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In Vitro Anti-Oxidant and *In Vivo* Hepatoprotective Activity of Mucuna Monosperma in Paracetamol Induced and Alcohol Induced Liver Injury in Experimental Rats

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ABSTRACT
Liver disease is a major public health threat, particularly in developing countries. Several medicinal plants and formulations have been claimed to have liver protective activities. The present study aimed to evaluate in vitro antioxidant and in vivo hepatoprotective activities of Methanolic extraction of Mucuna monosperma (MEMM).Free radical scavenging activity of crude extract was conducted using the DPPH assay method. Hepatoprotective activities of the crude extract and solvent fractions of the plant were carried out based on paracetamol and alcohol -induced liver damage in rats. Serum biomarkers (SGOT, SGPT, ALP, BIT, BID and ALB) were assessed to find out the effect. Antioxidant assay revealed that the crude extract, exhibited free radical scavenging activity with IC_{50} values of 213.42 µg/ml. Pre-treatment of the rat with the crude extract and significantly reduced all biochemical parameters levels at all the administered doses compared to the toxic group. The crude extracts total bilirubin level at doses of 250 mg/kg (P< 0.05) and 500 mg/kg (P< 0.001). From this study, it can be concluded that the MEMM demonstrated antioxidant and hepatoprotective activities.
***Key words**: Mucuna monosperma, Hepatoprotection, Free radical scavenging activity, antioxidant activity*

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INTRODUCTION

The role of free radical reactions in disease pathology is well established. It suggests that these reactions are necessary for normal metabolism but can be detrimental to health as well including outcome of various diseases like diabetes, immunosupression, neurodegenerative diseases and others [1]. Free radicals lead to cellular necrosis, which is implicated in some membrane pathophysiological conditions, including atherosclerosis, rheumatoid arthritis as well as toxicity of many xenobiotics [2]. Liver diseases remain a serious health problem. It is well known that free radicals cause cell damage through mechanisms of covalent binding and lipid peroxidation with subsequent tissue injury. Antioxidant agents of natural origin have attracted special interest because they can protect human body from free radicals [3]. Numerous medicinal plants and their formulations are used for liver disorders in ethnomedical practices as well as in traditional systems of medicine in India. Liver diseases are among the global health problems; In which, liver cirrhosis is the ninth leading cause of death in western Nations. [4] Toxic chemicals, xenobiotics, alcohol consumption, malnutrition, anemia, medications, autoimmune disorders, and viral infections [5, 6] are some major causes of liver disease; among which, medications are the most common contributing factors. [7] Drug-induced liver damage accounts for more than 60% of all cases in the United States of America, and it is the leading cause of acute liver failure. [8]. Paracetamol is a commonly available over-the-counter analgesic and antipyretic drug which is associated with acute liver damage. The hepatotoxicity associated with paracetamol is mainly due to excessive accumulation of its toxic metabolite, N-acetyl-p-benzoquinone imine (NAPQI), which oxidizes liver tissue macromolecules such as lipid or -SH group of protein causing oxidative stress and hepatic necrosis. [9-11]. The modern drugs available for the treatment of liver ailments are less effective, less safe and, expensive. [12] This indicates the need for new better drugs. Several medicinal plants and formulations have been claimed to have liver protective activities, about 160 phytoconstituents from 101 plants have been suggested to possess hepatoprotective activity. [13]. *Mucuna monosperma*, commonly known as Negro beans in India, or deer-eye beans, donkey-eye

beans, or ox-eye beans, is a large woody climber from the family Fabaceae. The plan has three layers; a brown pod covered in small hairs, curved petals usually colored purple and black round-shaped beans. Small hairs on the pod can irritate the skin. The petals of the *Muncuna monnosperma* flower open at night and closes in the morning. It is found in India, including the eastern Himalayas, the northeastern states, and the Andaman and Nicobar Islands, Sri Lanka, Bangladesh, Myanmar, and Thailand. Its natural habitat is evergreen forests, and it commonly grows near swamps or along streams. The present study was aimed for *in vitro* anti-oxidant and in vivo hepatoprotective activity of *mucuna monosperma* in paracetamol induced and alcohol induced liver injury in experimental rats.

MATERIAL AND METHODS

Chemicals

The entire chemicals used were of analytical grade. The chemicals and reagents used were purchased from Sigma Aldrich (St. Louis, MO, USA). We used an enzyme-linked immunoassay kit for cytokines from liver and blood of rats (R & D Systems Inc., Minneapolis, Minnesota, USA).

Plant material

Seed of *Mucuna monosperma* were obtained from the local places of Tirupati, AP. The plant was authenticated by Dr. K. Madhava Chetty M.Sc., M.Ed., M.Phil., Ph.D., PG DPD.,

Extraction by Maceration

Fresh *Mucuna monosperma* seeds were dried in the shade after being water-washed to remove contaminants like dirt and other impurities. To get a consistent, coarse powder, these dry seeds were crushed and sieved. One kg of powdered plant material was weighed, immersed in methanol, and left to macerate for seven days while being occasionally stirred. The solvent was filtered on the eighth day by squeezing it through a muslin cloth, and it was then heated to 40°C in a rotary evaporator to evaporate. The end product was placed in a desiccator to get rid of any remaining methanol. For additional research, the dried methanolic extract of *Mucuna monosperma* (MEMM) was placed in an airtight container and kept in a dry location.

Preliminary Phytochemical Analysis

All the extract/fractions of *Mucuna monosperma* were analyzed for their primary and secondary metabolites to confirm the presence of various primary metabolites, such as carbohydrates, amino acids, proteins, and lipids, and secondary metabolites, such as alkaloids, tannins, phenols, flavonoids, saponins, steroids, glycosides, and resins, according to standard methods.

In vitro antioxidant screening assays

DPPH radical scavenging assay

DPPH radical scavenging activity was assessed according to the method of Blois, 1958. Various concentrations of the plant extract or standard (2 ml) were added to 6 ml of methanolic solution of DPPH (33 mg/l) in a test tube. The reaction mixture was kept at 25^0C for an hour in an incubator. The absorbance of the residual DPPH solution was determined at 517 nm in a UV-Visible Spectrophotometer. The experiment was performed in triplicate. Ascorbic acid was used as standard. The inhibition was calculated in terms of percentage inhibition (I %) using following formula and lower IC_{50} value indicates high antioxidant capacity. [14]

$$I\ \% = (Abs_{control} - Abs_{sample}) / Abs_{control} \times 100$$

ABTS radical scavenging Activity

ABTS radical scavenging ability was assessed according to the method of Roberta *et al*., 1999. Initially, ABTS 2 mM (0.0548 gm in 50ml) and potassium per sulphate 70 mM (0.0189 gm in 1ml) were prepared in distilled water. Next, 200 ml of potassium per sulphate and 50 ml of ABTS were mixed and kept aside for 2 hrs. This solution was used for assessing ABTS radical scavenging activity. To the 1 ml of various concentrations of plant extract or standard, 0.6 ml of ABTS radical cation and 3.4 ml of phosphate buffer pH 7.4 were added and the absorbance was measured at 734 nm. The experiment was performed in triplicate. Ascorbic acid was used as standard. The percentage of inhibition (I %) was calculated using following formula and lower IC50 value indicates high antioxidant capacity [15].

$$I\ \% = (Abs_{control} - Abs_{sample}) / Abs_{control} \times 100$$

Metal chelating assay

Metal chelating ability was carried out according to the Dinis *et al*., 1994. In this assay, 10 ml of plant extract or standard, 0.2 ml of 2 mM ferric chloride and 0.4 ml of ferrozine solution were mixed and kept aside for 10 min at room temperature with continuous shaking. The absorbance was measured at 562 nm. The experiment was performed in triplicate. EDTA was used as standard. The percentage inhibition was calculated using following formula and lower IC_{50} value indicates high antioxidant capacity.

$$I\ \% = (Abs_{control} - Abs_{sample}) / Abs_{control} \times 100$$

Animals

Male Wistar Rats of 180-200mgs, housed maximum of 6 per cage in a polypropylene cage with maintained room temperature of 20.2- 23.5°C, relative humidity of 30 - 70% with 12h fluorescent light and 12h dark cycle were used in the studies. Animals were fed with rodent feed and purified water that was provided ad libitum. Animals were kept in sterilized rice husk beddings changed along with the cage twice a week during acclimatization and entire experimental study period. The study protocol was approved by IAEC with Ethical No: **45/SRCP/CPCSEA/2022**

Evaluation of hepatoprotective activity: [16**]**

Experimental Design

Evaluation of hepatoprotective activity in alcohol-induced hepatotoxicity:

Wistar Albino Rats (Wistar Strain) of either sex weighing 150-200g were selected and divided into five groups of six animals each. Group I: Vehicle treated rats were kept on normal diet and served as control for 15 days. Group II: Rats orally received 30% alcohol (1.5 ml/ Rat / twice a day) for 15 days. Group III: Rats orally received Silymarin (25 mg/kg b. w/day) and alcohol as group II, for 15 days. Group IV: Rats orally received *MEMM* (250 mg/kg b. w/day) and alcohol as group II, for 15 days. Group V: Rats orally received *MEMM* (500 mg/kg b. w /day) and alcohol as group II, for 15 days.

Evaluation of hepatoprotective activity in paracetamol induced hepatotoxicity.

In case of paracetamol-induced hepatotoxicity, the rats were divided into 5 groups of 6 rats each: Group I: Vehicle treated rats were kept on normal diet and served as control for 15 days. Group II: Rats received paracetamol (500 mg/kg b. w/day, orally) for 15 days. Group III: Rats received Silymarin (25 mg/kg b. w/day, orally) and paracetamol as group II, for 15 days. Group IV: Rats received MEMM (250 mg/kg b. w/day, orally) and paracetamol as group II, for 15 days. Group V: Rats received MEMM (500 mg/kg b. w/day, orally) and paracetamol as group II, for 15 days.

This period of treatment, the rats were maintained under normal diet and water. The blood was collected from the retro orbital plexus of the rats of all groups 24 h after the last dose administration, under light anesthetic ether. The blood samples are centrifuged at 3000rpm for 30min to separate the serum. The serum was analyzed for various biochemical parameters such as SGOT, SGPT, ALP, BIT, BID and ALB. Liver was dissected out and subjected for morphological study such as liver weight and liver volume of each animal. Further the liver was placed in 10% formalin solution for histopathological study.

Estimation of serum bio-chemical parameters [17-19]

Different biochemical parameters were estimated like SGOT, SGPT, ALP, bilirubin and albumin

RESULTS

In the present study, the investigation of Methanolic extraction *Mucuna Monosperma* revealed the presence of various presences of various phytoconstituents like flavonoids, phenolic compounds,triterpenoids, tannins, saponins, amino acids, proteins, Steroids,Gums and carbohydrates results were showed in Table 1

Table 1: Results of Phytochemical screening

S. No	Name of the Phytochemical	MEMM
1.	Carbohydrates	+
2.	Amino acids	+
3.	Proteins	+
4.	Alkaloids	+
5.	Cardiac glycosides	+
6.	Triterpenoids	+
7.	Saponins	+
8.	Flavonoids	+
9.	Phenolic compounds	+
10.	Tannins	+
11.	Steroids	+
12.	Gums	-

Where, + means positive and - means negative.

***In vitro* antioxidant assays**

DPPH radical scavenging assay

It is an extensively used, relatively rapid and accurate method for the assessment of free radical scavenging activity. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable

diamagnetic molecule. Antioxidant donates the electron or hydrogen atom after interaction with DPPH radical and thus neutralizing free radical character of the DPPH and convert it to 1-1,diphenyl-2- picryl hydrazine and the degree of discoloration indicates the scavenging activity of the drug. The reduction capacity of DPPH radical is determined by the decrease in its absorbance at 517 nm induced by antioxidants. The decrease in absorbance of DPPH radical caused by antioxidants because of the reaction between antioxidant and radical progress which results in the scavenging of the radical by hydrogen donation. It is visually evident as change in color from purple to yellow. Hence DPPH is usually used as a substance to evaluate the antioxidant activity. The IC_{50} values of the Methanolic extraction of *Mucuna Monosperma* (MEMM) was found to be 213.42 µg/ml. IC_{50} value for the Vitamin C (Standard) was found to be 6.8µg/ml and also presented in Table 2.

Table 2: DPPH radical scavenging assay of MEMM

Extract/Standard	Concentration(µg/ml)	% Inhibition	IC_{50} Value
Methanolic extraction of *Mucuna Monosperma* (MEMM)	100	25.85±2.01	213.42 µg/ml
	200	43.37±2.26	
	300	79.44±3.46	
	400	84.75±1.50	
	500	90.87±2.14	
Ascorbic acid	1	4.16 ± 0.27	6.8 µg/ml
	2	16.22± 2.09	
	4	28.88± 3.95	
	6	44.95± 2.96	
	8	57.02± 3.98	
	10	66.12± 2.76	

ABTS radical scavenging assay

It is one of the most commonly used assays in food industry for the measurement of antioxidant ability of foods. In this, ABTS is converted to its radical cation by addition of potassium per sulfate. This radical cation is blue in color and absorbs light at 734 nm. The ABTS radical cation is reactive towards most antioxidants including polyphenols, thiols and ascorbic acid. During this reaction, the blue ABTS radical cation is converted rear to its colorless neutral form. The IC_{50} values of the Methanolic extraction of *Mucuna Monosperma* (MEMM) were found to be 520µg/ml respectively. IC_{50} value for the Vitamin C (Standard) was found to be 14.1µg/ml and also presented in Table 3.

Table 3: ABTS radical scavenging assay of MEMM

Extract/Standard	Concentration (µg/ml)	% Inhibition	IC_{50}value
Methanolic extraction of *Mucuna Monosperma* (MEMM)	100	13.59±1.14	520 µg/ml
	200	17.73±1.87	
	300	28.64±4.11	
	400	35.97±1.02	
	500	47.26±4.04	
	750	60.33±3.73	
	1000	76.52±3.46	
ASCORBIC ACID	10	36.70 ±2.19	14.1 µg/ml
	20	72.63± 3.91	
	30	88.69± 2.85	
	40	92.18± 1.02	
	50	98.11± 0.97	

Metal chelating assay

Ferrous iron can initiate lipid peroxidation by the Fenton reaction as well as accelerating peroxidation by decomposing lipid hydro peroxides into peroxyl and alkoxyl radicals. Ferrozine can make complexes with ferrous ions. From the result it was evident that BOB and ASP extracts possessed Fe^{2+} chelating activity and might play a protective role against oxidative damage induced by metal catalyzed decomposition reactions.

The IC_{50} values of the Methanolic extraction of *Mucuna Monosperma* (MEMM) were found to be 320µg/ml respectively. IC_{50} value for the EDTA(Standard) was found to be 76.19µg/ml and also presented in Table 4.

Table 4: Metal chelation assay of MEMM

Extract/Standard	Concentration (µg/ml)	% Inhibition	IC_{50}value
Methanolic extraction of *Mucuna Monosperma* (MEMM)	100	23.17±0.16	320 µg/ml
	200	39.14±0.29	
	300	48.73±1.43	
	400	61.74±1.85	
	500	74.28±1.65	
EDTA	10	16.20± 0.83	76.19 µg/ml
	20	27.11±1.05	
	40	35.39±0.05	
	60	42.73±1.64	
	80	54.18±1.21	
	100	61.32 ± 0.26	

Alcohol induced hepatotoxicity

Liver weight and liver volume

Alcohol treatment in rats resulted in enlargement of liver which was evident by increase in the liver weight and volume. The groups treated with Silymarin showed good restoration of liver weight and liver volume where as test groups treated with MEMM showed significant effect on liver weight and liver volume compared to toxic control group results were showed in Table 5.

Table 5: Liver weight and liver volume in alcohol induced hepatotoxic rats

Group	Liver weight gm/100gm	Liver volume ml/100gm
Control	3.68±0.18	6.95±0.65
Toxic control	4.82±0.16	8.95±0.65
Silymarin (10mg/kg)	3.81±0.48**	7.23±0.72**
MEMM(250mg)	4.23±0.20*	7.63±0.14**
MEMM(500mg)	4.13±0.09**	7.51±0.04**

Values are expressed as mean ± SEM; n=6

* p≤0.05, **p≤0.01 and ***P<0.001. Comparison with toxic control

Bio chemical parameters

Effect of MEMM on SGOT, SGPT & ALP levels in alcohol induced hepatotoxic rats

Rats treated with alcohol developed a significant hepatic damage observed as elevated serum levels of hepatospecific enzymes like SGPT, SGOT and ALP when compared to normal control. Treatment with Silymarin had showed good protection against alcohol induced toxicity to liver. Groups treated with MEMM showed significant effect which can be comparable with toxic control. Dunnet's test indicates a significant reduction in elevated serum enzyme levels with extract treated animals compared to toxic control animals results were showed in Table 6.

Table 6: SGOT, SGPT & ALP levels in alcohol induced hepatotoxic rats

Group	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)
Control	113.15±5.05	106.11±4.13	130.75±3.97
Toxic control	396.93±0.93	391.77±7.87	378.62±3.59
Silymarin	148.22±1.79**	135.39±6.88**	181.04±11.49**
MEMM(250mg)	220.08±15.80**	200.78±5.67**	242.27±6.05**
MEMM(500mg)	185.24±6.06**	178.91±9.05**	224.52±11.91**

Values are expressed as mean ± SEM; n=6

* p≤0.05, **p≤0.01 and ***P<0.001. Comparison with toxic control

The total bilirubin concentration was found to increase in animals with liver damage by alcohol. In standard group, Silymarin administration reduced total bilirubin and animals treated with MEMM have exhibited dose dependent significant reduction in total bilirubin compared to toxic control group. Alcohol treated groups significantly elevated direct bilirubin concentration in animals by inducing hepatic damage compared to normal animals. But treatment with standard drug Silymarin showed good reduction in direct bilirubin concentration. Groups treated with MEMM significantly reduced direct bilirubin level in respective groups. Induction of liver damage by administration of alcohol significantly reduced serum albumin level in positive control group animals when compared to normal animals. But the treatment with

Silymarin has shown significant increase while MEMM have shown dose dependent increase in serum albumin level compared to toxic control group. Results were showed in Table 7.

Table 7: BIT, BID & ALB levels in alcohol induced hepatotoxic rats

Group	BIT (mg/dl)	BID (mg/dl)	ALB(g/dl)
Control	0.63±0.09	0.30±0.06	4.64±0.22
Toxic control	2.04±0.15	1.98±0.17	2.18±0.11
Silymarin	0.75±0.07**	0.41±0.02**	4.38±0.47**
MEMM(250mg)	1.13±0.03**	1.08±0.11**	3.405±0.30**
MEMM(500mg)	0.99±0.04**	0.75±0.04**	4.03±0.02**

Values are expressed as mean ± SEM; n=6

* p≤0.05, **p≤0.01 and ***P<0.001. Comparison with toxic control

Paracetamol induced hepatotoxicity

Liver weight and liver volume

Paracetamol treatment in rats resulted in enlargement of liver which was evident by increase in the liver weight and volume. The groups treated with Silymarin showed good restoration of liver weight and liver volume where as test groups treated with MEMM showed significant effect on liver weight and liver volume compared to toxic control group. Results were showed in **Table 8.**

Table 8: Liver weight and liver volume in paracetamol induced hepatotoxic rats

Group	Liver weight gm/100gm	Liver volume ml/100gm
Control	3.11±0.11	5.6±0.15
Toxic control	4.55±0.18	9.65±0.18
Silymarin	3.23±0.12**	6.06±0.10**
MEMM(250mg)	3.85±0.10**	7.25±0.06**
MEMM(500mg)	3.58±0.11**	6.76±0.08**

Values are expressed as mean ± SEM; n=6

* p≤0.05, **p≤0.01 and ***P<0.001. Comparison with toxic control

Bio chemical parameters:

Effect of MEMM fruits on SGOT, SGPT & ALP levels in paracetamol induced hepatotoxic rats

Rats treated with paracetamol developed a significant hepatic damage observed as elevated serum levels of hepatospecific enzymes like SGPT, SGOT and ALP when compared to normal control. Treatment with Silymarin had showed good protection against Alcohol induced toxicity to liver. Groups treated with MEMM showed significant effect which can be comparable with toxic control. Dunnet's test indicates a significant reduction in elevated serum enzyme levels with extract treated animals compared to toxic control animals. Results were showed in Table 9.

Table 9: SGOT, SGPT & ALP levels in paracetamol induced hepatotoxic rats

Group	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)
Control	109.20±2.66	96.49±5.35	96.68±5.21
Toxic control	388.98±2.49	302.46±7.49	379.94±4.79
Silymarin	123.86±4.67**	106.83±2.21**	128.64±1.87**
MEMM(250mg)	194.62±2.19**	193.6±1.6**	201.75±8.67**
MEMM(500mg)	161.93±2.30**	150.93±1.83**	165.38±5.66**

Values are expressed as mean ± SEM; n=6

* p≤0.05, **p≤0.01 and ***P<0.001. Comparison with toxic control

The total bilirubin concentration was found to increase in animals with liver damage by paracetamol. In standard group, Silymarin administration reduced total bilirubin and animals treated with MEMM have exhibited dose dependent significant reduction in total bilirubin compared to toxic control group. Paracetamol treated groups significantly elevated direct bilirubin concentration in animals by inducing hepatic damage compared to normal animals. But treatment with standard drug Silymarin showed good reduction in direct bilirubin concentration. Groups treated with MEMM significantly reduced direct bilirubin level in respective groups. Induction of liver damage by administration of paracetamol significantly reduced serum albumin level in positive control group animals when compared to normal animals. But the treatment with Silymarin has shown significant increase while MEMM have shown dose

dependent increase in serum albumin level compared to toxic control group.Results were showed in Table 10.

Table 10: BIT, BID & ALB levels in paracetamol induced hepatotoxic rats

Group	BIT (mg/dl)	BID (mg/dl)	ALB(g/dl)
Control	0.66±0.03	0.29±0.01	4.67±0.25
Toxic control	2.95±0.48	2.08±0.32	2.38±0.07
Silymarin	0.75±0.01**	0.39±0.02**	4.41±0.43**
MEMM(250mg)	1.24±0.02**	1.07±0.01**	3.62±0.29**
MEMM(500mg)	1.08±0.01**	0.79±0.01**	3.95±0.45**

Values are expressed as mean ± SEM; n=6

* p≤0.05, **p≤0.01 and ***P<0.001. Comparison with toxic control

DISCUSSION

As many of the chemicals pass through the liver to enter the general circulation; the liver is at a higher risk to be damaged than other organs. Hepatotoxicity is a significant problem in patients taking paracetamol intentionally or accidentally; causing acute liver failure. [20]

The evaluation of protective activity in liver damage induced by paracetamol has been widely used for hepatoprotective drug screening. Hepatotoxicity with paracetamol is due to its highly reactive metabolite, NAPQI.[21] Increase in NAPQI quantity leads to glutathione depletion, which finally causes an alteration in homeostasis, an increase in the permeability of the cell membrane with a consequent cellular swelling, karyolysis, vacuolization of hepatocytes and an elevation of liver enzymes. [22]

The main enzyme levels elevated during liver injuries are SGOT, SGPT, ALP, BIT, BID and ALB. The reason is that these enzymes are mainly found in the liver and are released into the blood as a result of liver injury. There is also a reduction in the total protein and albumin levels due to disruption and dissociation of polyribosomes on endoplasmic reticulum resulting in decreasing the biosynthesis of protein.

In the present study, the plant extracts showed a dose-dependent free radical scavenging activity; by which the crude extract showed antioxidant activity with IC_{50} of 520µg/ml.The antioxidant activity may be attributed due to the presence of secondary metabolites including flavonoids, polyphenols, and coumarins. This is in agreement with the previous findings reported by Alghazeer et al (2018); Sharma et al (2016); Teshome et al (2015); Torres et al (2006). [23-25] the variation in antioxidant activities of the plant extracts could be due to a difference in the amount and kind of phytochemicals present in the crude extract and solvent fractions of the plant. [26]

An increase in liver weight is an indication of liver injury. [27] In the present study, liver weight of the mice administered with paracetamol and Alcohol alone significantly increased compared to the normal control group (P<0.01). The reason is that water is retained in the cytoplasm of hepatocytes leading to enlargement of liver cells, resulting in increased total liver mass. [28] In our study, the crude extract at a dose of 500 mg/kg reduced liver weight of the mice significantly (P<0.05).

Pretreatment of the rat with different doses of the crude extract and solvent fractions combined with paracetamol and alcohol demonstrated hepatoprotective activities against paracetamol-induced liver injury; reducing the elevated levels of ALT, AST and ALP (P<0.001). Total bilirubin was reduced significantly in a dose dependent manner. The crude extract and the aqueous fraction showed a significant elevation in the total protein at a dose of 400 mg/kg compared to toxic group (P<0.05).

Suppression of the elevated serum levels of ALT and AST by the crude extract and solvent fractions of ***Croton macrostachyus*** is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damages caused by paracetamol. This is consistent with what has been found in previous studies done by Gutiérrez and Solís (2009). [29] On the other hand, the suppression of increased ALP and the subsequent depletion of elevated bilirubin total level states that the crude extract and solvent fractions of the plant have the potential to stabilize biliary dysfunction. The negative effect of paracetamol on total protein was reversed with the administration of the crude extract and aqueous fraction which indicates an improvement of the functional status of the liver cells to synthesize proteins. These findings corroborate the ideas of Okokon et al (2017); Shrivastava et al (2017). [30, 31]

As explained by Trifunschi et al (2015), the hepatoprotective activities of the plant extracts could be due to their free radical scavenging activity. [32] Additionally, their anti-inflammatory activity could contribute to their hepatoprotective activity, as paracetamol toxicity produces inflammatory mediators such as monocytes, neutrophils and cytokines (interleukin-6 and tissue necrosis factor α). [33]

Other liver protective activities of the plant extracts could be; (1) inhibition of metabolism of paracetamol; because, metabolism of paracetamol with cytochrome P450 enzymes specifically, CYP2E1, is implicated in the hepatotoxicity of paracetamol by producing toxic metabolite, NAPQI. As a result, inhibition of this

enzyme by the plant extracts could possibly reduce the toxic effects of paracetamol; [34] and (2) an increase in glutathione level, which is reduced during paracetamol overdose due to production of excess NAPQI level. Excess NAPQI alkylates and oxidizes intracellular glutathione resulting in liver glutathione depletion subsequently leads to increased lipid peroxidation and liver damage. [35] Thus, administration of the crude extract and solvent fractions might increase the glutathione level as a mechanism to protect paracetamol-induced liver damage in mice.

Hepatoprotective activities of the plant extracts could be due to the presence of phytochemicals such as polyphenols, alkaloids, flavonoids, saponins and coumarins which posses' hepatoprotective activity either alone or in combination. These findings agree with other studies that have shown the hepatoprotective activity of these phytoconstituents. [36-38]

The mechanism of these phytochemicals for their hepatoprotective activity is mainly due to their free radical scavenging activity since the plant extracts contain different phytoconstituents which possess free radical scavenging activity. Additionally, the phytochemical flavonoid could maintain cell membrane stability or could protect cell membrane leakage up on damage by paracetamol as evidenced by a reduction in the liver biomarkers. This is in line with the findings of Tarahovsky et al (2014). [39] Furthermore, the anti-inflammatory effect of the secondary metabolites such as alkaloids, saponins, coumarins and flavonoids could be the means for their hepatoprotective activity.

CONCLUSION

In conclusion, the present results show that the crude extract demonstrated antioxidant and hepatoprotective activities. The hepatoprotective activities of the plant extract could be due to their free radical scavenging and antioxidant activities, resulting from the presence of some phytochemicals including polyphenols, flavonoids, saponins and alkaloids. Furthermore, the exact phytoconstituents and their mechanism of hepatoprotection should be studied. Additionally, these findings could justify the traditional use of the MMEM in liver disorders; and suggest the possible utilization of the MEMM as a source of new compounds for hepatoprotective activity.

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