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Effect of Ashwagandharista on Different Hepatic Enzymes at Three Different Dose Level Utilizing Male and Female Rat Model

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ABSTRACT

Ashwagandharista (ASG) is a classical Ayurvedic preparation which is used as an anabolic. The key objective of this study was to analyze the effect of ASG on different enzyme profile i.e; Aspartate Aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline Phosphatase (ALP) and Lactate dehydrogenase (LDH). A total of 40 males and 40 females were randomly assigned to the four groups, namely group I (Control: water), group II (0.625 ml/kg BW of ASG), group III (5.0 ml/kg BW of ASG), and group IV (40.0 ml/kg BW of ASG) consisting of 10 males and 10 females in each group. To detect the outcome of ASG on different enzyme profile, it was administered chronically to both male and female Sprague-Dawley rats for 51 days. The results showed significant decrease of serum AST level in ASG treated male rat groups ($p < 0.05$) than control counterpart. In females, serum AST level in mid dose (group III) was significantly higher as compared to control ($p < 0.05$). The females from mid dose group showed significant increase in serum ALT level ($p < 0.05$) whereas other groups from male and female showed no significant changes. For the enzyme Lactate dehydrogenase, only the male rat high dose showed a significant decrease ($p < 0.05$) than the corresponding control group. No statistically significant change was noted in Alkaline Phosphatase level for both the male and female rats at three different doses.

Keywords: Ashwagandharishta, Aspartate Aminotransferase, Alanine aminotransferase, Alkaline Phosphatase, Lactate dehydrogenase.

INTRODUTION

Acute and chronic liver injury is a growing worldwide problem which in turn had augmented the number of tests available for measurement of liver function parameters. Most liver enzymes reside within cells and only small quantities of them are present in the serum. Myocardial infarction, acute hepatitis and other hepatic injury can cause cellular damage and some intracellular content will discharge into extracellular fluid and ultimately reach the serum in high concentrations. Elevated concentrations of serum enzymes may be the sole indicator of the presence of disease [1]. In this study, the Serum Aspartate Aminotransferase (AST), Serum Alanine aminotransferase (ALT), Serum Alkaline phosphatase (ALP) and Serum Lactate dehydrogenase (LDH) were studied to evaluate the effect of Ashwagandharista on the above mentioned enzymes (Table 1).

Table 1: Tabular presentation of the ingredients along with their botanical name and amount used to prepare Ashwagandharista (ASG)

No	Sanskrit Name	Botanical Name	Amount of ingredient
1	Ashwagandha	*Withania somnifera*	2.400 kg
2	Sweta Musli	*Asparagus adscendens*	960 g
3	Manjishtha	*Rubia cordifolia*	480 g
4	Hareetaki	*Terminalia chebula*	480 g
5	Haridra	*Curcuma longa*	480 g
6	Daruharidra	*Berberis aristata*	480 g
7	Yashtimadhu	*Glycyrrhiza glabra*	480 g
8	Rasna	*Pluchea lanceolata*	480 g
9	Vidarikanda	*Pueraria tuberose*	480 g
10	Arjun Tvak	*Terminalia arjuna*	480 g
11	Mustaka	*Cyperus rotundus*	480 g
12	Trivrit	*Ipomoea turpethum*	480 g
13	Anantamool	*Hemidesmus indicus*	384 g
14	Krishna Sariva	*Cryptolepis buchanan*	384 g
15	Rakta Chandan	*Pterocarpus santalinus*	384 g
16	Chandan	*Santalum album*	384 g
17	Vacha	*Acorus calamu*	384 g
18	Chitrak Mool	*Plumbago zeylanica*	384 g
19	Water for decoction	98.304 L reduced to	12.288L
20	Dhataki puspa	*Woodfordia fruticosa*	768 g
21	Madhu	*Honey*	14.400 kg
22	Shunthi	*Zingiber officinale*	96 g
23	Maricha	*Piper nigrum*	96 g
24	Pippali	*Piper longum*	96 g
25	Tvak	*Cinnamomum zeylanicum*	192 g
26	Tejpata	*Cinnamomum tamala*	192 g
27	Elach	*Elettaria cardamomum*	192 g
28	Priyangu	*Callicarpa macrophylla*	192 g
29	Nagakeshar	*Mesua ferrae*	96 g

According to the World Health Organization (WHO), about 80 percent population of the world presently uses herbal medicines for some aspect of primary health care. There is a new interest in traditional medicines because of a perception of lower incidence of side effects [2].

Ashwagandharishta is a classical ayurvedic formulation used in the treatment of *Murchha* (syncope), *Mandagni* (poor digestive power), etc. *Ashwagandha* (*Withania somnifera* D.), the key component of *Ashwagandharishta*, [3], has anti-stress and anxiolytic activities. Further, it also affects brain-derived neurotrophic factor (BDNF) that boosts synaptic plasticity, delivers neuro-protection, augments neurotransmission, and has antidepressant effects [4]. It also has many actions on body like anti-ageing, adaptogenic, immune-modulatory, cardiovascular protection, hypothyroidism etc. [5].

Withania somnifera, also recognized as Ashwagandha, Indian ginseng, or winter cherry, has been an important herb in the Ayurvedic and indigenous medical systems. The roots of the plant are categorised as *Rasayanas* which promote health and longevity by enhancing defenses against disease, arresting the ageing process, revitalizing the body in debilitated conditions. It contains alkaloids (withanine, withasomnine), steroidal lactones and glycosides (withanolides and sitoindosides). It is also used as a general tonic, to increase energy and improve health and longevity. Some studies suggest that, it may promote growth in

children and improve hemoglobin level, red blood cell count, and physical performance in adults [2]. It also increases heart weight and glycogen in myocardium and liver representing intensification of the anabolic process and enhances the duration of contractility as well as coagulation time [6].

To execute further clinical studies with this liquid preparation, this study was performed to evaluate the effects of ASG on the hepatic marker enzymes utilizing laboratory animals.

MATERIALS AND METHODS

Drugs, chemicals and reagents

For the toxicological study, *Ashwagandharishta* was collected from Sri Kundeswari Aushadhalaya Limited, Chittagong. Ketamine injection was obtained from ACI Pharmaceuticals Limited, Bangladesh. All other reagents, assay kits and chemicals used in this research work were obtained from Human GmbH, Wiesbaden, Germany.

Experimental Animals

Albino rats (*Rattus novergicus* : Sprague-Dawley strain, 48 weeks old, 70-80 g) of both sexes bred and maintained at the animal house of the Department of Pharmacy, Jahangirnagar University, were used in this toxicological experiment. They were kept in a well-ventilated hygienic experimental animal house under constant environmental and adequate nutritional conditions throughout the period of the experiment. They were fed with rat chow which was prepared according to the formula developed by Bangladesh Council of Scientific and Industrial Research (BCSIR). Water was given *ad libitum* and the animals maintained at 12 hours day and 12 hours night cycle. All experiments on rats were carried out in absolute compliance with the ethical guide for care and use of laboratory animals approved by Ethical Review Committee, Faculty of Life Sciences, Department of Pharmacy, Jahangirnagar University.

Experimental design

Acute toxicity study: The acute oral toxicity test was performed for *Ashwagandharishta* [7]. Sixteen male & female mice (30-35 g body weight) were divided into four groups of four animals each. Different doses (1000 mg/Kg, 2000 mg/Kg, 3000 mg/Kg and 4000 mg/Kg) of experimental drug (ASG) were administered to them. The dose was divided into two fractions and given within 12 hours. Then all the experimental animals were observed for mortality and clinical signs of toxicity at 1, 2, 3 and 4 hours and thereafter once a day for the next three days following ASG administration.

Chronic toxicity studies: A total of 40 males and 40 females were randomly assigned to the four groups, namely group I (Control: water), group II-Low Dose (0.625 ml/kg BW of ASG), group III-Medium Dose (5.0 ml/kg BW of ASG), and group IV-High Dose (40.0 ml/kg BW of ASG) consisting of 10 males and 10 females in each group. The animals of control group were administered with distilled water only as per the same volume as the drug treated group for 51 days. Ayurvedic medicinal preparation was administered to the rats by intra-gastric syringe at fixed time daily soon after acclimatization. All the experiments were carried out in absolute compliance with the ethical guideline for care and use of laboratory animals (Table 2).

Table 2: The effect of ASG on the serum aspartate aminotransferase (AST) level

Group	Serum AST (U/L)			
	Male		Female	
	Mean ± SEM	% Change	Mean ± SEM	% Change
Control	382.10 ± 14.01	-	105.70 ± 1.95	-
ASG (0.625 ml/kg)	302.00 ± 12.18*	↓20.96%	106.10 ± 2.90	↑0.378%
ASG (5.0 ml/kg)	316.00 ± 22.96*	↓17.29%	134.60 ± 14.85*	↑27.341%
ASG (40.0 ml/kg)	256.70 ± 11.66*	↓32.82%	118.30 ± 4.71	↑11.920%

Values are presented as mean ± SEM (n=10). One-way ANOVA followed by Dunnett's multiple comparison was performed to

analyze this data set. *p <0.05 was considered statistically significant when compared against control. ↑: increase, ↓: decrease.

Blood samples collection and preparation of serum*:* At the end of treatment period (51 days), after 18 hours fasting, blood samples were collected from post vena cava of the rats anaesthetizing with Ketamine (500 mg/Kg body, intra peritoneal). Blood samples were transferred into plain sample tubes immediately for serum generation. It was then centrifuged at 4,000 g for 10 minutes using Bench top centrifuge (MSE Minor, England). The supernatant plasma samples were collected using dry Pasteur pipette and stored in the refrigerator for further analyses. All analyses were completed within 12 hours of sample collection.

Biochemical studies: Serum samples were analyzed for Serum Aspartate Aminotransferase (AST), Serum Alanine aminotransferase (ALT), Serum Alkaline phosphatase (ALP) and Serum Lactate dehydrogenase (LDH) using spectrophotometer (Vitros–250, Johnson & Johnson) Random Access Multibatch Chemistry Analyzer (USA).

Statistical analysis: Data were expressed as Mean ± SEM (Standard Error of the Mean). One-way ANOVA followed by Dunnett's multiple comparison was performed to analyze this data set. $P < 0.05$ was considered statistically significant. Statistical programs used were SPSS (version 16, IBM software Inc, USA).

RESULTS

Acute toxicity study

The high dose of *Ashwagandharishta* (4000 mg/kg body weight) produced no death of animals. So, it is clear that the LD_{50} value of ASG was greater than 4000 mg/kg body weight. Therefore, it can be decided that ASG when administered at single dose is non-toxic and can be used safely in oral formulations.

Chronic toxicity study

Effect of ASG on aspartate aminotransferase (AST) level of Male & female rat: All the doses of ASG showed statistically significant decrease in serum AST level of male rat. There were a highly significant decrease in serum AST level at low dose (20.96% decreases) and medium dose (17.299% decrease). Also at the high dose level, there was a very highly significant (32.82% decrease) decrease in the AST level. On the other hand, in case female rat, there was a statistically significant **(**27.34% increase**)** increase in the AST level at the medium dose level. The high and low dose exhibited no significant change in the serum AST level.

Effect of ASG on alanine aminotransferase (ALT) of male & female rat: All the doses of ASG showed no significant change in serum ALT level of male rat. On the other hand, at the medium dose level, there was a statistically significant **(**67.08% increase**)** increase in the ALT level of the female rat. The low and high dose of ASG showed no significant change in ALT level of female rats (Table 3).

Table 3: The effect of ASG on the serum alanine aminotransferase (ALT) level

Group	Serum ALT (U/L)			
	Male		Female	
	Mean ± SEM	% Change	Mean ± SEM	% Change
Control	311.40 ± 18.41	-	48.90 ± 1.61	-
ASG (0.625 ml/kg)	320.50 ± 7.13	↑2.92%	48.50 ± 2.86	↓0.82%
ASG (5.0 ml/kg)	305.00 ± 10.98	↓2.06%	81.70 ± 14.09*	↑67.08%
ASG (40.0 ml/kg)	283.68 ± 32.55	↓8.90%	53.20 ± 1.88	↑8.79%

Values are presented as mean ± SEM (n=10). One-way ANOVA followed by Dunnett's multiple comparison was performed to analyze this data set. *p <0.05 was considered statistically significant when compared against control. ↑: increase, ↓: decrease.

Effect of ASG on alkaline phosphatase (ALP) of male & female rat: All the doses of ASG showed no significant change in serum ALT level of male and female rat.

There was a minor decrease in the ALP level of the male and

female rat, the decrease though not significant yet it was prominent (Table 4).

Table 4: The effect of ASG on the serum alkaline phosphatase (ALP) level

Group	Serum ALP (U/L)			
	Male		Female	
	Mean ± SEM	% Change	Mean ± SEM	% Change
Control	401.60 ± 25.73	-	260.70 ± 19.69	-
ASG (0.625 ml/kg)	365.80 ± 17.85	↓8.91%	238.80 ± 10.78	↓8.40%
ASG (5.0 ml/kg)	368.90 ± 29.67	↓8.14%	247.60 ± 15.54	↓5.02%
ASG (40.0 ml/kg)	336.00 ± 22.82	↓16.33%	232.30 ± 18.84	↓10.89%

Values are presented as mean ± SEM (n=10). One-way ANOVA followed by Dunnett's multiple comparison was performed to analyze this data set. *p <0.05 was considered statistically significant when compared against control. ↑: increase, ↓: decrease.

Effect of ASG on lactate dehydrogenase (LDH) of male rat: All the doses of ASG showed no significant change in serum LDH level of male and female rat. There was a minor change in the serum LDH level of the male and female rat, the increase or decrease though not significant yet it was prominent (Table 5).

Table 5: The effect of ASG on the serum lactate dehydrogenase (LDH) level

Group	Serum LDH (U/L)			
	Male		Female	
	Mean ± SEM	% Change	Mean ± SEM	% Change
Control	9141.00 ± 513.47	-	1756.90 ± 150.91	-
ASG (0.625 ml/kg)	7624.44 ± 365.04	↓16.590%	1196.40 ± 191.00	↓31.902%
ASG (5.0 ml/kg)	8897.78 ± 525.72	↓2.660%	1905.80 ± 156.94	↑8.475%
ASG (40.0 ml/kg)	6598.00 ± 601.36*	↓27.819%	2183.80 ± 446.68	↑24.298%

Values are presented as mean ± SEM (n=10). One-way ANOVA followed by Dunnett's multiple comparison was performed to analyze this data set. *p <0.05 was considered statistically significant when compared against control. ↑: increase, ↓: decrease.

DISCUSSION

Hepatic injury results in the leakage of cellular enzymes into the bloodstream, resulting in augmented levels of serum AST, ALT and LDH [8]. Thus, serum levels of these enzymes are excellent indicators of hepatic parenchymal damage and dysfunction [9]. In a recent study on Ashwagandharishta, showed that it significantly prohibited the ISO-induced adverse fluctuations in the level of serum enzymes such as creatine kinase (CK-MB), lactate dehydrogenase (LDH), aspartate aminotransferase (AST) and alanine aminotransferase (ALT). It also improved serum lipid profile [10]. In this present study, for the ASG treated group of male rats, the serum aspartate aminotransferase (AST) was decreased by 20.963%, 17.299% and 32.818% for the low, medium and high dose respectively. And interestingly all the rate of decrease was highly statistically significant. And the above mentioned results are supported by the following study published previously utilizing Ashwagandharishta preparation. For the serum Alanine aminotransferase (ALT), the ASG treated group of male rats, was decreased by 2.055% and 8.901% for the medium and high dose respectively. Serum Lactate dehydrogenase (LDH) was decreased by 16.590%, 2.660% and 27.819% for the low, medium and high dose respectively in ASG treated male rats.

CONCLUSION

The present study was limited to just 51 days period on healthy male and female rats. It should be further tested in long term randomized placebo controlled trial to establish its clinical use. Further studies are also required to assess whether the drug can improve other physical parameters or not.

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