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Total Phenolic Content, Antioxidant, Cytotoxicity and Hepatoprotective Activities of Aqueous Extract of *Channa striatus* (Haruan)

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Abstract: *Antioxidant has been the important approaches to reduce the development of cancer disease and play a role as a protective against liver toxicity. Channastriatus (haruan) have been used traditionally oral remedy for wound healing among women after child birth. However, there is little scientific evidence or research yet regarding the cytotoxicity and hepatoprotective activity of C.striatus. Thus, the aims of this study are to determine potential total phenolic content (TPC), antioxidant, cytotoxicity and hepatoprotective effect of C.striatus (Haruan) extract. For this present study, aqueous and lipid extract of was prepared using chloroform and methanol solvent in a ratio of 2:1. Folin-Ciocalteu was used to quantify TPC and three antioxidant assays were used to determine antioxidant activity, including 2,2-diphenyl-picrylhydrazyl (DPPH) assay, azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) and ferric reducing ability of power (FRAP). For cell viability assay, HepG2 cell lines were seeded in 96-well plates and were treated with various concentration of aqueous extract of C.striatus, AECS (0, 0.00001, 0.0001, 0.001, 0.01, 0.1, 1, and 10 mg/ml) at 24, 48 and 72 hours. MTT assay has been used to measure HepG2 viability by using ELISA microplate reader. For hepatoprotective study, thirty six of adult male Sprague-Dawley rats will be divided into six groups of six rats each (n=6): G1:control (10%DMSO), G2:negative control (10% DMSO), G3:positive control (silymarin-100 mg/kg),G4: C.striatus (50mg/kg), G5:C.striatus (150mg/kg) and G6:C.striatus (450 mg/kg). The extract was given orally for 1 week. Acetaminophen,AAP (3g/kg) was induced orally from group 2 to 6 after 7 days of treatment. Blood collection was analyzed for liver function test and then the rats were sacrificed for histopathologically study. In TPC assay, AECS was observed to have higher content of phenolic (12799.33±237.90) compared to lipid extract of C.striatus, LECS (515.33±160.75). This indicates that, AECS has higher scavenging activity in DPPH and ABTS assay with EC_{50} (64.93±10.78) and (4687±0.67)respectively in comparison to LECS with EC_{50} (0.1513±0.046) and (93333.33±11.25) respectively. Additionally, AECS also consists of reducing potency since it has higher ability to reduce ferric ion compared to lipid extract of Channa striatus.For MTT assay, a significant decrease the percentage of HepG2 viability in a dose dependent manner was observed after HepG2 treated with various concentration of AECS at 24, 48 and 72 hours. The result showed no IC_{50} value of HepG2 obtained at 24 hours while IC_{50} value of AECS were 0.85 ± 0.26 and 0.1 ± 0.04mg/ml at 48 and 72 hours respectively. In vivo study, all groups pretreated of rats with AECS has shown significant decrease ($p < 0.05$) in the level of ALT, AST, AST and histological scoring of liver when comparing with acetaminophen group. In conclusion, AECS has higher TPC and antioxidant activity than LECS. Besides that, AECS exhibited potential cytotoxicity effect towards HepG2 cell lines in both dose and time dependent manner and also hepatoprotective effect at lowest dose of AECS (50 mg/kg). Further studies are required for fully elucidation on the mechanism of cytotoxicity and hepatoprotective activity of AECS.*

Keywords: *AECS, TPC, antioxidant, cytotoxicity hepatoprotective.*

I. Introduction

Free radical plays most pivotal role in disease pathology. This free radical reaction is important for normal metabolism and also can be harmful to the human health [1]. They are electrically charged molecules where they have an unpaired electron which causes them to find out other substances in order to neutralize themselves. Cell damage caused by free radicals appears to be major contributor to aging and to degenerative disease, cataracts, and brain dysfunction. Auspiciously, the free radical formation is controlled naturally by various beneficial substances known as antioxidant. These antioxidants scavenge free radicals by preventing and repairing damages caused by the reactive oxygen species (ROS), and therefore can enhance the immune defense and lower the risk of cancer and degenerative diseases [2].Liver cancer ranks number six among the most

common cancer worldwide and it has been estimated that, the burden of liver cancer is continuously rising up until 2030. The most common type of liver cancer is hepatocellular carcinoma (HCC) which accounts about 85% - 90% of liver cancer [3]. Whereas, liver disease is classified as fatal and able to become life threatening. There are various factors that contribute liver disease such as infection or exposure to various toxic substances such as drugs or alcohol. Sometimes over intake of certain drugs also able to lead to liver damage. Unfortunately, available drugs in market used in treatment of liver disease are inadequate and sometimes cause bad side effects. So, it becomes necessary to search natural products for treatment liver disease with more efficacy and safe [4].

Channa striatus belongs to family Channidae. There are around thirty species *C.striatus* around the world and eight was found in Malaysia [5]. It becomes popular today used by local peoples to treat mother after birth if the hospital is far from the village. This fish always consume because the ability to reduce pain and inner inflammation. It is also believed by rural peoples to having good medicinal benefits which able to access naturally [6].A study showed that *C.striatus* is beautifully build up by many nutritional values such as aspartic acid, glutamic acid, arachidonic acid and also DHA [7]. This significantly explained why haruan has been widely used to heal wounds. This finding was supported by a study which successfully showed that *C.striatus* helped in wound healing process due to present of various essentials amino acids such as glutamic acid, methionine, alanine and aspartic acid [8]. These amino acids play an important role in the synthesis of collagen fibers, which is a fundamental in wound healing process. This is proved by the formulation of centrimide cream and *C.striatus* showed the highest tensile strength, which indicated the properties of wound healing process compare to *C.striatus* formulation alone and centrimide cream alone.

The highest antinociceptive activity of *C.striatus* fillet was found in chloroform: methanol extract, followed by methanol, ethanol, water and chloroform extract. The best activity of antinociceptive was found in chloroform: methanol extract due to the synergistic effects of both polar and non polar compound present in the solvent [9]. There is a study reported that *C.striatus* having anti-inflammatory property [10]. In previous study, *C.striatus* possessed antimicrobial and antifungal property. The anti-fungal activity demonstrated only partial growth inhibition of the respective fungus, but the extracts is not strong enough to kill those funguses[11]. Today, there are a number of medicinal plants that have been discovered which have potential for treatment liver toxicity and liver cancer. However, relatively little scientific studies have been reported on the pharmacology and toxicity from the fishes. Thus, the present study is conducted to investigate TPC, antioxidant, cytotoxicity and hepatoprotective activity of AECS.

II. Materials And Methods

Preparation Of *C.Striatus* Aqueous And Lipid Extract

Aqueous and Lipid extract of *C.striatus* (Haruan) were prepared based on the method described[12]. Medium size *C.striatus* (250g-400g/fish) was bought from Pasar Borong Selangor, Malaysia. The fish were then cleaned. Subsequently, the fish was cut lengthwise along the backbone to obtain fillets with maximum amount of flesh without any spine. Then, the fillets were cut into cubes and weighed. The fillets then were soaked in the ratio of 1:2 (w/v) in chloroform methanol solution (2:1) for 5 minutes in a beaker which was wrapped in aluminium foil. The beaker was left on orbital shaker for 48 hours to allow the fillets and solvent to mix thoroughly. At the end of 48 hours, the solution was filtered by using muslin cloth followed by Whatman No.1 filter paper. After that, the solution was placed on separation tunnel for 30 minutes until two layers were clearly seen. The top layer was aqueous whereas the bottom was lipid. For aqueous layer, it was lyophilized into powder form by using freeze dryer whereas for lipid layer was evaporated using rotary evaporator.

Total Phenolic Content

The TPC in Haruan was determined by using the modified Folin-ciocalteu method[13]. Folin-ciocalteu reagent was prepared (previously by diluted with distilled water in the ratio of 1:9).Gallic acid with various concentrations was prepared ranging from (0.01-0.05mg/ml) and mixed with 0.75ml of Folin-ciocalteu reagent. The mixture was allowed to react for 5 minutes, followed by the addition of 0.75ml of (6%v/w) sodium carbonate Na_2CO_3. The mixture was then incubated in room temperature for 90 minutes. Absorbency was measured at 765nm using a spectrometer.

Ferric-Reducing Power (FRAP) Assay

The reducing power of extracts was evaluated by described method[14]. The FRAP reagent was prepared by mixing acetate buffer with pH 3.6 at concentration of 300mM,10mM of TPTZ(2,4,6-tri[2-pyridyl]-s-triazine(diluted in 40mM of HCl) , 20mM of $FeCl_3$ and distilled water with the ratio of [10:1:1:1.2]. 2 ml of standard stock solution of FRAP ($FeSO_4$, 7 H_2O) with various concentration from 100µM-1000µM was prepared. In a 96 multiwell plate 50ul of stock solution was added. Then 150ul of freshly prepared FRAP

reagent was added. The plate was then wrapped with aluminium foil. Absorbance was measured using plate reader at 590 nm.

Scavenging Activity Of 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) Radical

The method was conducted based on the procedure described[15]. The DPPH stock solution was prepared by mixing 2.1 mg of DPPH powder in 50 ml of methanol. It was then incubated for 2 hours in the dark at room temperature to form radicals. Aqueous extract, lipid extracts and trolox solution were prepared with a concentration of 100mg/ml with different concentrations ranging from 100/ml-1.5625mg mg/ml. Trolox was dissolved in methanol, aqueous extract was dissolved in distilled water (dH_2O) whereas lipid extract was dissolved in ethanol. For present study, 50µl of trolox was mixed with 195µl of DPPH solution into 96-well plate. This step was repeated by using aqueous and lipid extract of *C.striatus.* The solution was incubated for 1 hour at room temperature in dark room. The assay was done in triplicate. Then, the absorbency was measured in plate reader at 517nm. The radical scavenging activities of antioxidants were expressed in term of % effective concentration, $EC_{50.}$ The percentage of Effective concentration ($EC_{50)}$ was calculated by the following equation: EC_{50} %=(Abs blank - Abs sample / Abs blank x 100), where Abs blank is absorbance of DPPH without the extract of *C.striatus* and Abs sample is the absorbance of DPPH in the presence of the extract. The EC_{50} value was concentration of the antioxidant needed to scavenge 50% DPPH radical

2, 2'-Azino-Bis (3-Ethulbenzthiazoline-6-Sulphonic Acid (ABTS) Scavenging Activity (ABTS)

The method was carried out based on the procedure described[16]. Firstly, the ABTS solution was prepared by mixing two stock solution of 7mM ABTS solution and 2.4mM potassium persulphate solution in same amount. This solution was left in room temperature for 12-16 hours in the dark. Fresh ABTS solution was prepared for each assay. Aqueous, lipid extract and trolox solution was prepared at various concentrations from 100-1.5625mg/ml and allowed to react with 300µl of ABTS solution. Then, 200µl of absolute ethanol was added. The absorbency was measured at 540nm.

Cell Culture

HepG2 was grown in RPMI medium supplemented HepG2 cell lines supplemented with 10% of foetal bovine serum (FBS) and 1% antibiotics (100 IU/ml penicillin and 100 µg/ml streptomycin) in 75 cm^3 tissue culture flask and incubated at 37^oC in a humidified atmosphere of 5% carbon dioxide. The cells were subcultured when the cells reach about 70-80% confluency.

***In Vitro* Cytotoxicity Assay**

The cytotoxic effect of AECS was evaluated in human liver hepatoma HepG2 cells by the MTT assay[17].Briefly, cells were seeded at concentration of 1 x 10^5 cells/ml into 96-well plates (100µl/well) in triplicates. After 24 hours, the cells were treated with various concentrations of AECS from 0.00001, 0.0001, 0.001, 0.01, 0.1, 1 and 10 mg/ml for 24, 48 and 72 hours. At the end of the treatment, cells were added with 10µl of MTT (5 mg/ml in PBS) in fresh medium and incubated for 3 hours at 37°C. After 3 hours, formazan crystals were formed by mitochondrial reduction of MTT and solubilized in DMSO (100 µl/well). The absorbance was read at 570 nm after 10 minutes incubation. Percent inhibition of cytotoxicity was calculated and IC_{50} value was determined.

***In Vivo* Study**

The 36 adult male Sprague-Dawley rats (180-220 g) were purchased from animal house, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia. They were kept in stainless steel cages which housed in air conditioned room with temperature maintained at 25±1°C and humidity at 55±5°C under a regular 12:12 hours light/dark cycle for 1 week prior to treatment. The rats were fed the standard rodent chow and water ad libitum. At all times the experiment were carried out in strict condition according to Animal Ethics rules and regulation, followed by Universiti Putra Malaysia's ethical guideline for investigation of experimental pain in conscious animals. All rats received humane care in accordance to the Animal Ethics rules and regulation of Universiti Putra Malaysia. The rats were fasted 48 hours, without food given although free access to water were given before two days prior to administration of test solution. The rats were randomly divided into 6 groups of 6 animals per each group. Group I treated with vehicle (distilled water) was kept as control. Group II treated with distilled water for 7 days before acetaminophen (APAP) (3 g/kg, p.o) intoxication and served as negative control. Group III, IV, V and VI were treated with (200 mg/kg, p.o) silymarin drug (positive control), 50,150 and 450 mg/kg p.o of AECS respectively once daily for seven days. After 3 hours of last test solution was given, the liver toxicity inducer of 3g/kg p.o of acetaminophen (AAP) was given in group II to VI. All the treated rats were fasted for 24 hours. After 24 hours fasting, the rats were sacrificed by exposure to diethyl ether and blood

samples were collected by cardiac puncture in heparinized tubes. Then, the rats were dissected and the liver was immediately taken out. The liver was washed with normal saline, weighed and directly fixed in 10% formaldehyde. The blood and liver samples were assessed for their biochemical and histological studies.

Serum Analysis Of Liver Function Test

The serum were sent to the chemical pathology laboratory in Universiti Putra Malaysia for assessment of the liver function tests including the determination of the level of alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in the rat's sera.

Histological Observation Of The Liver

The fixed liver was subsequently dehydrated in ethanol (50-100%), cleaned in xylene and embedded in paraffin wax. 5-6 mm thick sections were prepared and stained with hematoxylin and eosin for photomicroscopic observation. Then, liver sections were evaluated by a pathologist using light microscope. The severities of histological change were scored based on their specific morphological change of the liver section such as injury, necrosis, and inflammation, which they were graded on scale 0-8[18].

Statistical Analysis

The result obtained was analyzed using SPSS software, version 16.0. For TPC, antioxidant assay and cytotoxicity assay, all data were expressed as mean ± SEM. The data of TPC and antioxidant assay were analysed by one-way analysis variance (ANOVA) followed with Duncan test for comparisons. Meanwhile, data of cytotoxicity assay was analyzed by two-way analysis of variance (ANOVA) followed with Duncan test for comparisons. For serum analysis, the results were presented in mean±S.D. The data was analyzed by one-way analysis of variance (ANOVA) followed with Dunnet post hoc test to compare the data between negative group treatment. $p< 0.05$ was considered as statistically significant.

III. Results And Discussion

TPC And Antioxidant Assay

Table 1: Tpc Was Expressed As Mg GAE/100 G Of Dry Weight Of Sample.

Sample	TPC (mg GAE/100g dry weight)	FRAP (mg $FeSO_4$/1mg dry weight	DPPH EC_{50} value (µg/ml)	ABTS EC_{50} value (µg/ml)
AECS	12799.33± 237.90^{b}	1250±0.617^{c}	0.2804±0.035^{e}	4687±0.67^{g}
LECS	515.33±160.75^{a}	>100 000	0.1513±0.046^{e}	>100 000
Trolox	1611.33±304.77^{c}	71.5±0.005^{d}	2.1778±0.032^{f}	2973.33±0.055^{g}

Values are represented as mean ± S.E.M (n = 3); those with different superscripts are significantly different at $p<0.05$, analysed by multiple comparison (Duncan test), ANOVA to compare the values between samples.
AECS = Aqueous extract of *C.striatus*; LECS= Lipid extract of *C.striatus.*

Cytotoxicity Assay

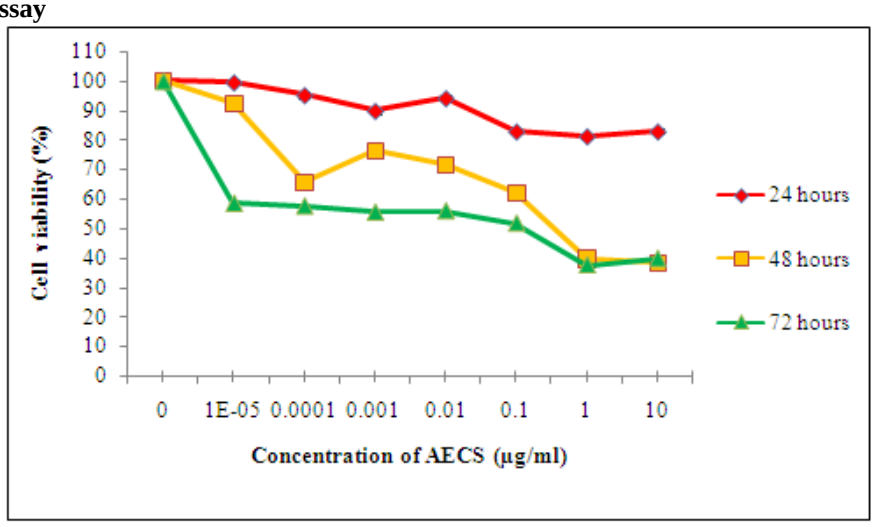

Figure 1:The Effect Of Different Concentration Of AECS On The Percentage Viability Of HepG2 Cell Lines In Various Incubation Times As Determined By MTT Assay. Data Represent As mean ± SEM.

Table 2: Ic_{50} Values Of AECS Towards HepG2 At Various Incubation Time As Determined By MTT Assay.

Incubation time (hours)	IC_{50} (µg/ml)
24	>10
48	0.85 ± 0.26^{a}
72	0.1 ± 0.04^{b}

Values were presented as mean ± SEM (n=3) and values with the different letters are significant different ($p < 0.05$).

Biochemical Test Analysis Of Liver Enzyme.

Table 3: Effect Of Administration Of Distilled Water, Aap, Silymarin And AECS On Serum ALT, AST And Alp Levels.

Treatment group	Control	AAP (3 g/kg)	Silymarin (200 mg/kg) + AAP (3 g/kg)	AECS (50 mg/kg) + AAP (3 g/kg)	AECS (150 mg/kg) + AAP (3 g/kg)	AECS (450 mg/kg) +AAP (3 g/kg)
ALT	100.8 ±20.36	1675 ±259.3	153.7 ±34.09*	247.4 ±15.25*	531.8 ±151.9*	1288 ±295.5
AST	167.3 ±52.00	1816 ±163.3	283.5 ±59.20*	187.3 ±85.09*	500.6 ±344.0*	589.7 ±350.2*
ALP	146 ±1.155	291.0 ±22.71	140.3 ±9.415*	126.3 ±15.96*	162.3 ±6.381*	183.0 ±21.54*

Values represented as mean ± S.E.M (n = 6). Serum ALT, AST an ALP levels were determined 24 hours after AAP intoxication.*$p<0.05$ is significantly different from AAP.

Histopathology Analysis of Liver

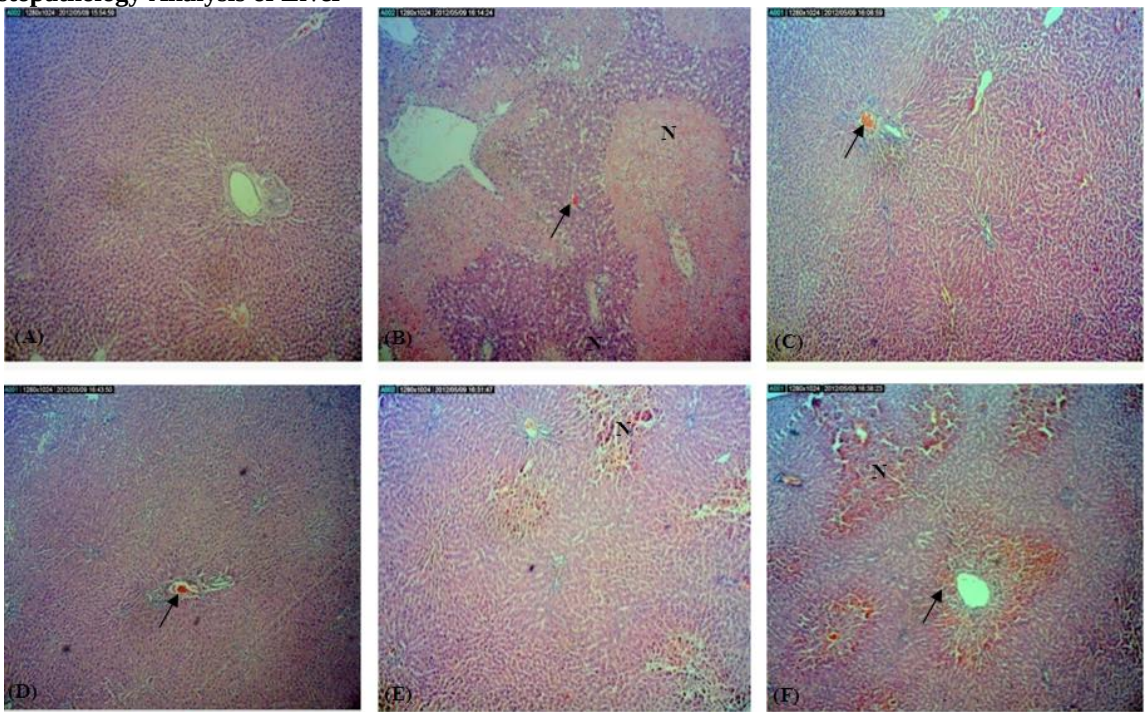

Figure 2: Rat liver sections (40 x magnifications) stained with haematoxylin and eosin (H&E) stained hepatic tissue section for the evaluation of general microscopic morphology of liver. (A) Normal group show normal architecture of hepatocytes. (B) AAP alone show large area of necrosis. (C) Silymarin + AAP show similar histological changes with normal group with mild inflammation cells confine to perivenular area. (D) AECS (50 mg/kg) +AAP show mild inflammation at perivenular area. (E) AECS (150 mg/kg) +AAP show mild of necrotic area. (F) AECS (450 mg/kg) +AAP show massive infiltration of inflammation cells and present of necrotic area.

Liver Histological Section Scoring in AAP-induced liver damage

Table 4: Effect Of Aecs On Histological Changes Of Aap-Induced Liver Damage.

Control	AAP (3 g/kg)	Silymarin (200 mg/kg) + AAP (3 g/kg)	AECS (50 mg/kg)+ AAP (3 g/kg)	AECS (150 mg/kg)+ AAP (3 g/kg)	AECS (450 mg/kg)+ AAP (3 g/kg)
0.33 ±0.21	7.17 ±0.40	1.33 ±0.21*	4.17 ±0.60*	5.17 ±0.60*	6.33 ±0.33

Values are reported as mean + SD of 6 animals per group. *$p<0.05$ is significantly different as compared to AAP.

The amount of total phenolic content in extract was determined with Folin-Ciocalteu's reagent. Phenols in the extract react with phosphomolybdic acid in Folin-Ciocalteau reagent in alkaline medium to produce a blue-coloured complex. Standard for TPC test was gallic acid. A linear calibration curve of gallic acid was obtained with R^2=0.9867. Table 1 showed TPC mean of the extracts measured using GAE equation of y=5.1603 + 0.0133 (R^2=0.09948), whereby y= absorbance at 725nm and x= concentration of total phenolic content in mg per ml of the extract (0.01mg/ml-0.05mg/ml). AECS (12799.33±237.90mg GAE/100g) has higher phenolic content than LECS(515.33±160.75mg GAE/100g). Total phenolic content in the extract depends on the type extract, specifically the polarity of solvent used in extraction. The high solubility of phenols in polar solvents provides high concentration of these compounds in the extracts obtained using polar solvents for the extraction[19].Several studies reported on the relationships between phenolic content and antioxidant activity. Some authors found a correlation between the phenolic content and the antioxidant activity. A study reported a strong relationship between TPC and antioxidant activity in selected fruits, vegetables and grain products [20]. Thus, TPC in Haruan showed a strong relationship with antioxidant activity.

FRAP assay is one the most widely used antioxidant assay. As revealed in a linear calibration curve of $FeSO_4$ with R^2 = 0.9867 was obtained. At low pH,when a ferric tripyridyltriazine complex is reduced to the ferrous form, an intense blue colour form with an absorbtion maximum at 593 nm[21]. Table 1 showed AECS (0.2804±0.035 mg $FeSO_4$/1 mg) had higher reducing power than LECS (0.1513±0.046 mg $FeSO_4$/1 mg). The standard trolox showed highest reducing power (2.1778±0.032mg $FeSO_4$/1 mg) which showed a significant reducing power among the samples. DPPH assay a widely used method to evaluate the free radical scavenging ability of various samples. DPPH is a stable nitrogen-centred free radical the colour of which changes from violet to yellow upon reduction by either the process of hydrogen- or electron- donation. Table 1 showed thatAECShas higher DPPH scavenging activity than LECS. But both the extract has lower DPPH scavenging activity than the standard trolox. AECS (EC_{50} value = 1250±0.617µg/ml) showed lower EC_{50} value than LECS (EC_{50} value >100 000±2.02µg/ml). In fact, lower value EC_{50} means higher antioxidant activity.

ABTS is a decolouration assay used widely as antioxidant assay. ABTS forming a relatively stable radical($ABTS^{+\bullet}$) upon one-electron oxidation, has become a popular substrate for estimation of total antioxidant capacity[22]. Table 1 showed AECS (EC_{50}=4687±0.67µg/ml) has higher ABTS radical scavenging activity than LECS (EC_{50}>100 000±11.25µg/ml). The standard, trolox (EC_{50}= 2973.33±0.055 µg/ml) showed highest antioxidant activity against the ABTS radical. Thus, the trolox has highest ABTS scavenging activity followed AECS and LECS.

In cytotoxicity assay, the result clearly showed that no IC_{50} is obtained (>10 mg/ml) at 24 hours treatment. However, IC_{50} value of HepG2 were 0.85 ± 0.26and 0.1 ± 0.040 mg/ml are obtained at 48 and 72 hours incubation respectively (Table 2). The line graph (Fig 1) showed the percentage of cell viability decreasing as concentration of AECS increase. This study showed there was significant different ($p < 0.05$) between IC_{50} value at 48 and 72 hours. It suggested that AECS efficacy depend on the dose itself and also the incubation time.

Based on the result obtained, cytotoxic activity of AECS increases as the concentration of AECS increasing. It believed that antioxidant molecules inside AECS helped in the reducing the proliferative activity of HepG2. Fish has its own natural antioxidative defense system, including enzymes (catalase, peroxidase, gluthathione and superoxide dismustase), carotenoids, peptides, amino acids, and phenolic compounds[23-24]. These components act as free radical propagation inhibitor, synergistic effect to reinforce the antioxidant activity, hydroperoxide reduction, metal inhibitor to catalyze production of free radicals and singlet oxygen quencher. Furthermore, *C.striatus* contain polar-uncharged (glycine, serine, threonine, cysteine, aspargine and glutamine), non-polar (organic) (Alanine, valine, leucine, isoleucine, phenylalanine, tryptophan and methionine), positively charged (lysine,arginine and histidine) and negatively charged (aspartic acid and glutamic acid) dicarboxylic amino acids[25]. All of this amino acid have antioxidant properties as synergists or primary antioxidant and important to be metal chelators and significant antioxidative potential in linoleic acid and methyl esters of linoleic acid system[26].

In this study, the protective effect of AECS acetaminophen (AAP)-induced liver damage in rats was also conducted. Toxicity from AAP can initiate an inflammatory process and increase tissue damage[27]. The present study showed administration of toxic doses of AAP resulted in the development of necrosis and inflammation. Excess production of free radicals attacks the biological molecules such as DNA, proteins and lipid and lead to hepatocellular injury. In addition, the depletion in intracellular GSH in AAP toxicity condition caused high levels of reactive electrophilic metabolites bind massively to the biological molecules. AAP toxicity led covalent binding of NAPQI with sulphydryl group of proteins resulted in the protein oxidation and cell necrosis[28]. From the result obtained, rats treated with single dosage of AAP show significant liver damage from the elevation serum of ALT, AST and ALP levels. The present study exhibited that silymarin and different dosage of AECS showed significant ($p<0.05$) in reducing the AST and ALP level when comparing to the AAP group. However, only 200 mg/kg of silymarin, 50 mg/kg and 150 mg/kg of AECS show significant ($p<0.05$) in reducing the ALT level when comparing with the AAP group (Table 4). Serum liver enzyme performed in this study supported the result obtained by histopathological observations.

According to the light microscopic evaluation, rats treated with a single dose of AAP show liver necrosis in the centrilobular regions, severe inflammatory cell at perivenular area and degenerated hepatocytes were observed. On the other hand, pre-treated with different dosage of AECS, less inflammatory cell at perivenular area, less area of necrosis without extension of necrosis bridges and well-preserved hepatocytes were observed (Figure 2). This shows an improvement in the preservation of tissue structures caused by AAP. From the study, lower dosage of AECS (50 mg/kg and 150 mg/kg) show significant ($p<0.05$) in reduction the level of ALT, AST and ALP and histological scoring (Table 4) when comparing with the AAP group It suggested that hepatoprotective effect AECS against AAP-induced liver damage which most probably mediated through its antioxidant properties. Previous study found that any compound with antioxidant properties can be effectively towards partial or total alleviation of liver damage[29]. As mentioned above, *C.striatus* possesses essential amino acid which has antioxidant properties. It postulated that these amino acids possess antioxidant properties which can reduce enzyme level in serum and protect liver against AAP-induced toxicity. This study showed that lowest dosage (50 mg/kg) more effective than higher dosage (150 mg/kg and 450 mg/kg) in protecting liver against AAP induced toxicity. Thus, protective effect of AECS against AAP-induced toxicity is not dose dependent manner. The present study demonstrated that enzyme level in serum and histological scoring increasing as the dosage of AECS increase. It assumed that AECS may have some compound which can cause liver toxicity. This hypothesis was supported by a study which proved that *C.striatus* contain high level of tyrosine in amino acid composition[30]. Previous study proved that pre-treated of tyrosine to the liver damage rat model resulted in increase toxicity and died in rats[31]. Besides, it is also possible that tyrosine involved in AAP-toxicity mechanism. In AAP toxicity mechanism, detoxification of peroxynitrite, is highly toxic agent that able to attack cells is impaired as depletion of GSH level. In this mechanism, tyrosine actas mediator that react with peroxynitrite to form nitrotyrosine that correlates with necrosis formation[32].

IV. Conclusion

In a nutshell, AECS have higher total phenolic content and antioxidant activities than LECS by showing higher phenolic content, ferric reducing ability power and scavenging activity towards DPPH and ABTS radicals. The present study demonstrated that AECS is a potential antioxidant activity as it has ability to scavenge free radical even at very high dose. AECS also exhibited cytotoxicity towards HepG2 at different incubation time and more effective hepatoprotection against AAP-induced hepatic damage at lowest dosage (50 mg/kg) with significant reducing ALT, AST, ALP levels and pathological changes of AAP-induced liver damage. However, the precise mechanism for cytotoxicity and hepatoprotective activity of AECS is still unclear to be explained and further detailed studies are required to elucidate the mechanism.

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