

LIVER FUNCTION ENZYMES AT THE LD₅₀ LEVEL OF THE WATER AND METHANOL EXTRACTS OF STREBULUS ASPER ON MICE

ANNIE MATHAI and K.S. DEVI

Department of Biochemistry, University of Kerala, Kariavattom P.O., Trivandrum – 695 581, Kerala.

Received: 19 October, 1997

Accepted: 7 January, 1996

ABSTRACT: The LD₅₀ of the water and methanolic extractable portion of *S. asper* was 230mgm/kgm body weight and 200 mgm/kgm body weight respectively. Mice treated with SaW₁ and SaM₁ portion of *S. asper* showed an increase in GOT, GPT acid phosphatase, alkaline phosphatase and β -glucuronidase in the liver after 24hrs of drug treatment, the increase being more in SaM₁ treated group. The enzyme activity slowly recovered at 48 and 72 hrs. But were not reaching the corresponding control values after 72 hrs.

INTRODUCTION

Helminthiasis is a disease caused by infestation with parasitic worms living in the alimentary canal or in other tissues of the host, this disease is one of the major problems in the world, particularly in the tropical countries. The stem bark of *S. asper* was found to be effective against filariasis¹ and the anti-parasitic activity of the methanolic extractable portion of *S. asper* was studied.² Present study was done to determine the LD₅₀ of the water and methanolic extractable portion of strbulus and their biochemical effect on the liver of mice.

Materials and Methods

Determination of LD₅₀ of the water extract (SaW₁) of *S. asper* on mice.

LD₅₀ of the drug was determined according to the method adopted by british toxicology society.³ Selected animals of both sex (6each) and tested a dose level of

10mgm/kgm body weight, 50 mgm/kgm and 500mgm/kgm body weight of the 5% solution of asper. About 90% of the animals survived without signs of toxicity at 10mgm and 50 mgm level. It was found that at a dose level of 500mgm/kg body weight about 95% of the mice died, so the experiment was repeated with different doses. It was observed that at 230 mgm/kgm body weight about half of the mice survived. The LD₅₀ was taken as 230 mgm/kgm body weight. The result sis given in Table I.

Determination of LD₅₀ of the methanolic extractable portion (SaM₁) of *S. asper* on mice.

The LD₅₀ was determined by the same procedure as described for the water extract of *strebulus*. It was observed that at 200mgm/kgm body weight about half of the mice tested survived. The LD₅₀ of the methanolic extractable portion of *strebulus*

was taken as 200mgm/kgm body weight. The result is given in table 2.

Effect of SaW₁ and SaM₁ in mice at LD₅₀ Level

Healthy adult male albino mice weighing about 30-35 gm obtained from the animal house of the department were maintained on pellet diet (Lipton India Ltd) and water ad libitum. They were divided into three groups of 18 animals each. Group -I: received 1ml of normal saline vehicle 1p (Control group). Group-II: 1ml of water extractable portion of *S. asper* in saline (5% solution, 1ml of methanolic extractable portion of *S. asper* in saline (1ml = 7gmg) given 1p. Group-III: 1 ml of methanolic extractable portion of *S. asper* in saline (1ml=7gmg) given 1P.

Animals from each group were sacrificed on 24, 48 and 72nd hours after the treatment and liver was collected and used for various analysis.

Assay of GOT, GPT acid phosphatase, alkaline phosphatase, LDH and β-glucuronidase

Freshly removed liver was trimmed free from extraneous materials using chilled saline and homogenized in 0.25 Mice cold sucrose solution (10%w/v) in a potter Elvehjem type homogenizer. The homogenate was centrifuged (700g) at room

temperature for 10mnts to remove cell debris and used for the assay of enzymes. Glutamic oxaloacetic transaminase (GOT, EC: 2,6.1.1) glutamic pyruvic transmission (GPT, EC: 2,6.1.2) acid phosphatase (EC:3.1.3.2) and alkaline phosphatase (EC:3.1.3.1) were estimated calorimetrically as described by wooten,⁴ Activity of lactate dehydrogenase (LDH, EC: 1.1.1.27) was assayed by the method of bergmeyer and Bernt.⁵ β – glucuronidase (EC: 3.2.1.31) was assayed by the method of Kawai and anno.⁶ Protein was estimated by the method of Lowry et al.⁷

RESULTS AND DISCUSSION

Results are given in Tables 3and 4. Mice treated with SaW₁ and SaM₁ portion of *S. asper* showed an increase in GOT, GPT, Acid Phosphatase, Alkaline Phosphatase and β-glucuronidase in the liver after 24 hrs of drug treatment, the inverse was more in SaM₁ treated group. The activity of these enzymes was slowly recovered at 48hrs. But was not reaching the corresponding control values after 72 hrs.

It is felt that many environmental factors chemicals, drugs and contaminated food affect the liver physiology upto a certain extent which may lead to other secondary physiological changes. The increase of transaminers and phosphatase activities might be the sequela of either cellular alteration and or stress condition.

Table 1
LD₅₀ Determination of the water extract (SaW₁) of *S.asper* in mice.

Test dosage	Result	Action /Classification
10mgm/kgm	>95% Survival	Non-toxic
50 mgm/kgm	>95% Survival	Non-toxic
500 mgm/kgm	>95% dead	Toxic
200 mgm/kgm	>80% Survival	Non-toxic
230 mgm/kgm	About 50% Survival	LD ₅₀
250 mgm/kgm	>50% dead	Toxic

Table 1I
LD₅₀ Determination of the methanolic extract (SaW₁) of *Strebulus* on mice.

Test dosage	Result	Action /Classification
10mgm/kgm	>95% Survival	Non-toxic
50 mgm/kgm	>95% Survival	Non-toxic
500 mgm/kgm	>95% dead	Toxic
200 mgm/kgm	>80% Survival	Non-toxic
230 mgm/kgm	About 50% Survival	LD ₅₀
250 mgm/kgm	>50% dead	Toxic

Table-III

Concentration of GPT,GOT, and LDH in the liver of mice injected with normal saline, water and methanolic extractable portion of Strebulus at 24,48 and 72 hours. Values are mean ±SEM of estimations.

Extracts used	Concentration of GPT (μgm of pyruvate liberated mnt/mgm protein) at			Concentration of GOT (μgm of pyruvate liberated mnt/mgm protein) at			Concentration of LDH (μgm of pyruvate liberated mnt/mgm protein) at		
	24hr	48hr	72hr	24hr	48hr	72hr	24hr	48hr	72hr
Normal	19.8 ±1.7	19.6 ±1.7	19.3 ±1.7	8.1 ±0.72	7.9 ±0.71	7.8 ±0.70	8.5 ±0.76	8.6 ±0.77	8.3 ±0.74
Water	20.5± 1.8	20.3 ±1.8	20.4 ±1.8	9.3 ±0.83	8.7 ±0.78	8.5 ±0.76	10.9 ±0.98	9.6 ±0.86	9.0 ±0.81
Methanol	21.7 ±1.9	21.5 ±1.9	21.3 ±1.9	11.3 ±1.01	10.7 ±0.96	8.3 ±0.74	12.7 ±1.14	11.9 ±1.07	10.8 ±0.97

Table-IV

Concentration of GPT,GOT, and LDH in the liver of mice injected with normal saline, water and methanolic extractable portion of Strebulus at 24,48 and 72 hours. Values are mean ±SEM of estimations.

Extracts used	Concentration of GPT (μgm of pyruvate liberated mnt/mgm protein) at			Concentration of GOT (μgm of pyruvate liberated mnt/mgm protein) at			Concentration of LDH (μgm of pyruvate liberated mnt/mgm protein) at		
	24hr	48hr	72hr	24hr	48hr	72hr	24hr	48hr	72hr
Normal	21.1 ±1.8	21.0 ±1.8	20.0 ±1.8	26.5 ±2.3	26.3 ±2.3	26.1 ±2.3	29.7 ±2.6	29.1 ±2.6	28.1 ±2.5
Water	27.8 ±2.5	25.6 ±2.3	23.9 ±2.1	37.6 ±3.3	29.4± 2.6	27.3 ±2.4	32.5 ±2.9	32.1 ±2.8	31.1 ±2.7
Methanol	25.7 ±2.3	24.1 ±2.1	23.3 ±2.0	29.1 ±2.6	27.2 ±2.4	25.7± 2.3	31.3 ±2.8	30.7 ±2.7	29.7 ±2.6

References

1. Annie Mathai and K.S. Devi, Ancient science of Lift, Vol No. XII Nos 1 and 2 (1992) Pages 271-273
2. Annie Mathai and K.S. Devi, Ancient science of Lift, Vol No. XIV Nos 4 and 2 (1995) Pages 248-252
3. Van den Heuvel M.J. et al, Human Toxicology, 6 (1987) 279.
4. Wootton I.D.P. Microanalysis in Medical biochemistry, 4th ed Churchill, London (1964) 107.
5. Bergmeyer H.U. and Bernt E, Methods in enzymatic analysis, Academic press, New York (1965).
6. Kawai Y and Anno K, Biochem Biophys Acta, 342 (1971) 428.
7. Lowry O.H. Rosenbrough N.J., Farr A.L. and Randall R.J J Biol Chem, 193 (1951) 265.