Research Article **CODEN: AJPCFF** **ISSN: 2321 – 0915**

Asian Journal of Phytomedicine and Clinical Research

Journal home page: www.ajpcrjournal.com

ETHANOL INDUCED PHYSIOLOGICAL CHANGES AND PROTECTIVE EFFECT OF *ANDROGRAPHIS PANICULATA* USING RAT MODEL

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ABSTRACT

Andrographis paniculata is a folk medicine for protection against liver damage induced by alcohol intoxication. However, no report has been presented in this respect. In this rat study, we examined whether the *Andrographis paniculata* protect against liver damage induced by ethanol (EtOH). Rats were orally administered with ethanol (20% v/v) followed by *Andrographis paniculata* for 28 days. Rats were divided into six groups (G1-G6) and except for Group1, all rats were administered with alcohol. G1: Control; G2: EtOH control; G3: Silymarin (50mg/kg bw., p.o.); G4:100mg/kg; G5:200mg/kg; G6:400mg/kg. The results showed that changes of body weight,water intake and food intake. The aqueous extract of *Andrographis paniculata* showed reduction in physiological changes. In this present study, it has shown that *Andrographis paniculata* demonstrate a strong hepatoprotective activity against ethanol induced rats.

KEYWORDS

Andrographis paniculata, Ethanol and Hepatoprotective.

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INTRODUCTION

Andrographis was selected by the ministry of health as one of the medicinal plant to be included in “the national drug list of essential drugs A.D 1999” (list of herbal medicinal products) in Thailand[1]. Basically the taste is very bitter. This bitterness is related with its various pharmacological properties such as antiviral, anti-inflammatory, antivenom, immune stimulatory, anticancer, anti-HIV, anti-allergic and hypoglycemic activity[2]. Mostly the leaves and roots have been traditionally used over the countries for different medicinal purpose in Asia and Europe as a

folklore remedy for a wide spectrum of ailments or as an herbal supplement for a health promotion. The Indian pharmacopoeia narrates that it is a prominent constituent more than 26 Ayurvedic formulation[3]. It grows abundantly in south eastern Asia, i.e., India, Sri Lanka, Pakistan, Malaysia and Indonesia, while extensively in India, China and Thailand[3, 4]. Previous investigations on the chemical composition of *Andrographis paniculata* showed that it is a rich source of diterpenoids and 2'-oxygenated flavanoids including andrographolide, neoandrographolide, 14-deoxy-11,12-didehydroandro grapholide, 14-deoxyandrographolide, isoandro grapholide, and 19 β-D- glucoside, homoandr ographolide, andrographan ,andrographosterin and stigmasterol[5, 6]. Silymarin administration has demonstrated normalization of serum liver enzyme and total bilirubin levels in patients with alcoholic liver disease; there was also improvement in liver tissue histology[7]. Administration of ethanol produces a decrease in the hepatic content of glutathione (GSH), which is an important biomolecule that affords protection against chemically induced cytotoxicity[8].

MATERIALS AND METHODS

Plant material

The plant material of *Andrographis paniculata* was purchased from Natural Remedies and further identification has also been done on this plant.

Preparation of extract

The freshly collected leaves of *Andrographis paniculata* were washed thoroughly for 3 times, shade dried and size reduced into coarse powder. The coarse powder was macerated in 500ml of distilled water for 7 continuous days at room temperature. Extract were filtered and concentrated by using rotary vacuum evaporator under reduced pressure and the residues were weighed[9].

Chemicals

All the reagents used in this study were of high purity in content. Chemicals which have been used such as ethanol, formalin and chloroform were purchased from Sigma Aldrich Chemical (Malaysia). On the other hand, silymarin was purchased from a Sigma Aldrich (China).

Experimental animals

Adult albino wistar male rats weighing 180±20 grams were used in these pharmacological and toxicological studies. The animals were maintained in well ventilated room temperature 25± 2^{o}C with 12:12 hours light and dark cycle in polypropylene cages with stainless steel grill top[10]. The rats were fed a standard diet of pellets and tap water ad libitum. Rats were routinely acclimatized to laboratory conditions for 7 days prior to experiments. To ensure that the selected animals are in good state of health, after acclimation the animals was subjected to a gross observation. For the final allotment in the study, the animals were randomly selected. In order to use the laboratory animals, prior authorization was obtained from the University College Animal Ethical Committee.

Acute toxicity study

According to the OECD guidelines the acute toxicity in this present study[11] was followed. Fifteen male wistar rats weighing 180±20g were used for this study. The animals were divided into five different groups each consisting of 3 animals. Single dose of 5 mg/kg was administered separately to all the three rats in each group which were fasted over night with water *ad* libitum. To monitor for severity of any toxic sign and mortality the animals were observed for the period of 1 hour, occasionally for 3 hours. If there are no any signs of mortality at this dose, the dose will be increased to 50, 400 and 2000 mg/kg of extracts of the same procedure for newer groups. The animals were observed up to 7 days after drug administration to find out for any delayed mortality.

Experimental design

Animals were divided into six different groups containing of six rats each.

- Group I- (control) were administered single daily dose of normal saline (10ml/kg bw po) for 28 days.
- Group II - received ethanol (20%v/v/kg bw po) were administered to induce hepatotoxicity.
- Group VI, V and VI - received *Andrographis paniculata* at three different doses (100mg/kg, 200mg/kg and 400mg/kg bw po) respectively.

- Group III received silymarin (50mg/kg bw po)

Determination of body weight, food and water intake

For the daily basis weigh the animal before giving drug substance and measure the intake of food and water respectively for 28 days.

Statistical analysis

The experimental data were indicated by mean ± S.D., and were analyzed by an analysis of variance using ANOVA followed by Duncan's test, significant difference was assumed for $p< 0.05$.

RESULTS

Acute toxicity study

High dose up to 2000 mg/kg or low doses aqueous extract of *Andrographis paniculata* were found to be safe in all the rats that received it. During the entire duration of the study no mortality or toxic symptoms were observed. Aqueous extract of *Andrographis paniculata* showed a steady compliance towards the rats and proved to be safe.

The results of body weight, water intake and food intake in six different groups were recorded in Table No.1, 2 and 3.

Effect of aqueous extract of *Andrographis paniculata* on body weight in ethanol induced hepatotoxicity in albino wistar rats

Figure No.1 showed the effect of *Andrographis paniculata* on body weight and ethanol induced physiological changes in male albino wistar rats. The body weight in the control group shows the reading of 166.300 ± 1.633^{a} IU/L, whereas after ethanol treatment, it has decreased to 120.300 ± 0.816^{b} IU/L. daily for 28 days.

The body weight level starts increasing to 145.300 ± 1.751^{d} IU/L and 153.700 ± 1.862^{e} respectively at the doses of 200 mg/kg and 400 mg/kg bw po in ethanol intoxicated rats, after administration of *Andrographis paniculata*.

Effect of aqueous extract of *Andrographis paniculata* on food intake in ethanol induced hepatotoxicity in albino wistar rats.

Figure No.2 showed the effect of *Andrographis paniculata* on animal food intake and ethanol induced food intake variation in male albino wistar rats. The food intake in the control group shows the reading of 84.00 ± 2.58^{a} IU/L, whereas after ethanol treatment, it has decreased to 43.00 ± 2.97^{d} IU/L. The body weight level starts increasing to 65.00 ± 2.47^{d} IU/L and 79.00 ± 2.87^{e} respectively at the doses of 200 mg/kg and 400 mg/kg bw po in ethanol intoxicated rats, after administration of *Andrographis paniculata*.

Effect of aqueous extract of *Andrographis paniculata* on water intake in ethanol induced hepatotoxicity in albino wistar rats.

Figure No.3 showed the effect of *Andrographis paniculata* on animal water intake and changes of ethanol induced male albino wistar rats. The water intake in the control group shows the reading of 70.00 ± 3.14^{a} IU/L, whereas after ethanol treatment, it has decreased to 29.00 ± 3.25^{b} IU/L. The water intake starts increasing to 49.00 ± 3.03^{e} IU/L and 65.00 ± 3.23^{c} respectively at the doses of 200 mg/kg and 400 mg/kg bw po in ethanol intoxicated rats, after administration of *Andrographis paniculata*.

Table No.1: Changes in the Body weight of control and experimental rats

S.No	Groups	0 day (gm)	7th day (gm)	14th day (gm)	21st day (gm)	28th day (gm)
1	Normal control	117.800±1.329	130.700±1.862[a]	143.800±1.941[a]	155.000±1.673[a]	166.300±1.633[a]
2	Toxicity control	119.000±0.894	123.300±2.582[b]	125.200±2.639[b]	120.500±3.782[b]	120.300±0.816[b]
3	Standard drug	118.700±1.033	125.200±1.941[c,d]	131.500±2.074[c]	137.800±2.483[c]	141.300±2.733[c]
4	Low dose	118.700±1.211	126.300±0.816[d,e]	134.300±1.211[d]	141.300±1.033[d]	146.000±0.894[d]
5	Medium dose	118.500±1.378	126.500±1.378[d,e]	134.200±1.835[d]	141.200±1.722[d]	145.300±1.751[d]
6	Higher dose	118.800±1.169	128.200±1.472[e]	137.300±1.211[e]	145.700±1.506[e]	153.700±1.862[e]

Values are means ± S.D. of six rats in each group. Values that have a different superscript letter (a, b, c, d, e) differ significantly with each other ($p< 0.05$).

Table No.2: Changes in the Food intake of control and experimental rats

S.No	Groups	0 day (gm)	14th day (gm)	28th day (gm)
1	Normal control	48.00±2.58[a]	76.00±3.14[a]	84.00±2.58[a]
2	Toxicity control	23.00±2.97[b]	36.00±3.25[b]	43.00±2.97[d]
3	Standard drug	46.00±1.84[a]	70.00±3.04[c]	82.00±1.84[a]
4	Low dose	30.00±1.99[c]	49.00±3.27[d]	60.00±1.99[c]
5	Medium dose	34.00±2.47[d]	52.00±3.03[d]	65.00±2.47[d]
6	Higher dose	45.00±2.87[a]	66.00±3.23[e]	79.00±2.87[e]

Values are means ± S.D. of six rats in each group. Values that have a different superscript letter (a, b, c, d, e) differ significantly with each other ($p< 0.05$).

Table No.3: Changes in the Water intake of control and experimental rats

S.No	Groups	0 day (ml)	14th day (ml)	28th day (ml)
1	Normal control	42.00±3.14[a]	51.00±2.58[a]	70.00±3.14[a]
2	Toxicity control	15.00±3.25[b]	20.00±2.97[b]	29.00±3.25[b]
3	Standard drug	40.00±3.04[a]	48.00±1.84[c]	68.00±3.04[a,c]
4	Low dose	20.00±3.27[c]	31.00±1.99[d]	39.00±3.27[d]
5	Medium dose	29.00±3.03[d]	41.00±2.47[e]	49.00±3.03[e]

Values are means ± S.D. of six rats in each group. Values that have a different superscript letter (a,b,c,d, e) differ significantly with each other ($p< 0.05$).

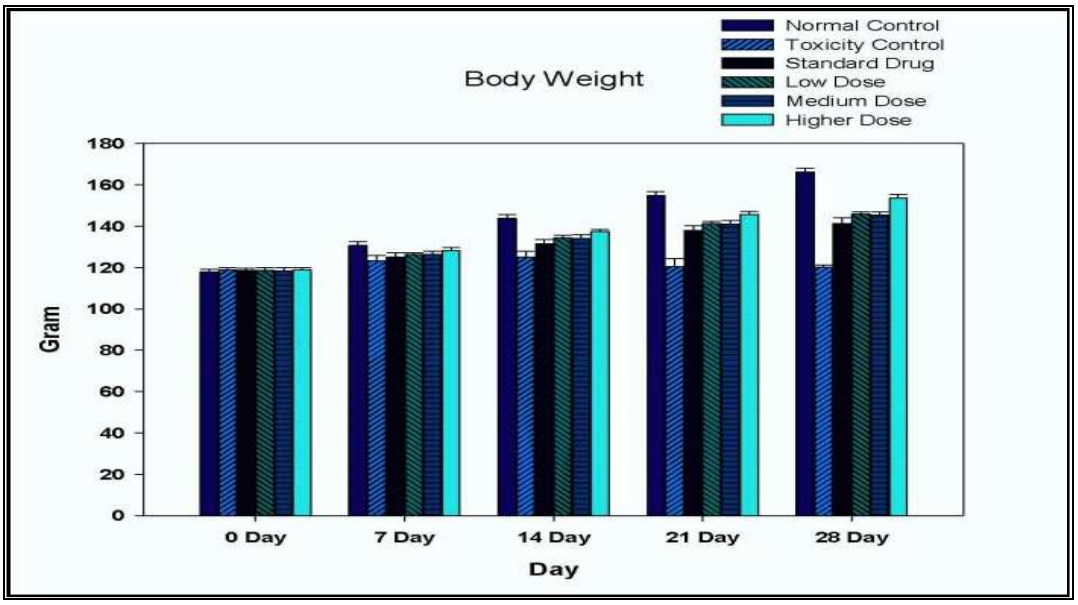

Figure No.1: Changes in the Body weight of control and experimental rats

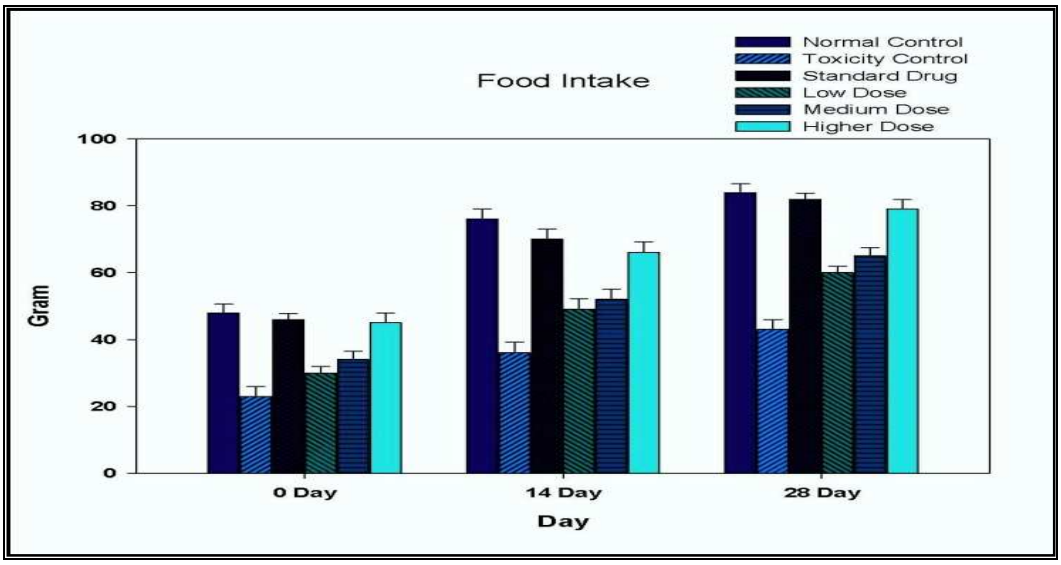

Figure No.2: Changes in the Food intake of control and experimental rats

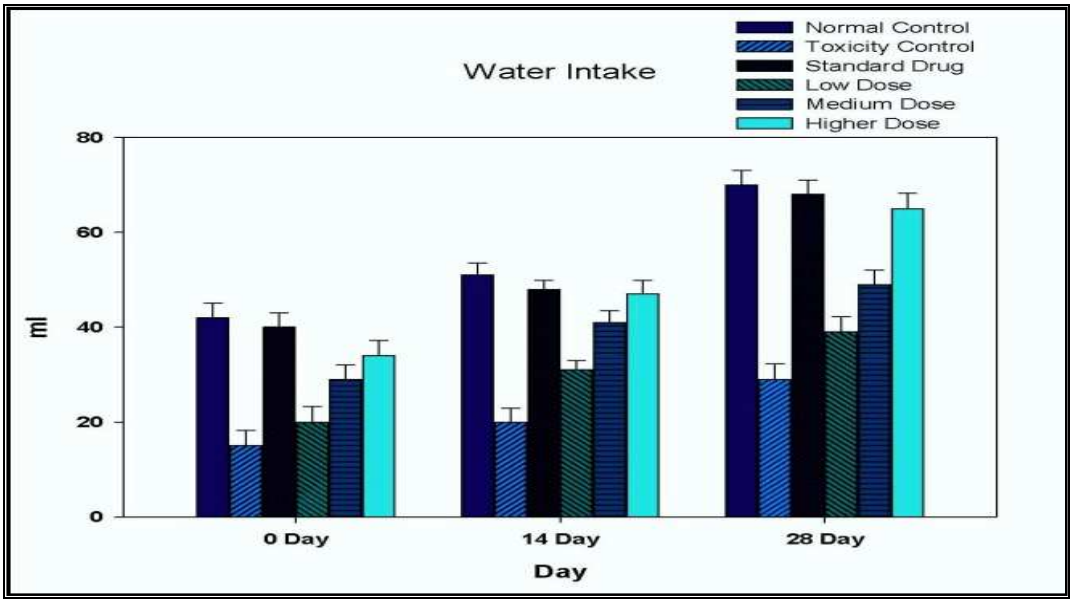

Figure No.3: Changes in the Water intake of control and experimental rats

CONCLUSION

Average food intake among the control rats was 84± 2.58 g/day (Figure No.2), which was similar to the consumption in the high dose (400mg/kg) of *Andrographis paniculata* with EtOH group (79 ± 2.87g/day). The alcohol group showed an important reduction in food intake (43±2.97), these differences between the group with alcohol and the groups without alcohol were statistically significant ($p<0.05$). Similar observations applied to liquid consumption, which on average was similar in the control group and in the high dose of *Andrographis paniculata* with EtOH group (70 ±3.14 ml/day and 65±3.23 ml/day, respectively). While the liquid consumed in the alcohol group was comparatively less 29±3.25ml/day (Table No.3). Each rat on the normal groups did ingested 12ml/day and alcohol groups did ingested approximately 4.8ml/day. Body

weight in the control and *Andrographis paniculata* corresponding values in the alcohol groups were seen to decrease ($p<0.05$) (Figure No.1). The drop in body weight was were pronounced in the group administered alcohol only, although there was no significant differences compared with the alcohol plus high dose of *Andrographis paniculta* group. From the above study concluded that, aqueous extract of *A.Paniculata* proved hepatoprotective activity against ethanol and its activity is comparable with the standard drug of silymarin.

ACKNOWLEDGEMENT

I wish to express my sincere thanks and hearty gratitude to my esteemed guide Dr.U.Subasini for their guidance and valuable suggestions and encouragement while carrying out this ebtire research activity.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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Please cite this article in press as: Vetriselvan S and U. Subasini. Ethanol induced physiological changes and protective effect of *andrographis paniculata* using rat model, *Asian Journal of Phytomedicine and Clinical Research*, 1(1), 2013, 57-63.