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Hepatoprotective Activity of Aqueous Leaf Extract of Pako (*Diplazium esculentum*) Plant against Chloroform Induced Albino Rats.

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Abstract:
Background
In this study, we wanted to determine the hepato-protective property of pako leaves extract (*Diplazium esculentum*) in Swiss albino rats and compare the effect of different rates of fiddle fern (*Diplazium esculentum*).
Methods
The in-vivo hepatoprotective effect of the aqueous extract of *Diplazium esculentum (Retz.) Sw.* against chloroform induced hepatotoxicity in albino rats was studied. The test rats were brought from PITAHC-DOH animal facility, the plant extraction was carried out at ROCO buildin, Cagayan State University (CSU) Carig Campus, College of Arts and Science, Carig Sur, Tuguegarao city, and the test was conducted at the PITAHC-DOH animal facility, after obtaining clearance from Institutional Ethics Committee.
Results
In the mean total protein, there was no significant mean difference between the treatments with the increase in dose rate of Fiddle fern extract given to the animals (P = 0.338), Significant differences were observed for the SGPT between the control group (T2) and the group treated with 20 g/kg extract (P = 0.024). There were no significant differences detected for the (T1) negative control group with T4-extract with 40 g/kg and T2-treated with essential.
Conclusion
The 40 g/kg of the fiddlehead fern extract prevents liver damage comparably similar to those with essential and the normal control group. To conclude, the death was prevented with administration of the extract 20 and 40 g/kg.
Keywords: Hepatoprotective Activity, Aqueous Leaf Extract, Pako *(Diplazium esculentum),* Chloroform, Induced Albino Rats.

1. Introduction

The liver is a vital organ of paramount importance involved in the maintenance of metabolic functions and detoxification of the exogenous and endogenous challenges like xenobiotics, drugs, viral infections and chronic alcoholism. Liver disease is a worldwide problem. Conventional drugs used in the treatment of liver diseases are sometimes inadequate and can have serious adverse effects **(Alessandro 2000 &**[1] **Mageswari**

2010).[2] Acute and chronic liver diseases are of global concern (**Sung-Hwa Kim 2009**).[3] In the absence of reliable liver protective drugs in modern medical practices, the medicinal values of pteridophytes against bacteria, fungi, virus, cancers, rheumatism, diabetes, inflammation, consultant fertility diuretic pesticides and sedative have been reported. Besides sugars, starch, proteins, and aminoacids; ferns contain variety of alkaloids, glycosides, flavinoids, terpinoids, sterols, phenols, sesquitorpens etc. as potential components used in various industries (**Kulandairaj and John de Britto, 2000)**.[4] Young leaves of ferns *Diplazium esculentum* are cooked as vegetables by the tribes in India and Philippines (**Yumkham and Singh, 2011;**[5] **Sen and Ghosh, 2011)**.[6] Scientists revealed the anthelmintic property of *Diplazium esculentum* and studied the antimicrobial property of different diplazium species against the poultry pathogens. With this knowledge, the present investigations were under taken to explore the phyto-constituents of Diplazium esculentum for further studies on different properties. Diverse homeostatic mechanisms are affected if liver function is impaired, with potentially serious consequences. About 20,000 deaths occur every year due to liver diseases. Hepatocellular carcinoma is one of the ten most common tumours in the world with over 2, 50,000 new cases each year. Although viruses are the main cause of liver diseases, excessive drug therapy, environmental pollution and alcoholic intoxication are not uncommon. Plants play an important role in the management of various liver disorders. A number of plants have shown hepatoprotective property (Scott Luper 1998). Developing therapeutically effective formulation from natural products may reduce the risk of toxicity when the drug is used clinically and can give the benefit of synergetic effect of many medicinal plants. Therefore, considerable efforts to formulate useful plant medicines from documented medicinal plants for hepatoprotection are successfully made and some plant formulations are marketed under various brand names. The aim of the present study is to evaluate hepatoprotective activity of one such plant formulation of pakoonoral administration in albino rats using chloroform as hepatotoxic agents. These compounds induce hepatotoxicity by various mechanisms. Plants and natural products have been used traditionally worldwide for the prevention and treatment of liver disease. Scientific research has supported the claims of the medicinal efficacy of several of these herbal compounds, as evidenced from the voluminous work on their hepatoprotective potentials. Hence, further study must be conducted.

2. Aims And Objectives

- The study is aimed to determine the hepatoprotective property of pako leaves extract (*Diplazium esculentum*) in Swiss albino rats.
- And also compare the effect of different rates of fiddle fern (*Diplazium esculentum)* on the total protein level and SGPT levels of rats with previous intake of chloroform liver toxicant.

3. Materials And Methods

The in-vivo hepatoprotective effect of the aqueous extract of *Diplazium esculentum (Retz.) Sw.* against chloroform induced hepatotoxicity in albino rats was studied. The test rats were brought from PITAHC-DOH animal facility, the plant extraction was carried out at ROCO building, Cagayan State University (CSU) Carig Campus, College of Arts and Science, Carig Sur, Tuguegarao city, and the test was conducted at the PITAHC-DOH animal facility, after obtaining clearance from Institutional Ethics Committee.

Inclusion Criteria

The study which involves extraction and evaluation of its hepatoprotective activity was done employing the completely randomized design.

Exclusion Criteria

Twenty-five (25) albino rats were assigned to different treatments with 5 replicates as follows:

- Distilled water
- Essential forte (300 mg/kg)
- Chloroform (0.5ml/kg) control group
- Fiddle fern extract (20 g/kg daily)
- Fiddle fern extract (40 g/kg daily)

Plant Collection

Diplazium esculentum was collected from Don Domingo Public Market. The plant was taxonomically identified and confirmed by the National Museum, Manila Philippines.

Preparation of Plant Extract

The aqueous extraction of the plant was prepared by collecting leaves which is washed thoroughly with distilled water and the leaves of pako were naturally air dried until all moisture is removed. Ujowundu CO, Igwe CU, Eremor VA, Nwaogwu LA, Okafor OE. Nutritive and anti-nutritive properties of *Boerhaviadiffusa* and *Commelinanucliflora* leaves. Pak J Nutr. 2008;7(1):90–2).[7] 100 gms of dried leaves are boiled in 1000 ml of distilled water (Okonogi, et al., 2007) for 30 mins and the extract is collected. The collected extract is again boiled until we get 100 ml of final extract (Nurliyana, et al., 2010). Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin Phenol reagents. J Biol Chem. 1951;193:265–75. PubMed).[8] The final extract is collected in a conical flask and stored in the refrigerator.

Acclimatization of the animals

Twenty-five (25) albino rats weighing (150 - 230g) were obtained from the Cagayan Valley Herbal Processing Center, Tuguegarao city. The animals were acclimatized under standard laboratory conditions (relatively 55 – 65 %, room temperature 23.0 ± 2.0 C and 12 h light: dark cycle). The animals were fed with standard diet and water as libitum. Research permit was secured prior to the conduct of the study from the Bureau of Animal Industry. Department of Agriculture Region 02.

Induction of liver damage with chloroform

Liver damage was induced using chloroform at dose rate of 0.5 ml/kg (CHEMTREC USA, 2009). Using one (1) ml sterile syringe and gavage needle, the chloroform was administered orally and introduced directly on the gastrointestinal tract. (6Imray, J. et al. (2007).[6] Effect of brief administration of chloroform on the liver anaesthesia. Vol 19 issue 01.)

Administration of essential forte

The commercial medicine was administered to test animals by gavage. The phospholipids in capsule were withdrawn using sterilized toothpick and placed into a sterilized petri dish. The phospholipids were dissolved in palm oil in 300 mg with 2 ml oil. Using one (1) ml sterile syringe and gavage needle, the essential forte was administered orally as before (Sook Yee Hor., et al, 2012).

Administration of *Diplazium esculentum* extract

The extract of *Diplazium esculentum* leaves was administered orally to rats at 20 g/kg and 40 g/kg for 3 days (Sook Yee Hor., et al, 2012). Using a ten (10) ml of sterile syringe and a gavage needle, the extract was directly administered orally.

Blood collection

Blood sample are collected after giving chloroform shock. Blood is collected through the cardiac puncture technique and analysed for the liver function enzymes of total protein and SGPT levels using Bio system automated analyser at Cagayan State University, college of veterinary medicine.

Statistical Methods

The data for mean and standard deviation was analysed using graph pad stat software using one-way analysis of variance (ANOVA). 0.05 level of significance.

4. Results

Table 1:

Treatments		Total Protein N.V 3.5-7.5g/dl	SGPTN.V 35-80 u/l	Remarks
T1.	1	16.7	83	
	2	145.	87	
	3	15.2	162	
	4	15.9	127	
T2	1	14.3	81.7	
	2	14.1	78.7	
	3	13.8	86.7	
	4	16.2	64.3	
	5	14	92.3	
T3	1	**15.5**		Insufficient sample
	2			Dead
	3			Dead
	4			Dead
	5			Dead
T4	1			Failed blood collection
	2	14.5	129	
	3	14.2	125.7	
	4	13.4	127	
	5	16.1	127.3	
T5	1	13.2		Insufficient sample
	2	13.6	101.3	
	3	13.9	110.3	
	4	12.5	116.7	

Total Protein and SGPT of Rats After Different Rates of Pako Extract

Treatment	Treatment Mean	Statistical Significance	Rank
T1-Negative Control	**15.58**	a	1
T2-Positive Control	**14.05**	bc	3
T4-20 g/kg Pako Extract	**14.55**	ab	2
T5-50 g/kg Pako Extract	**13.30**	c	4

Note: Any two means having a common letter are not significantly different at 5 % level;

Bold face means significantly different at 1 % level to the preceding letter.

With respect to the mean total protein in general, there was no significant mean difference between the treatments with the increase in dose rate of Fiddle fern extract given to the animals (P = 0.338),

Table 2:

Total Protein (g/dl)							
Treatment	**R1**	**R2**	**R3**	**R4**	**Total**	**Mean**	**Rank**
T1-Negative Control	16.7	14.5	15.2	15.9	**62.30**	**15.575**	1
T2-Positive Control	14.3	14.1	13.8	14.0	**56.20**	**14.050**	3
T4-20 g/kg Pako Extract	14.5	14.2	13.4	16.1	**58.20**	**14.550**	2
T5-50 g/kg Pako Extract	13.2	13.6	13.9	12.5	**53.20**	**13.300**	4
Total	**58.70**	**56.40**	**56.30**	**58.50**	**229.90**	**57.48**	
Mean	**14.68**	**14.10**	**14.08**	**14.63**	**57.48**	**14.37**	
CRD-ANOVA							
					Tabular F		

Source of Variation	df	Sum of Squares	Mean Square	Observed F	5 %	1 %	
Treatment	3	10.927	3.64229	5.64	3.49	5.96	s
Experimental Error	12	7.7475	0.645625				
Total	15	18.67438					
					cv	5.5921	
ANOVA of the Mean Total Protein Levels of Rats							
LSD							
Treatment	**Treatment Mean**		**Statistical Significance**			**Rank**	
T1-Negative Control	**15.58**		a			1	
T2-Positive Control	**14.05**		bc			3	
T4-20 g/kg Pako Extract	**14.55**		ab			2	
T5-50 g/kg Pako Extract	**13.30**		c			4	
Note: Any two means having a common letter are not significantly different at 5 % level;							
Bold face means significantly different at 1 % level to the preceding letter.							

Table 3:

SGPT (u/l)						
Treatment	**R1**	**R2**	**R3**	**Total**	**Mean**	**Rank**
T1-Negative Control	87.0	83.0	127.0	**297.00**	**99.000**	3
T2-Positive Control	78.7	86.7	81.7	**247.10**	**82.367**	4
T4-20 g/kg Pako Extract	129.0	125.7	127.0	**381.70**	**127.233**	1
T5-50 g/kg Pako Extract	101.3	110.3	116.7	**328.30**	**109.433**	2
Total	**396.00**	**405.70**	**452.40**	**1254.10**	**418.03**	
Mean	**99.00**	**101.43**	**113.10**	**313.53**	**104.51**	
CRD-ANOVA						
					Tabular F	
Source of Variation	**df**	**Sum of Squares**	**Mean Square**	**Observed F**	**5 %**	**1 %**
Treatment	3	3183.829	1061.27639	6.33	4.07	7.59S
Experimental Error	8	1341.9000	167.737500			
Total	11	4525.72917				
					cv	12.3926
ANOVA of the Mean SGPT Levels of Rats						
LSD						
Treatment	**Treatment Mean**		**Statistical Significance**		**Rank**	
T1-Negative Control	**99.0000**		bc		3	
T2-Positive Control	**82.3667**		c		4	
T4-20 g/kg Pako Extract	**127.2333**		a		1	
T5-50 g/kg Pako Extract	**109.4333**		ab		2	
Note: Any two means having a common letter are not significantly different at 5 % level;						
Bold face means significantly different at 1 % level to the preceding letter.						

Significant differences were observed for the SGPT between the control group (T2) and the group treated with 20 g/kg extract (P = 0.024). There were no significant differences detected for the (T1) negative control group with T4-extract with 40 g/kg and T2-treated with essential

5. discussion

T3 (chloroform medicated rats were disregarded from the group as they all died due to liver damage because of chloroform. No blood samples were obtained for analysis).

The hazards of chloroform anaesthesia to liver damage were assessed with rise of SGPT in test animals and the immediate death to the chloroform administered animals. The present findings were opposed to the previous report in man anesthetized with chloroform without evidence of liver damage after 24 hours (Imray, J. et al. 2007).[9] The evident death of rats, 2 hours after oral administration of chloroform suggests toxicity effect which was deadly to rats and the reversal of this toxic effect with extract administration has prevented possible death. The protective effect of both the essentials, a phospholipid and vitamin formula has been observed with similar effect with the fiddlehead fern extract administration.

6. Conclusion

We conclude therefore that 40 g/kg of the fiddlehead fern extract prevents liver damage comparably similar those with essential and the normal control group. We also conclude that death was prevented with administration of the extract 20 and 40 g/kg.

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