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REGULAR ARTICLE

A STUDY ON THE HEPATOPROTECTIVE EFFECT OF *ANDROGRAPHIS PANICULATA* (BURM.F) NEES ON MICE

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SUMMARY

Andrographis paniculata is a well known medicinal plant of Ayurveda with various pharmacological as well as medicinal properties. The present study determined the hepatoprotective effect of crude methanolic extracts of *Andrographis paniculata* on mice. The phytochemical screening of the crude methanolic extracts of *Andrographis paniculata* plant was also determined followed by the confirmation of the active compound using Thin Layer Chromatography. The hepatoprotective activity of methanolic extracts of *Andrographis paniculata* was evaluated against paracetamol induced (500 mg/kg) hepatic damage in mice. The extracts at doses of 10 mg/kg and 100 mg/kg were orally administered at 24 and 72 hours time interval in each group. Histological analysis of the liver and the liver protein content was determined. The results of the study indicated that the crude extracts of *Andrographis paniculata* at both doses exhibited a significant protective effect in the liver morphology of the paracetamol induced hepatotoxicity in mice. There was also a significant decrease ($P<0.05$) the liver protein content of the hepatotoxic mice after the treatments. Thin Layer Chromatography confirmed the presence of active compound, diterpene lactone or andrographolide which has contributed to the hepatoprotective activity of *Andrographis paniculata*. Hence, the results of the present study indicated that *Andrographis paniculata* possess hepatoprotective effects which could compromise the medicinal use of this plant in folk medicine.

Key words: *Andrographis paniculata*, Hepatoprotective, Time, Liver protein

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1. Introduction

Andrographis paniculata (AP) is one of the medicinal plants that seem promising found throughout Southeast Asia. Basically, the taste of *Andrographis* is very bitter. This bitterness is related with its various pharmacological properties such as antibiotic, antiviral, antimicrobial, anti inflammatory, antivenom and immunostimmulatory, anticancer, anti-HIV, anti-allergic, and hypoglycemic activity [1]. These properties are due to the presence of distinct lactones and flavones in the respective plant [2].

AP is employed extensively as hepatoprotective agent proven by some research studies. This is functional since liver diseases appear to be a serious problem for the past few years. In general; liver plays an astonishing array of vital functions in the maintenance and performance of the body such as for metabolism, storage, biosynthesis and detoxification. Unfortunately, the liver is often abused by environmental toxins, alcohol and over-the-counter drug use (xenobiotics) which can damage the liver and eventually lead to hepatitis, cirrhosis and liver diseases [3].

Therefore, conventional medicine is now pursuing the exploitation of natural products such as herbs to provide the support that liver needs on a daily basis. One such herb is AP which encompasses a long history of traditional use in revitalizing the liver and treating liver dysfunction and diseases due to the presence of the main active compound, 'andrographolide' or diterpene lactone which

contributes to the hepatoprotective activity [4].

The present research project was undertaken in order to determine the hepatoprotective activity of the crude methanolic extracts of AP on the paracetamol-induced hepatotoxicity in mice. Along with this phytochemical screening and TLC analysis of the crude methanolic extracts of AP was also done.

2. Materials and Methods

Plant material

The plant material, *Andrographis paniculata* was collected at various locations around Gurun, Kedah. The leaves were used for the study.

Preparation of the extracts

The leaves of *Andrographis paniculata* were washed, dried under shade and powdered with a mechanical grinder and stored in air-tight container. The dried powdered material of the leaves was extracted with Soxhlet extraction method using methanol as solvent for 3 days. The extract was filtered and concentrated under reduced pressure on rotary evaporator to obtain the crude extract.

Animals

Thirty albino mice purchased from University Science Malaysia, Penang weighing about 25-30 g were used in this study. Animals were housed in a clean and well ventilated experimental unit of animal house and they were fed with clean water and food ad libitum. Experimental protocols and procedures employed in this study were approved by the Animal Ethics Committee of the AIMST University.

Phytochemical screening

Phytochemical screening of the *Andrographis paniculata* ethanolic extract was performed according to the procedures described by [5] [6] [7]. This experiment was carried out to detect the presence of distinct constituents such as reducing sugars, saponins, alkaloids, flavonoids, anthraquinone, tannins and terpenoids.

Hepatoprotective activity

Study design

After one week of acclimatization, animals were divided into six groups of six rats (n=6) in each. Group A was administered with cosolvent 5 mL/kg (p.o.).Group B was given 500 mg/kg of paracetamol via intraperitoneal injection. Group C and D were administered with *Andrographis paniculata* ethanolic extract (10 mg/kg) orally 1 day after paracetamol injection. While, Group E and F were given 100 mg/kg of *Andrographis paniculata* ethanolic extract for 1 day after paracetamol injection. All the groups were observed for 24 hours and 72 hours respectively.

Protein quantification

After 24 hours and 72 hours of observation, the mice were anaesthized and sacrificed. The livers were removed and washed with NaCl (saline).The livers were further homogenized using using phosphate buffer saline (PBS).The homogenized liver was then centrifuged at 25,000 rpm at 4 ^{o}C for 20 minutes. The supernatant were collected and subjected to Biuret Protein Assay. A standard calibration curve was prepared by using BSA standard at a concentration range of (10- 100 µg) protein. Based on the calibration table, the unknown protein sample was determined [8].

Histopathological observation

Immediately after sacrifice of rats, the livers were removed and fixed in 4% formalin. After processing, the tissues were embedded in paraffin wax with Histo-Centre II- N (Barnstead/ Thermolyne, Iowa, USA) and sectioned into 4-5 µm thickness using microtome. The sections were stained with hematoxylin and eosin (H&E) for microscopic observation, which includes fatty changes, hepatocytes disarrangements and necrosis symptoms [9].

Statistical analysis

The statistical analysis was performed by one-way ANOVA followed by Dunnett's multiple comparison tests in SigmaStat® version 3.5 Software. The results were expressed as mean ± S.E.M to show

differences in groups. The differences are considered significant when $P<0.05$.

3. Results and Discussion

Phytochemical screening

Phytochemical screening tests showed the methanolic extracts of AP contain tannin, flavonoids, alkaloids and terpenoid. While, reducing sugar, saponin and anthraquinone were absent in the AP extracts.

Table 1. Phytochemical profile of standardized ethanolic extract of *Curcuma xanthorrhiza* rhizome

Chemical constituents	Tests/Reagents	Results
Reducing sugar	Fehling's reagent	-
Saponin	Frothing test	-
Alkaloids	Dragendroff's reagent	++
Flavonoids	Acid-alcohol	+++
Terpenoid	Sulphuric acid reagent	+++
Tannins	Ferric chloride reagent	++
Anthraquinones	Borntrager's test	-

Key: +++ = abundance; ++ = moderately present; + = present; ± = weakly present; - = absent

Hepatoprotective activity

Liver protein content

The liver protein content significantly decrease in the both AP treated of low dose (10 mg/kg) and high dose (100 mg/kg) groups at 24 and 72 hours time intervals compared to the toxic group. The protein content is high in the paracetamol- induced (toxic) group at both time intervals, 24 and 72 hours as in Figure 1. This can be explained using a number of reasons. At normal therapeutic dosage of paracetamol, the liver is able to perform its normal function such as the protein metabolism. However, when overdosage of paracetamol is induced, the liver is not capable to perform its normal functions where the protein metabolism is affected. Therefore, deamination of amino acids in order to breakdown the protein fails to arise resulting in the protein accumulation in the liver [10].

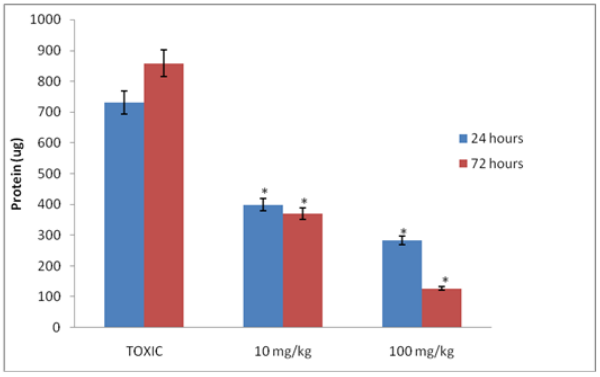
Besides, an obvious sign of hepatic injury is the leaking of cellular enzymes into the plasma due to the disturbance caused in the transport functions of hepatocytes [11]. When liver cell plasma is damaged, a variety of enzymes located normally in cytosol is released into the blood, thereby causing increased enzyme levels in the serum. Such enzymes are the serum ALT and AST which significantly increase in the paracetamol treated control group [12]. Since all enzymes are proteins, the enzyme increase is related to protein increase as well in the liver.

More to the point, the major portion of paracetamol is conjugated with either sulfate or glucuronide acid to form water soluble, readily excreted metabolites and only small amounts of the reactive intermediate, believed to be a quinoneimine, are formed by the P450 enzymes. When therapeutic doses of acetaminophen are ingested, the small amount of reactive intermediate formed is efficiently deactivated by conjugation with glutathione, an antioxidant compound. When large doses are ingested, however, the sulfate and glucuronide co factors become depleted, resulting in more of the acetaminophen being metabolized to the reactive metabolized. As long as glutathione (GSH) is available most of the reactive intermediate can be detoxified. When the concentration of glutathione in the liver also becomes depleted, covalent binding to sulfhydryl (-SH) groups of various cellular proteins increases, resulting in accumulation

of the cellular proteins in the liver as well hepatic necrosis [13].

However, the protein level in the liver is significantly reduced in both low and high doses of the AP methanolic extracts at 24 and 72 hours time interval indicating the significant hepatoprotective activity of the AP extracts against the paracetamol-induced hepatotoxicity.

Fig 1: Graph of liver protein of the negative control (toxic) and AP treated (low and high dose) groups. Each bar represents mean values. The error bar indicates the standard deviation of the mean.*P<0.05 compared to the toxic group (ANOVA, Dunnett's test)



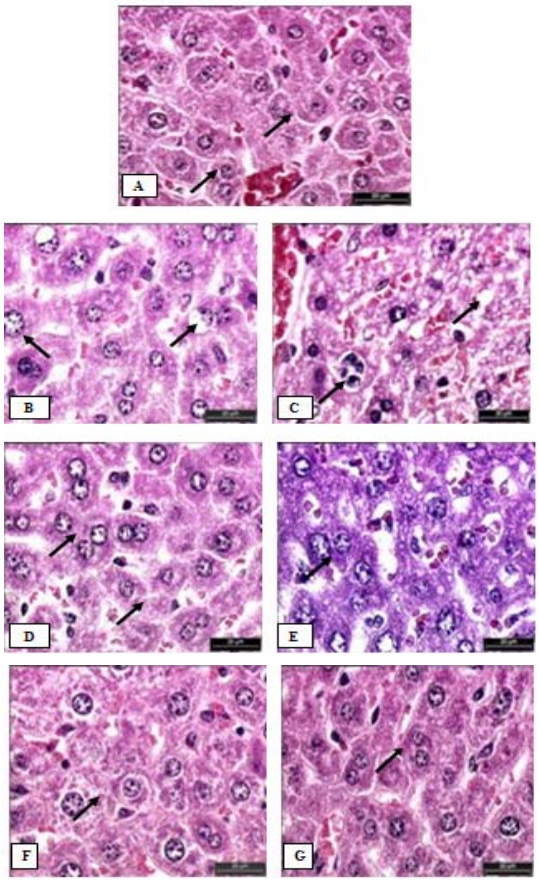
Liver morphology

Histologically, the normal liver tissue (Fig 2A) pointed out the presence of normal hepatocytes which are polyhedral in shape and distinct nuclei with one or two prominent nucleoli. The liver tissues of control group at 24 hours (Fig 2B) specified the presence of distorted shaped hepatocytes with undefined cell lining as well as vacuolated hepatocytes and vacuolated nucleus. The hepatotoxicity control group (72 hrs) demonstrated the degeneration of hepatocytes with more prominent and increased number of vacuoles in the hepatocytes and multinucleated giant-like cells (Fig 2C). Paracetamol or acetaminophen is a widely used analgesic that is normally safe when taken at therapeutic doses. Overdoses however may cause an acute centrilobular hepatic necrosis that can be fatal [14]. In the case of control liver tissue at 24 hours time interval, the hepatocytes are abnormal with distorted polyhedral shape and undefined cell lining due to the paracetamol toxicity effects. Besides, enlargement of nuclei or vacuolated nuclei were observed in this group due to the excessive fat accumulation in the nuclei. Other than that, fatty liver also occurs which is referred to the abnormal accumulation of fat or lipid in hepatocytes. This is addressed as vacuoles formation in hepatocytes. Basically, lipid accumulation related to disturbances in either the synthesis or the secretion of lipoproteins [15].The large vacuole in the cell forces the nuclei to the periphery of the hepatocyte and this condition is usually accompanied by nuclear atrophy [16].Whereas, the toxic liver tissue at a prolonged time, 72 hours illustrated severe liver cell damage. Here, the incidence of degeneration and disarrangement of hepatocytes are more prominent due to the excessive vacuoles formation in the hepatocytes [17].This indicates an undue accumulation of lipid or fat in the cytoplasm. Besides, multinucleated giant cells also exist in this liver tissue due to the clumping of two or more nuclei at one position which can lead to carcinogenesis [18]. Hence, paracetamol is a potential toxic drug which capable of causing liver cell damages that may direct to various liver diseases [19].

The AP treated liver (10 mg/kg) at 24 hours (Fig 2D) and 72 hours (Fig 2E) indicated the presence of hepatocyte which almost recovered to the normal polyhedral shape with the development of clear cell lining and reduced vacuolated nuclei. While liver tissue treated with 100mg/kg of AP extracts at both 24 hours (Fig 2F) and 72 hours (Fig 2G) displayed the complete

recovery of the hepatocytes with normal hepatocytes, reduced vacuolations, clear cell lining as well as absence of multinucleated giant cells. Hence, the normal liver cellular architecture is almost retained thereby confirming the hepatoprotective effect of AP methanolic extracts of low dose at 24 and 72 hours time period [20].

Fig 2 A- section of liver from normal control group indicating polyhedral hepatocytes with defined cell lining and round nuclei, with one or two prominent nucleoli , x 100. B –section of liver from toxic control group, 24 hrs indicating the presence of abnormal hepatocytes with a distorted shape and undefined cell lining as well as enlarged nuclei, vacuolation of hepatocytes, x 100, C section of liver from toxic control group, 72 hrs .indicating the presence giant nucleus vacuolation of hepatocytes, x 100. D & E - section of liver from low dose group, 24&72 hrs indicating the recovery of hepatocytes into normal shape and lesser vacuolated nuclei , F&G - section of liver from high dose group indicating more visible hepatocytes with defined cell linings and normal nuclei, x100. (H &E)



4. Conclusion

The present study shows the hepatoprotective activity of the crude methanolic extracts of the *Andrographis paniculata*. This is concluded using the results obtained from the histological studies as well as the liver protein content.

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