limit test;Superliv® DS; OECD 423; safety*

Received 21.05.2020 Revised 24.06.2020 Accepted 28.07.2020

INTRODUCTION

With poultry farming profits becoming marginal because of the increasing price of feed ingredients, enhancing farm productivity by improving feed utilization has become a core issue. The liver, being one of the most vital organs of the body, plays a major role in the digestion, metabolism and utilization of feed nutrients. Being the seat of a number of digestive, metabolic and productive activities, the liver is ever endangered by microbial and chemical toxins. These toxins cause varying degrees of damage to the liver and affect its functions, thereby resulting in poor health and production [6]. Nature has provided many herbs that are known to exert beneficial actions on the liver, thus helping improve farm productivity.Such herbs have also been indicated to exert immunomodulating action, which confers bird with better immune response against various diseases and tolerance against toxins leading to lower mortality, morbidity and enhanced productive adaptability [11]. Superliv® DS is one such liver formula for better growth and production in poultry,fish, prawns and shrimps. It contains herbs like *Andrographis paniculata, Azadirachta indica, Boerhaavia diffusa, Picrorhiza kurroa, etc.* known for their hepatoprotective [14, 4, 2, 3], anti-oxidant [15, 17], immunomodulatory [9] and choleretic action [12]. It is recommended for growth promotion in broilers, layer chicks and growers, for improving FCR and livability, and in fishes, prawns and shrimps for higher body weight gain, for better survival rate and for maintaining feed consumption. The present study aimed at determining the acute oral toxicity potential of Superliv® DS.

MATERIAL AND METHODS

The present study was undertaken at the Department of Pharmacology and Toxicology, Krantisinh Nana Patil College of Veterinary Science (KNPCVS), Shirwal, District Satara, India. The experimental protocol of the study was got approved from the Institutional Animal Ethics Committee of KNPCVS (Approval number: IAEC/16/KNPCVS/05/2019; dated: 23/08/19). Six healthy adult (3 males and 3 females) Swiss albino mice, weighing 20-25g, were used. The animals were procured from CPCSEA-registered breeding source *i.e.* National Institute of Biosciences, Pune. All animals were maintained as per the SOPs outlined in CPCSEA guidelines. The animals were identified by appropriate means. The number of animals per cage was kept at three for clear observation of each animal; housing conditions were conventional. The ambient temperature was 25°C and relative humidity was 70%. The animals were exposed to 12 hour light-dark cycle and provided with standard pelleted feed and water *ad lib* (OECD Test 423).

After procurement, the animals were kept in the cages for seven days for acclimatization. Thereafter, the animals were fasted overnight; food but not water was withheld for 3-4 hours. Following the period of fasting, the animals were weighed and the test substance was administered orally. After the administration of the test substance @ 2000 mg/Kg body weight, food was withheld for 1-2 hours. The animals were observed intensively for first 24 h, and then further for a period of 14 days for the manifestation of toxic effects and deaths; LD_{50} value was also assessed. The observations included changes in skin, coat and eyes; and changes in respiratory, circulatory, CNS, autonomic, somatic activity and behavior. Clinical signs like muscular tremors, convulsions, salivation, diarrhea, lethargy, sleep, and coma, if observed, were recorded. After 14 days of observation, the animals were euthanized and necropsy, along with the histopathological investigations of the liver, kidneys, spleen, heart, lungs, and reproductive organs, was performed.

RESULTS AND DISCUSSION

Individual body weights of mice were recorded on days 0, 7 and 14 of the study and body weights in both the groups (I and II) continued to increase throughout the study period (Table 1).

No mortality was seen throughout the period of observation. Since no mortality occurred in the mice receiving the limit dose of Superliv® DS at 2000 mg/Kg body weight *i.e.* the maximum dose which can be administered by oral route, therefore, the LD_{50} was inferred to be beyond this limit. Similarly, no abnormal symptoms, including lethargy, tremor, abdominal breathing or piloerection, were observed up to after 14 days of Superliv® DS administration. Necropsy after day 14 did not reveal any remarkable alterations in the gross appearance of the liver, kidneys, spleen, heart, lungs, and reproductive organs in any of the animals.Similarly, no abnormalities were detected in the histopathological appearances of the liver, kidneys, spleen, heart, lungs, and gonadal organs in any of the animals.

Superliv® DS contains parts of plants like *Andrographis paniculata, Azadirachta indica, Boerhaavia diffusa, Picrorhiza kurroa*, *etc*. that are Generally Regarded as Safe (GRAS). Diterpenoid constituents of *Andrographis paniculata* such as andrographolide (I), andrographiside (II) and neoandrographolide (III) protect against hepatotoxicity induced in mice by carbon tetrachloride or *tert*-butylhydroperoxide (tBHP) intoxication [8]. Andrographolide produces a choleretic effect in conscious rats and anaesthetized guinea pigs as evidenced by increase in bile flow, bile salt, and bile acids. The paracetamol-induced decrease in volume and contentof bile was prevented significantly by andrographolide pretreatment [12].Extract of dried *Andrographis paniculata* leaves exhibited anti-filarial activity against adult worms of sub-periodic *Brugiamalayi* [16].

Azadirachta indica possesses a significant hepatoprotective activity. The protective effect of *Azadirachta indica* leaf extract was investigated on hepatic lipid peroxidation and antioxidant status during *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG)-induced gastric carcinogenesis in male Wistar rats. Administration of neem leaf extracts significantly lowered the enhanced lipid peroxidation and increased the lowered hepatic levels of glutathione and glutathione dependent enzymes. The extract also altered cancer development at extra-hepatic sites by influencing hepatic biotransformation enzymes and antioxidants [1]. *Azadirachta indica* aqueous extract exerts chemopreventive effects on 7,12-dimethylbenz[a]anthracene (DMBA) induced buccal pouch carcinogenesis in hamsters by modulation of lipid peroxidation, antioxidants and detoxification systems [2].

An alcoholic extract of *Boerhaavia diffusa* plant given orally exhibited hepatoprotective activity against experimentally-induced carbon tetrachloride hepatotoxicity in rats and mice. The extract also produced an increase in normal bile flow in rats suggesting a strong choleretic activity [4]. Picroliv, the standardized active glycoside fraction of *Picrorhiza kurroa*, exhibited hepatoprotective action when given orally @ 6 and 12 mg/Kg body weight for 7 days to rats by causing significant reversal of the paracetamol-induced biochemical changes like increasedlevels of transaminases, alkaline phosphatase, bilirubin in serum,total lipids, lipid peroxides and changes in the glycogen and cholesterol content in the liver [7].

A composition based on GRAS constituents like *Andrographis paniculata, Azadirachta indica, Boerhaavia diffusa, Picrorhiza kurroa*, *etc*. is least likely to be toxic in practical doses.Superliv® DS also exerts multifarious benefits, including strengthening of immune system, improvement in feed consumption, weight gain, survival rate, FCR, livability, and productive performance of the birds due to the presence of multiple active ingredients.

Table 1: Individual body weights of experimental mice

Formulation and Dose	Mice No.	Body Weight (g) on Day		
		0	7	**14**
Superliv® DS @ 2000 mg/Kg body weight orally (Group I: Females)	1	20.0	21.0	**23.0**
	2	22.0	23.0	**24.0**
	3	20.0	22.0	**25.0**
Superliv® DS @ 2000 mg/Kg body weight orally (Group II: Males)	1	20.0	21.0	**23.0**
	2	22.0	24.0	**25.0**
	3	22.0	24.0	**25.0**
Mean ± S.E.		21±0.41	22.50±0.51	**24.17±0.37**

CONCLUSION

Superliv® DS did not produce acute oral toxicity as evident from the absence of mortality, toxic clinical symptoms, and gross and histopathological alterations, when administered up to a limit dose of 2000 mg/Kg body weight in mice. Based on these findings, the formulation was found to be safe for oral use.

REFERENCES

1. Arivazhagan S, Balasenthil S and Nagini S(2000). Garlic and neem leaf extracts enhance hepatic glutathione and glutathione dependent enzymes during N-methyl-N′-nitro-N-nitrosoguanidine (MNNG)-induced gastric carcinogenesis in rats. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, 14(4):291-293.
2. Balasenthil S, Arivazhagan S, Ramachandran CR, Ramachandran V and Nagini S (1999). Chemopreventive potential of neem (Azadirachta indica) on 7, 12-dimethylbenz [a] anthracene (DMBA) induced hamster buccal pouch carcinogenesis. *Journal of ethnopharmacology*, 67(2): 189-195.
3. Bhanwra S, Singh J andKhosla P (2000). Effect of Azadirachta indica (Neem) leaf aqueous extract on paracetamol-induced liver damage in rats. *Indian journal of physiology and pharmacology,* 44(1):64-68.
4. Chandan BK, Sharma AK and Anand KK (1991). Boerhaavia diffusa: a study of its hepatoprotective activity. *Journal of Ethnopharmacology*, 31(3):299-307.
5. Choudhury BRandPoddar MK (1984). Andrographolide and kalmegh (Andrographis paniculata) extract: in vivo and in vitro effect on hepatic lipid peroxidation, *Methods and findings in experimental and clinical pharmacology*,6(9):481-485.
6. Dutta M (2010). Healthy liver, healthy birds. https://www.poultryworld.net/ Breeders/General/2010/2/ Healthy-liver-healthy-birds-WP007031W/Accessed 28 October, 2020.
7. Dwivedi Y, Rastogi R, Garg NK and Dhawan BN (1991). Prevention of paracetamol-induced hepatic damage in rats by picroliv, the standardized active fraction from Picrorhiza kurroa. *Phytotherapy Research*, 5(3):115-119.
8. Kapil A, Koul IB, Banerjee SK and Gupta BD (1993). Antihepatotoxic effects of major diterpenoid constituents of Andrographis paniculata. *Biochemical Pharmacology*, 46(1):182-185.
9. Njiro S and Kofi-Tsekpo MW (1999). Effect of an aqueous extract of Azadirachta indica on the immune response in mice. *Journal of Veterinary Research*, 66(1):59-62.
10. No. OECD Test 423 (2001). Acute oral toxicity-acute toxic class method. OECD guidelines for the testing of chemicals (section 4: health effects) 1:14.
11. Sharma RK, Maini S and Ravikanth K (2008). Beneficial effects of Superliv DS and Xlivpro on growth promotion and carcass quality traits in broilers. *Veterinary World*,1(12): 363-365.
12. Shukla B, Visen PKS, Patnaik GK and Dhawan BN (1991). Choleretic effect of picroliv, the hepatoprotective principle of Picrorhiza kurroa1. *Planta Medica*, 57(1):29-33.
13. Shukla B, Visen PKS, Patnaik GK and Dhawan BN (1992). Choleretic effect of Andrographolide in Rats and Guinea Pigs1. *Planta medica*, 58(02):146-149.
14. Trivedi NP and Rawal UM (2001). Hepatoprotective and antioxidant property of *Andrographis paniculata* (Nees) in BHC induced liver damage in mice. *Indian Journal of Experimental Biology*, 39(1):41-46.
15. Wang HW, Zhao HY, Xiang SQ (1997). Effects of *Andrographis paniculata* component on nitric oxide, endothelin and lipid peroxidation in experimental atherosclerotic rabbits. *Chinese Journal of Integrated Traditional and Western Medicine,* 17(9):547-549.
16. Zaridah MZ, Idid SZ, Omar AW andKhozirah S (2001). In vitro antifilarial effects of three plant species against adult worms of subperiodicBrugiamalayi. *Journal of Ethnopharmacol*ogy, 78(1):79-84.
17. Zhang XF and Tan BKH (2000). Antihyperglycaemic and anti-oxidant properties of *Andrographis paniculata* in normal and diabetic rats. *Clinical and Experimental Pharmacology and Physiology,* 27(5-6): 358-363.

CITE THIS ARTICLE

V V Karande, V S Gagare, Sunidhi, R Kotagiri, B Ganguly. Evaluation of acute oral toxicity of a polyherbal liver stimulant. Res. J. Chem. Env. Sci. Vol 8 [4] August 2020. 15-17