

Hepatoprotective activity of methanolic extract of *Capsicum frutescens* (siling labuyo) against isoniazid and rifampicin-induced hepatotoxicity in Sprague-Dawley rats

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Abstract: Liver is the major organ involved in the metabolism and excretion of drugs in the body. Anti-tubercular drugs like isoniazid and rifampicin can cause hepatic injury. Folk remedies from plant origin are tested for their potential anti-oxidant and hepatoprotective activities. Extract of *C. frutescens* leaves was obtained through methanolic extraction which yielded 12.6%. Phytochemical analysis revealed the presence of flavonoids. Sprague-Dawley rats were grouped accordingly and were given saline (Group I), inducing agents (isoniazid and rifampicin) (Group II), and 100 mg/kg (Group III) and 200 mg/kg *C. frutescens* (Group IV). The LD₅₀ of the extract in mice was estimated to be 190 mg/kg. After 14 days of treatment, the obtained AST values for Group I, II, III and IV were 123.8±19.85 U/L, 151.6±17.31 U/L, 0.8±13.27 U/L, and 100.4±26.46 U/L, respectively; while ALT values for the said groups were 36±5.47 U/L, 39.4±8.57 U/L, 36±6.33 U/L, and 57.2±7.52 U/L, respectively. This shows that inducing agents can raise the liver enzymes that show hepatotoxicity, while *C. frutescens* extract can lower the liver enzymes. Furthermore, 100 mg/kg *C. frutescens* extract has significant effect on AST and ALT levels ($p=0.006$) since it renders lower AST and ALT levels compared to 200 mg/kg *C. frutescens* extract. Thus, 100 mg/kg of *C. frutescens* extract is a therapeutic agent against hepatotoxicity. However, the biochemical results were not supported in the histopathological findings.

Keywords: *Capsicum frutescens*, hepatoprotective, drug toxicity, isoniazid, rifampicin

INTRODUCTION

Liver is the largest internal organ of the human body and the chief site for metabolism and excretion. It maintains and regulates homeostasis in the body. Its major functions include carbohydrate, protein and fat metabolism, detoxification, excretion of bile and storage of vitamins (Ahsan, Islam, Musaddik, and Haque, 2009). Since this organ is involved in the removal of substances from the portal circulation, it becomes susceptible to foreign substances resulting to liver disorder (Adewusi & Afolayan, 2010).

Liver disease is ranked as top 14 cause of death worldwide (World Health Rankings, 2014). They are generally caused by chemicals in high doses, excess consumption of alcohol, infections and autoimmune disorders. Most of the hepatotoxic chemicals cause damage to liver cells mainly by inducing lipid peroxidation and other oxidative damages. Worldwide, alcohol is one of the main causes of end stage liver disease which requires liver transplantation as treatment. The spectrum of alcoholic liver disease ranges from fatty liver to alcoholic hepatitis and ultimately fibrosis and cirrhosis (Adewusi and Afolayan, 2010).

Moreover, liver-associated diseases in the Philippines ranked as number 70 worldwide with a death rate of 12.2 per 100,000 (World Health Rankings, 2014). According to De Lusong, Labio, Daez, and Gloria (2008), non-alcoholic fatty liver disease (NAFLD) has been recognized as one of the liver diseases in the Philippines. Recent studies have proposed that obesity and type II Diabetes mellitus, which are hyperinsulinemic states with decreased tissue sensitivity to insulin, are the major factors in the pathogenesis of NAFLD. The prevalence of obesity has been steadily rising in the Philippines, which may be due to changes in dietary habits and lifestyle.

Liver damage can also be caused by some drugs' toxicity (Vadivu, Krithika, Biplab, Dedeepya, Shoeb & Lakshmi, 2008). Some of these drugs when taken in therapeutic dose may cause liver damage especially in high doses resulting to a more severe liver injury (Shammi, Choudhry, Khan & Hossain, 2012). Anti-tubercular drug-induced hepatitis is caused by oxidative stress. This oxidative stress is accounted by the overproduction of oxidants and/or depletion of antioxidants (Jeyakumar, Rajesh, Meena, Rajaprabhu & Ganesan, 2008).

Rifampin may occasionally cause hepatocellular injury and may also potentiate the hepatotoxicities of other anti-TB drugs (Saukkonen, Cohn, Jasmer, Schenker, Jereb, et al., 2006). Additionally, Kalra, Aggarwal, Khurana and Gupta (2007) noted that rifampin contributes to the further production of toxic metabolites from

acetylhydrazine leading to the increase hepatotoxicity of isoniazid. It interacts with other drugs metabolized by these and other hepatic enzymes, including warfarin, prednisone, digitoxin, quinidine, ketoconazole, itraconazole, propanolol, clofibrate, sulfonylureas, phenytoin, HIV protease inhibitors, and HIV nonnucleoside reverse transcriptase inhibitors (Saukonnen et al., 2006).

The accumulation of free radicals in the body leads to oxidative stress from which different diseases arise. These free radicals and reactive oxygen species are produced in the body during energy production. Thus, the ingestion of different vegetables containing phytochemicals like phenolic acids, tannins and flavonoids which act as anti-oxidant is beneficial in the prevention of diseases (Hamzah, Jigam, Makun & Egwin, 2014).

Hence, liver diseases are now becoming one of the fatal diseases of our world today creating a serious challenge to public health. There has not been much drug available for the cure of such diseases. Modern medicines have little to offer for improvement of hepatic diseases as they may also contribute minimal damage to our liver. It is mainly the plant-based preparations which are employed for their treatment of liver disorders. For that reason, folk remedies from plant origin are tested for their potential anti-oxidant and hepatoprotective activities (Ahsan, Islam, Musaddik & Haque, 2009).

Flavonoids have a wide range of biological and pharmacological studies such as anti-oxidant, anti-hepatotoxic, and protection against cardiovascular mortality. They also exhibit anticoagulant, anti-hyperlipidase, vasodilative effects and human immune deficiency virus (HIV) type 1 integrase inhibition (Meenakshi, Gnanambigai, Tamil mozhi, Arumugan, and Basubramanian, 2009). In the study of Asif and Khodadadi (2013), it was found out that flavonoids (kaempferol, quercitin, myricetin, luteolin, and apigenin) are present in *Capsicum frutescens* (siling labuyo).

Capsicum is a genus of plant under the family of *Solanaceae* and this capsicum has varieties of names according to their location and type (Shaha, Rahman, and Asrul, 2013). According to Raimi, Shittu, and Oyetade (2014), *C. frutescens* is widely grown in Nigeria and commonly consumed in South West. This plant has many other names including bird pepper, cayenne pepper, and bird eye chilli pepper. It is also commonly known as siling labuyo (Maramag, 2013). Adding to this, Olufemi and Olusegun (2013) said that these species are the least common but largely cultivated of the other capsicums. Despite the fact that the species name *frutescens* means “bushy”, because they are described by compact growth and can grow even in the absence of winter frost and several seasons, they tend to grow into a large

perennial shrub. The peppers grow upright with a height up to 60 cm. The fruit may vary from green, yellow, or red colors. Furthermore, Wahua, Okoli, and Sam (2013) also described the fruits as linear and borne at nodes. Moreover, its leaves are simple, glabrous, lanceolate to ovate with apex being acutely acuminate, and the base is cuneate or abruptly acute.

C. frutescens is an excellent source of various anti-oxidant compounds like flavonoids, carotenoids and Vitamin C (Zhani, Elouer, Aloui, and Hannachi, 2012). Studies showed that these anti-oxidant compounds are concentrated in both leaf and fruit extracts of the plant which prevent free radical damage, reducing the risk of chronic diseases (Soumya and Nair, 2012).



Figure 1. *Capsicum frutescens* (siling labuyo)

In Central Africa, *C. frutescens* is used as anti-oxidative, hypocholesterolemic, hypolipidemic, immunomodulatory and anti-mutagenic (Ogbonnaya and Muritala, 2014). Moreover, Wangchareon and Morasuk (2007) reported that *C. frutescens* is believed to increase circulation, treat infected wounds, reduce blood clots, and aid in digestion by stimulating saliva and gastric juice flow. It has also biochemical and pharmacological properties including anti-oxidant, anti-inflammatory, anti-allergenic, anti-carcinogenic, and antimicrobial activities. Shaha et al. (2013) stated that the plant has also been used as folk remedies for dropsy, colic, diarrhea, asthma, arthritis, muscle cramps, and toothache.

Currently, there are no studies regarding the hepatoprotective activity of the leaves of *C. frutescens*. This is quite unfortunate given the wide array of reported benefits of the said plant and its potential hepatoprotective effect due to its flavonoid content. Thus, this study investigated the hepatoprotective activity of *C. frutescens* leaves. Specifically, it determined the effective dose of methanolic extract and at which dose it will be toxic. Also, the study evaluated the

histopathological morphology of the liver of Sprague-Dawley rats after the administration of the plant extract. If proven effective, *C. frutescens* can be a possible alternative agent in the pharmaceutical industry to protect the liver from oxidative stress.

MATERIALS AND METHODS

Plant Acquisition

Fresh leaves of green *C. frutescens* were obtained from Bgy. Conde Labac, Batangas City. Voucher of the plant specimen was submitted to Forest Products Research and Development Institute, Department of Science and Technology in Laguna for authentication.

Reagents

All the reagents used in the study were purchased from DKL laboratory supplies and Belman Laboratories.

Preparation of Methanolic Extract of *C. frutescens*

One hundred grams of powdered dried leaves were weighed and then soaked in methanol for 72 hours at room temperature in the ratio of 1:20 (w/v). The solutions were collected and filtered using cotton wool followed by Whatman No. 1 filter paper. This procedure was repeated three times.

Then, the collected supernatant was pooled together and evaporated using a rotary vacuum evaporator at 65°C under reduced pressure. The crude dried extract obtained was kept at 4°C prior to use (Kamisan, Yahya, Mamat, Kamarolzaman, Mohtarrudin, et al., 2014). Manifestation of dark green and oily mixture indicates that crude extract was obtained.

Phytochemical Screening

Phytochemical screening of the crude methanol extract of *C. frutescens* for flavonoids was carried out using standard phytochemical procedure by Sumathy, Lachumy, Zakaria and Sasidharan (2011).

Test for Flavonoids

Magnesium ribbon and few drops of concentrated HCl were added to 2 ml of methanolic extract. Pink or red color indicated the presence of flavonoids (Sumathy, et al., 2011).

Test Animals

Adult Sprague-Dawley rats (n=20) of both sexes weighing 200 to 225 g and albino mice (n=21) were purchased at the University of

the Philippines, Manila. The rats were grouped into four consisting of five rats per group. They were acclimatized for seven days prior to experimentation with free access to standard feed and tap water ad libitum and kept on a 12 hour light/dark cycle. All animals were housed in an air-conditioned room at $23\pm1^{\circ}\text{C}$ during the quarantine period (Ahmed, Azmat, Zafar, and Salahuddin, 2014).

Toxicity Study (LD50) of the Methanolic Extract of *C. frutescens*

The median lethal dose (LD50) of the methanolic extract was determined in mice intraperitoneally (i.p.) as described by Salawu, Chindo, Tijani, and Adzu (2008). Briefly, mice of either sex were fasted overnight and the evaluation of the LD was carried out in two stages. In the first stage, three groups, of three mice each, were treated with the extract at doses of 10, 100 and 1000 mg/kg, i.p. in order to determine the range in which the LD falls. In the second stage, another four groups of three mice each were further treated with the extract at doses 140, 225, 370 and 600 mg/kg. Animals were observed for 24 hours after treatment for signs and symptoms of toxicity. The number of deaths in each group within twenty-four hours was recorded and the final LD50 values were calculated as the geometric mean of the highest non-lethal dose (with no deaths) and the lowest lethal dose (where deaths occurred).

Experimental Procedures

The rats were randomly divided into four groups containing five animals each.

- | | |
|-----------|--|
| Group I | Control (saline) 5 ml/kg |
| Group II | Inducing agent (isoniazid of 50 mg/kg and rifampicin of 50 mg/kg) |
| Group III | Inducing agent (isoniazid of 50 mg/kg and rifampicin of 50 mg/kg), and <i>C. frutescens</i> extract, 100 mg/kg |
| Group IV | Inducing agent (isoniazid of 50 mg/kg and rifampicin of 50 mg/kg), and <i>C. frutescens</i> extract, 200 mg/kg |

Animals were fasted overnight; the methanolic extract of *C. frutescens* with concentrations of 100 mg/kg and 200 mg/kg was given orally for 14 days to Group III and IV, respectively, together with isoniazid (50mg/kg) and rifampicin (50mg/kg) to induce liver damage. The said agents were collectively injected intraperitoneally. At 15th day, 1 ml blood was collected from all animals by retro-orbital bleeding (Sreenu, Venkata and Delhiraj, 2013) for the evaluation of serum parameters like aspartate transaminase (AST) and alanine transaminase

(ALT) (Cengiz, Ozbek and Him, 2008). The animals were then sacrificed and liver tissues were used for histopathological study (Sreenu, et al., 2013).

Histopathological studies

The liver was excised quickly and fixed in ten percent buffered-formaldehyde at room temperature. After dehydration using graded ethanol, pieces of tissues were embedded in paraffin, cut in fine (5 μm) sections and mounted on glass slides. Sections were deparaffinized with xylene, counterstained with hematoxylin and eosin and viewed under a light microscope at X400 (Ravi, Patel, Verma, Dutta, and Saleem, 2010).

Statistical analysis

All the data were expressed as mean \pm SEM. Statistical significance was tested using one way ANOVA followed by the Dunnet's t test and Tukey method using computer based fitting program. Statistical significance was determined at $p<0.05$ (Sreenu, et al., 2013). One way ANOVA was used to determine if there is any difference between groups on AST and ALT levels. Moreover, Dunnet's t test was also used to compare the AST and ALT levels on Control Group. Meanwhile, the Tukey method was used to see multiple comparisons on the AST and ALT levels between each group.

RESULTS AND DISCUSSION

I. Plant Extract

From 100g powdered leaves of *C. frutescens*, 60ml of methanolic extract was obtained. After subjecting the extract to rotary evaporator, 12.6g of dark green and oily crude extract was produced. Total yield was 12.6%.



Figure 2. *C. frutescens* methanolic extract

II. Phytochemical Screening

In the detection for the presence of flavonoids from *C. frutescens* extract, a pink colored result was produced when magnesium ribbon and few drops of concentrated HCl were added to the extract. This confirms the presence of flavonoids as one of the main phytochemical components of *C. frutescens*. This is supported by the study of El-Kamali and Elshikh in 2015 which also produced pink color using the same method.

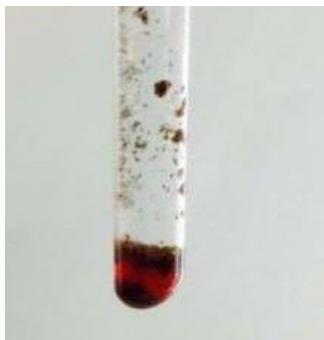


Figure 3. Positive result for the presence of Flavonoids

III. Lethal Dose (LD50) of the Methanolic Extract of *Capsicum frutescens*

For the lethal dose determination, mice were observed for behavioral signs of toxicity. LD50 is usually an initial step in the assessment and evaluation of the toxic characteristics of a substance. On the first stage, no signs and symptoms were observed after the administration of 10 mg/kg. At the same time, no deaths occurred. Meanwhile, decreased respiratory rate was observed following the administration of 100 mg/kg but no deaths occurred. On the other hand, after administration of 1000 mg/kg, signs of toxicity were observed like decreased respiratory rate and inactivity whereas 3 rats died. This shows that at a low dose of 10 mg/kg, *C. frutescens* extract is not toxic to rats while high dose such 1000 mg/kg is not only toxic but also lethal. Shammi, Choudhry, Khan, and Hossain (2012) stated that when therapeutic dose exceeds, it may result to damage. On the second stage, signs of toxicity were decreased respiratory rate and inactivity after administration of 140 mg/kg and above. However, 3 deaths occurred with doses of 370 mg/kg and 600 mg/kg. This shows that for as low as 370 mg/kg, it may already cause mortality. Additionally, LD50 was estimated to be 190 mg/kg. This implies that higher doses of *C.*

frutescens produce toxicity. In the study of Suresh Babu, Krishna, Maruthi, Shankarmurthy, and Babu in 2011, signs of toxicity such as ataxia and convulsion were observed. However, the mortality dose was observed at greater than 3000 mg/kg to 4000 mg/kg which is far greater than the observed mortality dose in the study.

Table 1
LD50 of the Methanolic Extract of *C. frutescens*

	Dose	Signs/Symptoms	No. of deaths
First stage	10 mg/kg	none seen	0
	100 mg/kg	decreased respiratory rate	0
	1000 mg/kg	decreased respiratory rate and inactivity	3
Second stage	140 mg/kg	decreased respiratory rate and inactivity	0
	225 mg/kg	decreased respiratory rate and inactivity	0
	370 mg/kg	decreased respiratory rate and inactivity	3
	600 mg/kg	decreased respiratory rate and inactivity	3

IV. Biochemical Analysis

Aminotransferases are enzymes that catalyze the interconversion of amino acids and α -keto acids by the transfer of amino groups. These enzymes are specific to the liver and are considered to be very sensitive. Thus, they become reliable for determining hepatotoxicity and hepatoprotectivity. When there is liver damage, both aspartate transaminase (AST) and alanine aminotransferase (ALT) levels increase. However, decreased levels of these enzymes indicate stabilization of plasma membrane as well as protection of hepatocytes against damage caused by hepatotoxin (Sreenu et al., 2013).

Serum ALT is a routine biochemical assay used in the clinical laboratory as it is readily available and inexpensive. This enzyme may be evaluated in cases of hepatitis, muscular dystrophy and other muscular diseases. In these conditions, the ALT level is observed to be elevated (Liu, Que, Xu and Peng, 2014).

Table 2 shows the mean values of the test results of AST and ALT. As shown, Group I has AST value of 123.8 ± 19.85 U/L and ALT value of 36 ± 5.47 U/L while Group II has AST and ALT values of 151.6 ± 17.31 U/L and 39.4 ± 8.57 U/L, respectively. This shows that the inducing agents were responsible to the elevation of the two enzymes. This implies that isoniazid and rifampicin were able to cause

hepatotoxicity. It can also be seen that Group III has lower AST and ALT values of 0.8 ± 13.27 U/L and 36 ± 6.33 U/L when compared to Group II. This shows that the methanolic extract of *C. frutescens* can lower the levels of AST and ALT. This may be due to the flavonoid content of *C. frutescens* since it is said that flavonoids have anti-hepatotoxic effect as revealed in the study of Meenakshi, Gnanambigai, Tamil mozhi, Arumugan, and Basubramanian in 2009.

Table 2
Mean values of Aspartate transaminase (AST) and Alanine transaminase (ALT)

	AST (U/L)	ALT (U/L)
Group I	123.8 ± 19.85	36 ± 5.47
Group II	151.6 ± 17.31	39.4 ± 8.57
Group III	0.8 ± 13.27	36 ± 6.33
Group IV	100.4 ± 26.46	57.2 ± 7.52

Legend: Group I (Control group), Group II (INF+RIF 50mg/kg), Group III (INF 50mg/kg +RIF 50mg/kg + MECF 100mg/kg), Group IV (INF 50mg/kg+RIF 50mg/kg + MECF 200mg/kg).

Group IV has AST value of 100.4 ± 26.46 U/L and has higher ALT value of 57.2 ± 7.52 U/L than Group I. This result was expected since dose of 200 mg/kg is near the LD50 which is 190 mg/kg. The results from Table 2 are related to the study conducted by Nasiru, Hafsat, Mohammad, and Sabo in 2012, wherein AST and ALT levels of negative control revealed higher values when compared to the normal control. Groups which received inducing agents and plant extract produced lower AST and ALT values when compared to the negative control.

Table 3 presents the comparison between the levels of AST and ALT on each treatment to the control group (saline group). In the dependent variable AST, Groups II, III, and IV have p-values of 0.640, 0.519, and 0.743, respectively which are not significant. On the other hand, in the dependent variable ALT, Groups II, III, and IV have p-values of 0.973, 1.00, and 0.121 which are also not significant. This was found out from the obtained p-values which were greater than 0.05 level of significance. This only shows that the effect is the same on each treatment both for AST and ALT. This implies that the dose of inducing agents used in the study caused liver damage using AST and ALT values of Sprague-Dawley rats. However, statistically speaking, the treatment, methanolic extract of *C. frutescens* did not show significant improvement of the damaged liver using AST and ALT values as bases.

Table 3
Comparison between the levels of AST and ALT on Control Group

Liver Enzyme	Group	p-value	Interpretation
AST	Group II	0.640	Not Significant
	Group III	0.519	Not Significant
	Group IV	0.743	Not Significant
ALT	Group II	0.973	Not Significant
	Group III	1.00	Not Significant
	Group IV	0.121	Not Significant

Dunnett t (2-sided); Legend: Significant at p-value < 0.05

This result is in contrast with the study of Eshwaraiah, Manasa, Kavitha and Bardalai in 2013 where there was significant decrease in hepatic enzyme levels observed in drug-treated animals with 150 mg/kg *Punica granatum* ethanolic root extract (PGERE) and 300 mg/kg PGERE. CCl₄ intoxication in normal rats significantly elevated the serum levels of AST and ALT. This only shows that the effect is different on each treatment both for AST and ALT levels. This implies that *Punica granatum* extract showed significant improvement of the damaged liver using AST and ALT values as bases.

Table 4 shows the comparison between AST and ALT on each group. Group I has a p-value of 0.003 which is significant since it is less than 0.05 level of significance. This shows that the effect on AST and ALT is different when compared with other treatments. This is due to the significant decrease of liver enzymes in Group I as compared with those in Group II. Since Group I was not induced and was only given saline, thus, it is suspected that the liver enzymes will not be elevated. It is assumed that the obtained AST and ALT levels were within the normal range. For Group II with p-value of 0.000 the significant effect of the inducing agents was observed with high AST and ALT levels. This proves the liver damaging effect of isoniazid and rifampicin which were used as the inducing agents in the study. Furthermore, significant effect (p=0.006) of 100 mg/kg of *C. frutescens* was observed in Group III. This implies that 100 mg/kg of *C. frutescens* can lower AST and ALT levels. The effect is more likely due to the flavonoid content of *C. frutescens* which was proven to have anti-hepatotoxic effects (Meenakshi, Gnanambigai, Tamil mozhi, Arumugan, and Basubramanian, 2009). Furthermore, Group IV has a p-value of 0.155 which is not significant since it is greater than 0.05 level of significance. This shows that the effect is the same when compared to other treatment because liver enzymes are higher than Group III. This implies that 200 mg/kg might be toxic since it is near the LD50 which is 190 mg/kg.

Table 4
Comparison between AST and ALT on each group

Groups	p-value	Interpretation
Group I	0.003	Significant
Group II	0.000	Highly Significant
Group III	0.006	Significant
Group IV	0.155	Not Significant

Legend: Significant at p-value < 0.05

This is in contrast with the study of Sreenu et al. in 2013 wherein groups treated with *Calycopteris floribunda* extract significantly lowered the elevated aminotransferase levels when compared to isoniazid-rifampicin group. Moreover, 200 mg/kg of *C. floribunda* extract showed a remarkable decrease in the enzyme levels than 100 mg/kg of *C. floribunda* extract indicating the greater hepatoprotective activity.

Table 5
Multiple comparisons on the AST and ALT levels between each group

Liver Enzyme		Group	p-value	Interpretation
AST	Group I	Group II	0.756	Not Significant
		Group III	0.649	Not Significant
		Group IV	0.837	Not Significant
	Group II	Group I	0.756	Not Significant
		Group III	0.174	Not Significant
		Group IV	0.297	Not Significant
	Group III	Group I	0.649	Not Significant
		Group II	0.174	Not Significant
		Group IV	0.986	Not Significant
ALT	Group IV	Group I	0.837	Not Significant
		Group II	0.297	Not Significant
		Group III	0.986	Not Significant
	Group I	Group II	0.986	Not Significant
		Group III	1	Not Significant
		Group IV	0.189	Not Significant
	Group II	Group I	0.986	Not Significant
		Group III	0.986	Not Significant
		Group IV	0.318	Not Significant
	Group III	Group I	1	Not Significant
		Group II	0.986	Not Significant
		Group IV	0.189	Not Significant
	Group IV	Group I	0.189	Not Significant
		Group II	0.318	Not Significant
		Group III	0.189	Not Significant

Tukey method; Legend: Significant at p-value < 0.05

Table 5 shows multiple comparison of Groups I to IV on ALT. ALT comparison of Group I to Group II produced a p-value of 0.756 which is greater than 0.05 level of significance. The same is for Groups III and IV when compared to Group I having p-values of 0.649 and 0.837, respectively, which means that there is no difference in the values. ALT comparison of Group II to Groups III and IV also produced no significant difference due to p-values of 0.174 and 0.297. In addition, Group III when compared to Group IV produced p-value of 0.986 which has also no significance.

On the other hand, AST comparison of Group I to Group II produced a p-value of 0.986. The same is for Groups III and IV when compared to Group I having p-values of 1 and 0.189, respectively which means that there is no difference in the values. Furthermore, AST comparison of Group II to Groups III and IV also produced no significant difference due to p-values of 0.986 and 0.318. Additionally, Group III when compared to Group IV produced p-value of 0.189. This shows that the effects were the same when compared to each group. This may be caused by the pair wise comparison considered only two variances while the multiple comparisons considered all variances affecting the given finding. Single-degree-of-freedom F test or the equivalent T test is used to basically consider contrasting data in both pair wise and general analyses (Saville, 1990). Multiple comparisons are not recommended if the study is confirmatory. For a confirmatory study where one difference is particularly important, consulting the literature or using other criteria to choose a multiple comparison method for means that appears to give the best chance to show this difference is advised (Rafter, Abell and Braselton, 2002).

V. Histopathological Results

Assessment of liver tissues was done to confirm the hepatoprotective activity of *C. frutescens* by observing healing of damaged tissues.

Figure 3a shows liver tissue from Group I which received only NSS. The result revealed sinusoidal dilatation and congestion. This result was quite surprising because it was suspected to demonstrate normal morphology of cells since NSS cannot induce damage to the liver. The result is more likely due to the exposure of the rats to chloroform during the experiment. According to Malaguarnera, Cataudella, Giordano, Nunnari, Chisari, and Malaguarnera (2012), acute exposure and toxicity to organic solvents like dimethylformamide (DMF), dimethylacetamide (DMA), trichloroethylene (TCE), tetrachloroethylene, carbon tetrachloride, xylene, toluene, and chloroform, have been associated with liver necrosis, and liver

steatosis, and chronic exposure has been associated with liver cirrhosis. The mechanism of injury is most likely the result of metabolic changes by the liver.

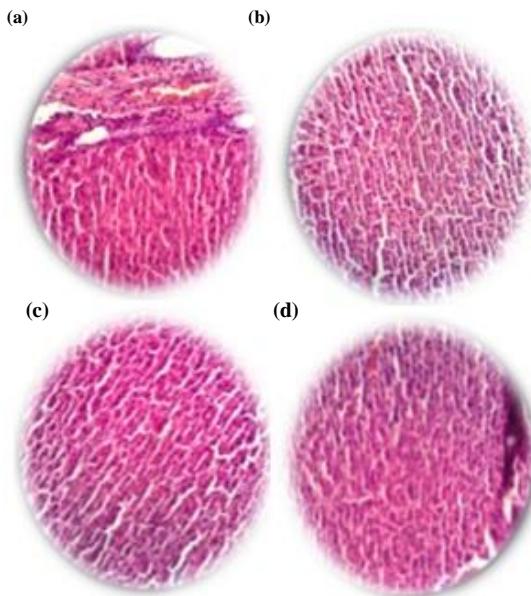


Figure 3. Histopathologic result of the liver tissues:
(a) Group I, (b) Group II, (c) Group III, (d) Group IV.

Figure 3b shows liver tissue from Group II which received high doses of isoniazid and rifampicin. There was a widespread coagulative necrosis mostly in the hepatocytes surrounding the central vein with multiple foci of sinusoidal dilation. There was random number of hepatocytes with pyknotic nuclei. Few small lymphoid aggregates along blood vessels and portal tracts are also seen. Final pathological diagnosis was centrilobular hepatocellular necrosis, moderate with intralesional sinusoidal dilation and vasculitis. This proves that the liver was damaged and the effect can be implied on the action of isoniazid and rifampicin. This implies that anti-tuberculosis drugs such as isoniazid and rifampicin can induce hepatotoxicity. According to Balakrishnan, Sangameswaran and Bhaskar (2010), during metabolism of isoniazid (INH), hydrazine is produced directly from INH or indirectly from acetyl hydrazine. The combination of isoniazid and rifampicin (RIF) was reported to result in higher rate of inhibition of biliary secretion and an increase in liver cell lipid peroxidation. Additionally, in the study of Saukkonen, Cohn, Jasmer,

Schenker, Jereb, et al. in 2006, it was found out that isoniazid is cleared mostly by the liver, primarily by acetylation by N-acetyl transferase 2 (NAT-2). Acetyl-isoniazid is metabolized mainly to mono-acetyl hydrazine (MAH) and to the nontoxic diacetyl hydrazine, as well as other minor metabolites. Rifampin appears to enhance a metabolic hepatocellular idiosyncratic reaction in patients receiving isoniazid, perhaps by promoting the formation of toxic isoniazid metabolites.

Figure 3c shows liver tissue from Group III which received inducing agents and 100 mg/kg of *C. frutescens* simultaneously. There is widespread coagulative necrosis mostly in the hepatocytes surrounding the central vein with widespread sinusoidal dilation. Congested blood vessels are also seen. Final pathological diagnosis was centrilobular hepatocellular necrosis, moderate with intralesional sinusoidal dilation. The results revealed a damaged liver. This shows that 100 mg/kg of *C. frutescens* was not enough to heal the damaged liver brought about by the inducing agents. Since Groups II and III showed almost the same histopathological result, this means that 100 mg/kg of *C. frutescens* is not effective to heal the damaged liver tissues.

Figure 3d shows liver tissue from Group IV which received same with Group III but with higher dose of 200 mg/kg of *C. frutescens*. There was a widespread coagulative necrosis mostly in the hepatocytes surrounding the central vein with widespread sinusoidal dilation. Prominent nuclear pyknosis and congested blood vessels are seen. These results show that *C. frutescens* was not able to restore the normal hepatic morphology. This is probably due to the nearness of the dose (200 mg/kg) with the LD50 which is 190 mg/kg. This implies that although higher dose of *C. frutescens* extract was used, still it cannot heal the liver from damage. This is supported by the study of Ravi, Patel, Verma, Dutta, and Saleem in 2010 wherein liver which was treated 150 mg/kg of *Bomba ceiba* extract showed minimal inflammation with moderate portal triaditis and normal lobular architecture. This shows that even at a low dose of 150 mg/kg, it cannot revert completely hepatic injury caused by isoniazid and rifampicin.

CONCLUSION

From all the results obtained, the researchers concluded that 100 mg/kg of *C. frutescens* extract is a therapeutic agent against hepatotoxicity since it renders lower AST and ALT levels. However, the biochemical results were not supported in the histopathological findings.

RECOMMENDATION

The researchers recommend conducting further studies for the

purification of flavonoids to obtain high probability of results and to confirm its hepatoprotective activity since the study used crude extract. They would also like to recommend the use large population experimental animals for more reliable and precise results. Furthermore, the use of baseline data is highly recommended to ensure the validity of the experiment.

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