



IN VITRO EFFECT OF AQUEOUS *CALOTROPIS PROCERA* ROOT EXTRACT ON AMMONIUM SULPHATE PRECIPITATED LIVER MARKER ENZYMES OF ALBINO RATS

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ABSTRACT

The in vitro effect of aqueous root extract of C. procera on liver marker enzymes; alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) of albino rats was evaluated. ALT was precipitated at 40% ammonium sulphate saturation whilst AST and ALP were precipitated at 35 % saturation from rat liver homogenate. The enzymes were assayed at varying concentrations (mg/ml) of the extract (0.00, 0.05, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35, 0.40, 0.45, 0.50) at 37°C. At 0.00µg/ml extract; ALT, AST and ALP activities of 5.91×10^4 , 2.70×10^4 and 3.74×10^5 µmol/min respectively. Upon incubation with extract, the enzymes had respective mean activities of $6.38 \pm 0.35 \times 10^4$, $4.07 \pm 0.62 \times 10^4$ and $2.80 \pm 0.44 \times 10^5$ µmol/min. The activities of ALT and AST were significantly increased ($P < 0.05$) in presence of C. procera extract with significant decrease ($P < 0.05$) in the activity of ALP. It indicates that the aqueous root extract of C. procera activated ALT and AST and inhibited ALP in vitro.

Keywords: *in vitro*, C. procera, liver marker enzymes, ammonium sulphate

INTRODUCTION

Calotropis procera is a species of flowering plant natured to North Africa, Tropical Africa, Western Asia and Indochina. It is commonly known as dead Sea fruit, desert wick plant milkweed Swallow-worth, "Tumfafiya" and Apple of Sodom (Ahmad and Beg, 1993; Trulat, 1997). It is a shrub or small tree reaching 2.5-6m in height, stems usually simple rarely branch woody at base and covered with a tissue corky bark. All part of the plant produces white latex when cut or broken, a toxic milky sap that is extremely bitter and turn into a gluelly coating resistant to soap (Hussein *et al.*; 1994).

Jam *et al* (1996) reported that *C. procera* was used in traditional medicine as a purgative, anthelmintic, anticoagulant, anticancer as well as antipyretic, analgesic and antimicrobial. Fluerentin and Pelt (1982) also reported that the plant was used as an antiseptic for skin infection. Several studies have been carried out on the effect of various extract of *C. procera* on different organs of animals (Al-Robalet *al.*; 199; Jam *et al.*, 1996; Basuet *al.*, 1997). *Calotropis procera* latex affords protection against Carbon Tetrachloride (CCl₄) induced hepatotoxicity in rats (Padhyet *al.*, 2007). Alhassan *et al* (2012) demonstrated the curative effect of aqueous root extract of *C. procera* on CCl₄ induced hepatotoxicity in rats. The root of *C. procera* was found to contain the chemicals; benzoylisolineoline, benzoyloneolone, isoloneolone and lineolone (Parrotta, 2011). The root bark is used to treat a variety of illnesses including leprosy, fever, menorrhagia, malaria, and snake bite (Parrotta, 2001).

Hepatic necrosis is associated with plasma increase of non-plasma specific enzymes; alanine aminotransferase (ALT), aspartate aminotransferase

(AST) and alkaline phosphatase, among others (Henderson and Moss, 2001; Alhassan *et al.*, 2012). Reversal of such increase was adopted by researchers as criteria of proving hepatocurative effect of acclaimed antihepatitis agents (Al-Robalet *al.* 1993; Alhassan *et al.*, 2012). However; neither *in vivo* nor *in vitro* documented information on the effect of such medicaments on the activities of these marker enzymes has been reported. This research work is to evaluate *in vitro* effect of aqueous root extract of *C. procera* on ALT, AST and ALP of albino rat liver.

MATERIAL AND METHODS

Collection and preparation of root extract

Root of *C. procera* was obtained from Biological Sciences garden Bayero University, Kano. The root of *Calotropis procera* was allowed to dry under the shade, it was then pulverized using mortar and pestle. The extract was prepared by weighing and soaking the root powder in water, then filtered and adjusted to concentration of 10.0g/cm³.

Liver tissue homogenate and enzyme pellet preparation

Albino rats were sacrificed by decapitation; the liver was removed and placed in cold 0.25M sucrose solution. The liver samples were blotted free from excess sucrose solution and were homogenized in a volume of 0.25M sucrose equal to 9 times their weight for ALT and AST isolation. While for ALP precipitation, the rats' liver was homogenized in 0.25M Tris buffer. All procedures were performed at 4°C. The homogenized tissue was filtered through 2 layers of cheese cloth before centrifugation at 17,000g for 15 minutes.

The crude tissue homogenate was placed in a beaker with magnetic stir bar and made to 40% ammonium sulphate saturation for ALT precipitation and to 35% for AST and ALP precipitation. The preparation was centrifuged at 17,000g for 15minutes. The enzyme precipitate (pellet) was kept and re-suspended in a respective buffer for subsequent analysis.

Effect of *C. procera* root Extract on Enzyme Activity

For each marker enzyme assay eleven triplicates test tubes (33) were labeled (I, II – XI), into each tube 1.0cm³ of enzyme substrate was pipetted for ALT and AST, 0.5cm³ substrate for ALP. Volumes of extract (0.00, 0.05, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35, 0.40, 0.45, 0.50 cm³) were serially pipetted to the test tubes and incubated at 37° for 10minutes. To the pre-warmed substrate 0.05ALT, AST and ALP re-suspended pellets were pipettes to their respective tubes and incubated at 37°C for 30 minutes. At the end of incubation, colour reagent was added and the absorbance recorded at 340nm for ALT and AST and at 450nm for ALP according to Ramnik (1999). The

enzyme activity was calculated using standard calibration plot.

Statistical analysis

The data was statistically analyzed using one – way Analysis of Variance (ANOVA) with P value < 0.05 considered significant, using a component of GraphPad Instat3 Software (2000) version 3.05 by GraphPad Inc.

RESULTS AND DISCUSSION

The result obtained (Table 1) show the activities of ammonium sulphate precipitated liver marker enzymes in the presence of aqueous root extract of *C. procera*. Unpaired t - test of mean values for ALT specific activities of the test in presence of different concentration of aqueous root extract of *C. procera* significantly increased with two-tailed value P < 0.05 (0.0182) compared to control experiment. ASTmean values of the test significantly increased with two-tailed value P< 0.05 (0.0011) compared to the control experiment. While unpaired t test of the ALPmean values of the test significantly decreased with two-tailed value P < 0.05 (0.0014).

Table 1: *In vitro* specific activities of liver marker enzymes incubated with aqueous root extract of *C. procera*

Extract (10mg cm ⁻³) cm ³	ALT × 10 ⁻⁴ μmol/min	AST × 10 ⁻⁴ μmol/min	ALP × 10 ⁻⁵ μmol/min
0.00	5.91 ± 0.06	2.70 ± 0.04	3.74 ± 0.12
0.05	6.25 ± 0.13	3.06 ± 0.08	3.72 ± 0.11
0.10	5.78 ± 0.35	2.89 ± 0.26	3.28 ± 0.23
0.15	5.91 ± 0.28	3.24 ± 0.13	3.60 ± 0.14
0.20	6.38 ± 0.09	4.18 ± 0.08	2.75 ± 0.16
0.25	5.85 ± 0.22	4.16 ± 0.15	2.65 ± 0.09
0.30	6.38 ± 0.13	4.00 ± 0.06	1.85 ± 0.02
0.35	6.65 ± 0.05	5.12 ± 0.08	2.55 ± 0.08
0.40	6.52 ± 0.06	5.04 ± 0.09	2.45 ± 0.16
0.45	6.45 ± 0.08	4.07 ± 0.11	2.35 ± 0.14
0.50	7.66 ± 0.16	4.94 ± 0.08	2.75 ± 0.06
Mean ± SD	6.38 ± 0.35	4.07 ± 0.62	2.80 ± 0.44

The mean values for ALT and AST activities of the control experiment were significantly lowered compared to the test incubated with the aqueous root extract of *C. procera* (p<0.05), this may indicates *in vitro* stimulatory effect of the extract on the specific activity of the two liver marker enzymes. The finding of this research work substantiates the reported hepatoprotective/hepatocurative effect of *C. procera* documented by Padhyet al (2007); Alhassan et al (2012) and many others by following serum level of these liver maker enzymes (ALT and AST) of CCl₄ induced hepatotoxicity rats treated with the plant extract. The reported activity lowering effect (Padhyet al., 2007; Alhassan et al.,2012) could not be by direct *in vivo* inhibitory effect, rather is on the healing effect of the extract on the hepatocytes, thereby reducing/preventing the release of the enzymes into the serum. The observed *in vitro* stimulatory effect of

the extract on the activity of ALT and AST could be associated with the photochemicalsand/or elemental content of the plant reported by Parrotta (2001) and Khanzadaet al (2008) respectively. The mean values for ALP specific activity of the control was significantly higher (p<0.05) compared with test, it shows *in vitro* inhibitory effect of the extract. This could be associated with either chemical contents or pH altering effect of the extract.

CONCLUSION

Conclusively this research work substantiates the reported hepatocurative of *C. procera* that the observed decrease serum activities of ALT and AST is not due to inhibitory effect but could be due to decreased release of these marker enzymes from damage hepatocytes by promoting the healing of the damaged hepatocytes.

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