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THE HEPATOPROTECTIVE ACTIVITY OF *ALSTONIA MACROPHYLLA* G. DON (APOCYNACEAE) AGAINST PARACETAMOL-INDUCED LIVER TOXICITY

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ABSTRACT

To evaluate the hepatoprotective activity of crude ethanolic leaf extract of *Alstonia macrophylla* G. Don in Paracetamol induced hepatotoxicity in Sprague Dawley rats. Hepatotoxicity was established by induction of Paracetamol 500mg/kg leading to elevated liver markers, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alanine phosphatase (ALP), and decreased Albumin levels. Single dose administration with *Alstonia macrophylla* crude extract (AmCE) on a 7-day duration treatment, resulted in decreased AST, ALT, and ALP and increased Albumin levels. Specifically the AmCE 600mg/kg resulted to significant effects at ($p<0.01$). Similarly, depleted levels of Glutathione, and Catalase levels were increased ($p<0.003$), ($p<0.000$), respectively. While Malondialdehyde – a measure of lipid peroxidation, was significantly decreased ($p<0.000$). Among the treatments, AmCE 600mg dose showed comparable effect with the standard drug N-acetylcysteine. This study demonstrated the hepatoprotective activity of the crude ethanolic leaf extract of *Alstonia macrophylla*.

Keywords: *Alstonia macrophylla*, Hepatoprotective, Liver toxicity, Paracetamol

INTRODUCTION

The liver is the fundamental organ for metabolism and is vital for maintaining normal body function. It aids nutrient absorption and importantly facilitates detoxification of our body. However, abused to the liver caused by exposure to environmental toxins, poor eating habits, alcohol and over-used of drugs, will lead to different liver diseases such as hepatitis, cirrhosis, alcoholic liver disease and hepatotoxicity [1, 2]. Liver diseases remain a serious health problem worldwide and oxidative stress plays a vital role in initiation and advancement of liver diseases [3]. Drug-induced hepatotoxicity is one of the most common forms of liver disease [4]. This is associated with an overload of hepatotoxicants, which are compounds that may include excessive doses of certain medicinal drugs. Paracetamol is a common over-the-counter drug worldwide that when taken in excess is deemed to be hepatotoxic. It produces a toxic metabolite, thus initiates oxidative stress, leading to hepatotoxicity [4, 5]. N-acetylcysteine (NAC), a derivative of amino acid cysteine, is a potent antioxidant, and the antidote of choice for Paracetamol toxicity [6]. It converts and synthesizes glutathione by producing more beneficial levels. But drug options are limited for the treatment of liver disorders, hence, the use of medicinal plants with high level of antioxidant constituents has been proposed as an effective therapeutic approach for hepatic damages [7, 8].

Alstonia macrophylla, a native plant in the Philippines, has been traditionally used in the treatment of diabetes, malaria, ulcers and liver diseases [9]. However, *A.macrophylla* has not been investigated for its potential *in vivo* hepatoprotective activity, hence, this study was done, focusing on the hepatoprotective activity using oxidative biomarkers such as Glutathione (GSH), Catalase (CAT) and Lipid peroxidation, Serum hepato-specific biomarkers including Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), and Albumin.

MATERIALS AND METHODS

Animals: A total of thirty (30) female Sprague-dawley rats were used in the experiment, approved by The University of Santo Tomas Institute of Animal Care and Use Committee (UST-IACUC) and was purchased from the Food and Drug Administration (FDA), certified by the Bureau of Animal Industry. The animals were kept at room temperature of 25±2^{o}C with 70% humidity and 12/12 hours of light to darkness ratio were provided for 1 week as part of the acclimatization period. Standard rodent pellet and water *ad libitum* were given to the test animals.

Paracetamol-induced liver toxicity: Rats were divided into six groups (5 rats / group) and were assigned with the following doses as shown in table 1. The Paracetamol was administered simultaneously with the treatments, the toxicant and the treatments were given four (4) hours apart through oral gavage, once daily for seven (7) days following the described protocol with minor modification. [10]

The animals were sacrificed 24 hours after the last treatment via cervical dislocation. Blood was collected and serum was separated at 10,000 rpm for 15 minutes. Liver excised from each group were used for histopathological studies and for the preparation of liver homogenates.

Liver Function Tests: Several clinical biomarkers were used in detection of liver toxicity such as, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and Albumin [11]. Standard commercial kits were used in the assays and were measured using Corona microplate reader SH-1000 (Hitachi, Japan).

Estimation of Liver's antioxidant capacity: The liver homogenates (10% w/v) were prepared from the samples using 50mM potassium phosphate buffer and a homogenizer. Moreover, the homogenates were subjected to centrifugation at 10,000 rpm x 15 minutes.

The supernatant obtained were used in determination of Malondialdehyde (MDA) a level of lipid peroxidation [12], Glutathione using 5, 5-Dithiobis-2nitrobenzoic acid (Ellman's reagent) [13] and Catalase levels [14]. All assays were done using Corona microplate reader SH-1000 (Hitachi, Japan).

Liver Index: The liver index is the ratio of the liver weight relative to the body weight and an indication of Paracetamol toxicity. Substantial changes observed in the liver weight with increase liver hemoglobin, decreased intrahepatic, portal vein pressure, and sinusoidal congestions accompanied with red blood cells are manifestations of toxicity and are computed as follows[15].

$$\text{Liver Index} = \frac{\text{Weight of excised liver (g)}}{\text{Body weight (g)}} \times 100$$

Histopathological Studies: Histopathological analysis and interpretation was done by a licensed veterinary pathologist. The liver was excised and fixed in 10% buffered formalin. Sections were prepared and stained with hematoxylin and eosin (H-E) dye for microscopic observation, including necrosis, hepatocyte swelling, fatty changes.

Statistical Analysis: Results were analyzed using SPSS version 21. Data were reported as mean ± SD and $p<0.05$ was statistically significant at 95% confidence. One-way ANOVA was used to measure the mean differences between treatment groups and Duncan test determined the variant groups.

RESULTS AND DISCUSSIONS

Serum biochemical markers: The Results of liver function tests are shown in Table 2. The Paracetamol significantly increased the levels of ALT, AST, ALP and decreased Albumin production in all treatment groups, indicating toxicity. The NAC and AmCE 600mg/kg among the treatment groups, had significantly decreased the ALT, AST, ALP and elevating albumin levels.

Oxidative stress parameters: The results of the following assay showed that Paracetamol significantly increased the lipid peroxidation as measured through Malondialdehyde, and a decreased in Glutathione and Catalase levels. Treatment with AmCE, reduced the levels of Lipid peroxidation, replenished GSH levels and improved catalase level. The crude extract has potentially shown its hepatoprotection capacity (Table 3).

Liver index: Table 4 below represents the mean liver index for the different treatment groups. Data shows that the Paracetamol group has the highest liver index among all groups followed by AmCE 200mg/kg, Normal control and AmCE 400mg/kg. While the NAC group (positive control) has the lowest mean liver index followed by AmCE 600mg/kg.

Histopathological Analysis: The results of the histopathological examination on the liver sections of the control group, NAC, AmCE 400mg and 600mg treatment groups showed normal hepatic cells with

observable well-preserved cytoplasm. Moreover, normal liver architecture of hepatocytes and central vein is not altered with well-defined hepatic cell lining of the liver tissues. The liver section of the Paracetamol group showed an increase liver weight, supported by the liver index measure, fatty change, necrosis, swelling of hepatocytes and presence of kupffer cells. While the normal group and AmCE 200mg/kg showed essentially normal lobular pattern with mild degree of fatty change, necrosis and infiltration of lymphocyte (Fig.1).

CONCLUSION

The crude extract from the leaves of *Alstonia macrophylla* showed strong potential hepatoprotective activity specifically at a dose of 600mg/kg which is significantly comparable with the clinical standard antidote, N-acetylcysteine.

CONFLICT OF INTEREST

The authors report no conflicts of interest in this work

Table 1. Treatment protocol used in the hepatoprotective assay

Group Description	Intervention Assignment
1. Normal Control	Distilled water
2. Paracetamol (Toxicant)	Paracetamol 500mg/kg/BW
3. Toxicant + Positive Control	Paracetamol 500mg/kg/BW +
4. Toxicant + Crude Extract	Paracetamol 500mg/kg/BW + AmCE 200mg
5. Toxicant + Crude Extract	Paracetamol 500mg/kg/BW + AmCE 400mg
6. Toxicant + Crude Extract	Paracetamol 500mg/kg/BW + AmCE 600mg/kg

Table 2. Effect of Alstonia macrophylla crude extracts on liver functions tests

Group	Treatment	ALT (U/L)	AST (U/L)	ALP (U/L)	ALBUMIN (G/L)
1	Normal Control	28.268 ± 1.712*	97.767 ± 3.315*	181.604 ± 3.042*	4.699 ± 5.143*
2	Paracetamol 500mg/kg (Toxicant)	98.114 ± 2.475**	208.473 ± 47.773*	245.470 ± 22.618**	245.470 ± 2.122**
3	Toxicant + NAC 100mg/kg	14.482 ± 0.399**	68.617 ± 9.844**	134.458 ± 11.779**	9.113 ± 0.597**
4	Toxicant AmCE 200mg/kg	60.277 ± 5.403*	122.702 ± 46.089*	195.068 ± 9.291*	2.435 ± 1.699*
5	Toxicant + AmCE 400mg/kg	31.512 ± 0.774*	111.773 ± 12.949*	168.496 ± 7.498*	3.930 ± 2.633*
6	Toxicant + AmCE 600mg/kg	20.884 ± 0.585**	83.375 ± 2.626**	149.920 ± 7.272**	8.348 ± 0.301**

** indicates non-significant difference (P > 0.05);** indicates significant difference (P < 0.05)*

Table 3. Measure of liver's antioxidant function

Group	Treatment	Reduced GSH	CAT	MDA
1	Normal Control	3.276 ± 0.522*	28.291 ± 10.574 **	0.027 ± 0.0123*
2	Paracetamol 500mg/kg (Toxicant)	2.190 ± 0.455**	24.016 ± 3.576 **	0.367 ± 0.301**
3	Toxicant + NAC 100mg/kg	5.882 ± 0.679**	51.407 ± 16.897 **	0.012 ± 0.008**
4	Toxicant + AmCE 200mg/kg	1.414 ± 0.817*	32.403 ± 6.710 **	0.252 ± 0.179*
5	Toxicant + AmCE 400mg/kg	2.710 ± 0.6310*	36.259 ± 3.606 *	0.137 ± 0.099*
6	Toxicant + AmCE 600mg/kg	5.384 ± 2.288**	47.483 ± 3.998 **	0.042 ± 0.017**

**indicates non-significant difference (P > 0.05); **indicates significant difference (P < 0.05)*

Table 4. Mean liver index of each group

Treatment Groups	**Mean ± SD**
Normal Control	2.16 ± 0.71
Paracetamol 500mg/kg (Toxicant)	3.00 ± 0.32
N-acetylcysteine 100mg/kg (positive control)	2.06 ± 0.67
Toxicant + AmCE 200mg/kg	2.40 ± 0.18
Toxicant + AmCE 400mg/kg	2.08 ± 0.54
Toxicant + AmCE 600mg/kg	2.03 ± 0.23
Significance within groups $p<0.028$	

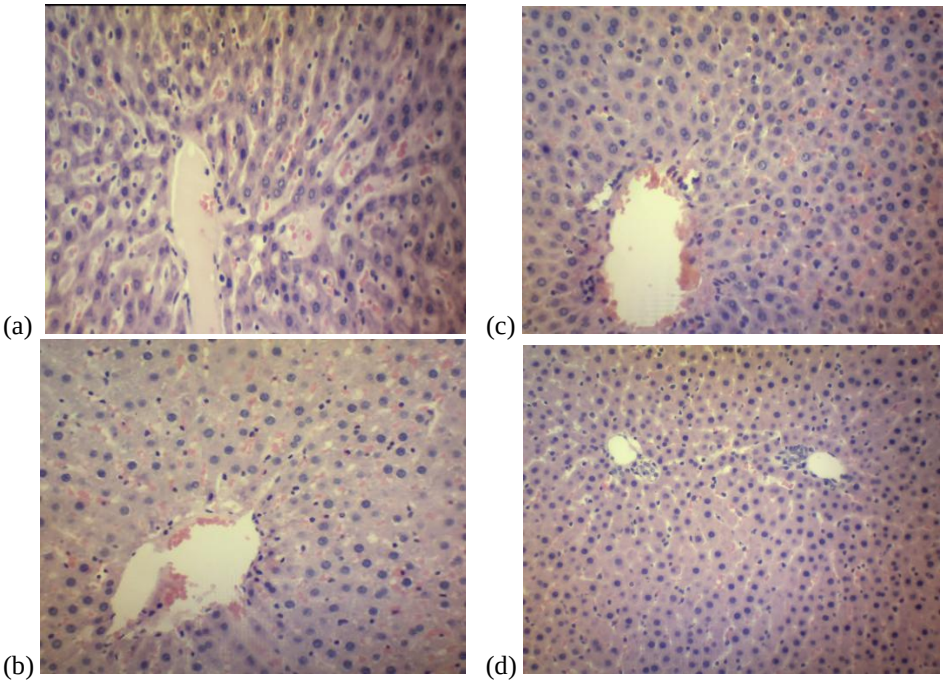

Figure 1. (a) Liver section of Paracetamol treated rats showing necrosis, hepatocyte swelling and presence of kupffer cells (b) liver sections from AmCE 600mg/kg, (c) N-acetylcysteine and (d) AmCE 400mg/kg has well preserved cytoplasm and central vein with no remarkable lesions noted.

REFERENCES

1. Treadway, Scott. Clinical Nutrition Insights (1998); 6(16): 1-3.
2. Kumar E, Harsha KN, Shaik S, Neelakanta Rao N, Giri Babu N. IOSR Journal of Pharmacy 2013; 3(1): 10-15.
3. Akanitapichat P, Phraibung K, Nuchklang K, Prompitakkul S. Food and Chemical Toxicology (2010); 48: 3017-3021.
4. Parmar SR, Vashrambhai PH, Kalia K. Journal of Herbal Medicine and Toxicology 2010; 4(2): 101-106
5. Heard KJ. N Engl J Med 2008; 359(3): 285–292
6. Laifer, S. Life Extension Magazine. 2004, http://www.lef.org
7. Al-Fartosi,KG., Khuon, OS., Al-Tae, HI. International Journal of Research in Pharmaceutical and Biomedical Sciences 2011; 2(4): 1795-1799
8. Ishola IO, Akinyede AA, Robert AK, Omilabu SA. DrugRes (2014); 64: 1-10
9. Khyade, M. S., Kasote, D. M., & Vaikos, N. P. Journal of Ethnopharmacology 2014; 153: 1-18.
10. Sabir SM, Ahmad SD, Hamid A, Khan MQ, Athayde ML, Santos DB, Boligon AA, Rocha BJT. Food Chemistry 2012; 131: 741-747
11. Singh, A., Bhat, T., & Sharma, O. Journal of Clinical Toxicology, 2011; 4:1-19
12. Nielsen F, Mikkelsen BB, Nielsen JB, Andersen HR, Grandjean P. Clinical Chemistry 1997. 43(7): 1209–1214
13. Saeed M, Amen A, Fahmi A, Garawani IE, Sayed S. Journal of Applied Pharmaceutical Science 2012, 4(12): 044-051.
14. Aebi, H.E. Catalase. In: Methods of Enzymatic Analysis. In: Bergmeyer, H.U. Verlag Chemie, Weinhem. 3rd ed., 1983, 273-286
15. Hinson, J. A., Roberts, D., & James, L. Handb Exp Pharmacol, 2010; 196: 369-405