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Effects of the ethanol root bark extract of *Cleistopholis patens* on the antioxidant status of doxorubicin-induced myocardial infarction in experimental rats

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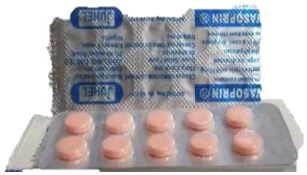
Graphical abstract

Crude extract

Cleistopholis patens root bark

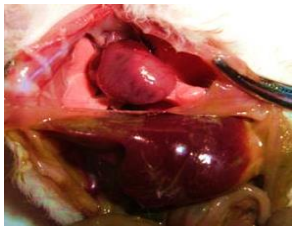
Experimental rat



Standard drug

Blood serum

Biochemical assay



Rat's heart

Doxorubicin

Histopathological examination

Abstract: Doxorubicin (DOX), a very active chemotherapeutic agent, has been implicated in the development of myocardial infarction. Oxidative stress is said to be one of the underlying mechanisms through which doxorubicin causes cardiotoxicity. This study evaluated the effect of the ethanol root bark extract of *Cleistopholis patens* against DOX-induced myocardial infarction in rats. Albino rats were randomly divided into six groups of five rats each. Groups I, II and III served as normal, positive and standard (Vasoprin 150 mg/kg bw) controls respectively while groups IV, V and VI were orally treated with the extract (200, 400 and 600 mg/kg bw) for two weeks prior to intraperitoneal induction of cardiotoxicity with Doxorubicin (20 mg/kg bw) on day 14. Biochemical indices such as cardiac troponin I (cTnI), malondialdehyde (MDA), glutathione (GSH) levels, superoxide dismutase (SOD), catalase (CAT), aspartate transaminase (AST) and alanine transaminase (ALT) were assayed for and histopathological examination of heart tissues was performed to validate results.
Results: Myocardial infarction was confirmed by significant changes ($p < 0.05$) in cTnI, MDA, GSH levels and SOD, CAT, AST, ALT activities in the DOX control group compared to the normal control. However, administration of the extract significantly ($p < 0.05$) restored all the biochemical alterations and reversed the histomorphological changes in the heart. The ethanol root bark extract of *Cleistopholis patens* demonstrated promising cardioprotective effect and can be exploited in the synthesis of novel therapeutic agents.

Keywords: Antioxidant; Cleistopholis patens; doxorubicin; myocardial infarction; oxidative stress.

Introduction

- Myocardial infarction, also known as heart attack is caused by low or no blood flow to a portion of the myocardium.
- In most cases, myocardial infarction has resulted from an underlying coronary artery disease.
- In some other cases, some chemotherapeutic agents like doxorubicin (DOX) has brought about onset of the disease.
- The generation of reactive oxygen species (ROS) and oxidative stress is observed as a result of myocardial injury.

- Over the years, there has been a radical shift in therapy from passive healing of the infarction through weeks of bed rest to early discharge, usually within 2-3 days as a result of immediate reperfusion strategies and other guideline-directed medical therapies.

- Plants have been shown to have medicinal properties, the reason why they are medicinal plants.

- *Cleistopholis patens* (Family: Annonaceae), a fast growing plant found in Africa has been observed to possess some medicinal properties.

Methods

- Plant material was collected and processed to obtain crude extract
- Crude extract was gotten through cold maceration
- Acute toxicity studies was carried out by the Lorke model, 1983.
- Qualitative and quantitative analysis were done according to Harbone, 1973; Trease and Evans, 1983
- Standard control and test groups were pre-treated with the extract prior to all groups being induced with DOX
- All experimental animals were sacrificed after induction and serum obtained was assayed for some biochemical indices

- The assay for Cardiac Troponin I was carried according to standardized methods on the ELISA kit
- The antioxidant parameters were assayed for, using standard methods as contained in the Randox kit
- ALT and AST were assayed for, using standard methods as contained in the Randox kit
- Histopathological examination was done according to Drury *et al*., 1976
- Statistical Analysis was done with the IBM Statistical Product and Service Solution (SPSS) version 21

Results and discussion

- Acute toxicity studies revealed that the crude extract had no lethal dosage, up to 5000 mg/kg body weight
- Phytochemical analysis showed the presence of steroids, terpenoids, phenols, flavonoids, alkaloids, glycosides, tannins and reducing sugar, in decreasing quantities
- Results of measurement of biochemical indices brought to the spotlight, significant changes ($p < 0.05$) in the levels of cTnI, MDA in the positive control group, in comparison with the normal control.
- Also, the activities of SOD and CAT were significantly altered ($p < 0.05$) in groups 2, when put side by side with group 1
- The same trend was observed for the GSH levels in both groups
- In the test groups (groups 4, 5 and 6) however, the biochemical indices were significantly ($p < 0.05$) restored to normalcy

Table 1: Quantitative phytochemical analysis of the ethanol root bark extract of *Cleistopholis patens*

Phytochemical constituents		Concentration (mg/100g)
Steroids	+++	1.6057 ± 0.00153
Terpenoids	+++	1.4783 ± 0.00058
Phenols	+++	1.2193 ± 0.02084
Flavonoids	++	1.1790 ± 0.02358
Alkaloids	++	1.1597 ± 0.00252
Glycosides	++	1.0237 ± 0.00153
Tannins	+++	0.6860 ± 0.00819
Reducing Sugar	+++	0.2060 ± 0.03643

Results are expressed in Means ± SD (n = 3)

Table 2: Effect of ethanol root bark extract of *C. patens* on the Cardiac Troponin I, AST and ALT in rats induced with myocardial infarction

Groups	Troponin Levels (ng/ml)	AST Activity (IU/L)	ALT Activity (IU/L)
Group 1	0.258 ± 0.054^{a}	81.400 ± 19.983^{b}	26.000 ± 7.842^{a}
Group 2	0.714 ± 0.133^{b}	96.800 ± 4.919^{b}	30.000 ± 9.247^{ab}
Group 3	0.343 ± 0.083^{a}	85.250 ± 16.500^{b}	28.750 ± 4.574^{ab}
Group 4	0.336 ± 0.070^{a}	24.000 ± 8.277^{a}	40.400 ± 13.145^{b}
Group 5	$0.246 \pm 0.043_{a}$	15.600 ± 3.286^{a}	33.200 ± 4.604^{ab}
Group 6	0.278 ± 0.058^{a}	20.800 ± 13.330^{a}	37.000 ± 9.979^{ab}

Results are expressed in Means ± SD (n = 4)

Mean values with different letters as superscripts down the column are considered significantly different at $p < 0.05$, while mean values with the same letters as superscripts down the column are considered non-significantly at $p > 0.05$

Table 3: Effect of ethanol root bark extract of *C. patens* on Enzymatic and Non-Enzymatic Anti-Oxidant Levels in rats induced with myocardial infarction

Groups	Anti-oxidant Levels			
	MDA conc. (mg/dl)	SOD Activity (IU/L)	Catalase Activity (IU/L)	GSH conc. (mg/dl)
Group 1	4.489 ± 0.527^{a}	10.689 ± 0.532^{b}	5.817 ± 0.899^{b}	0.866 ± 0.045^{c}
Group 2	8.625 ± 0.535^{b}	6.449 ± 0.438^{a}	2.186 ± 0.382^{a}	0.527 ± 0.045^{a}
Group 3	4.935 ± 0.603^{a}	10.189 ± 0.071^{b}	6.547 ± 0.505^{b}	0.638 ± 0.023^{b}
Group 4	4.147 ± 0.293^{a}	10.275 ± 0.201^{b}	6.200 ± 0.085^{b}	0.583 ± 0.050^{ab}
Group 5	4.613 ± 0.588^{a}	9.803 ± 1.758^{b}	5.820 ± 0.898^{b}	0.632 ± 0.063^{b}
Group 6	4.183 ± 0.612^{a}	10.662 ± 0.718^{b}	6.687 ± 0.628^{b}	0.623 ± 0.056^{ab}

Results are expressed in Means ± SD (n = 4)

Mean values with different letters as superscripts down the column are considered significantly different at $p < 0.05$, while mean values with the same letters as superscripts down the column are considered non-significantly at $p > 0.05$

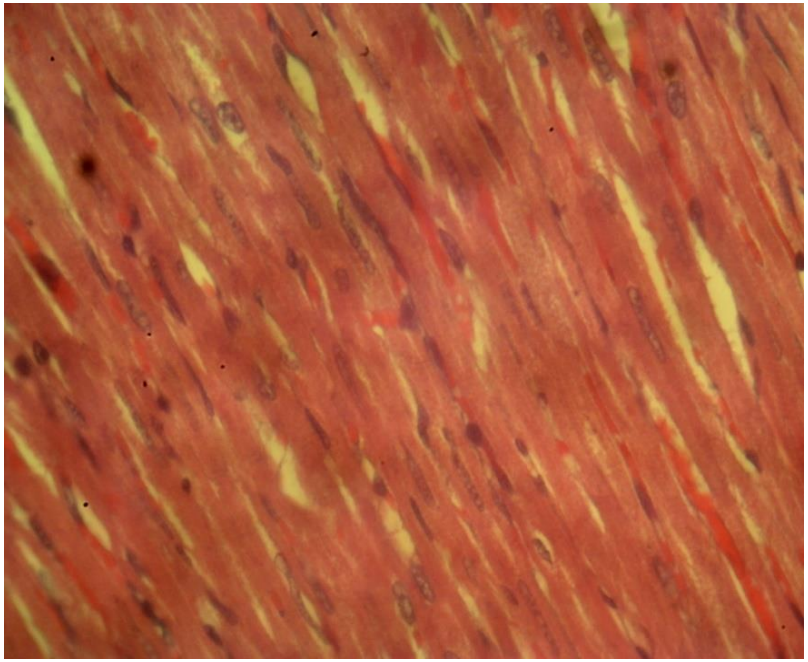


Fig 1: diagram of the myocardial histomorphology of the normal control group (group 1) with H&E x 400

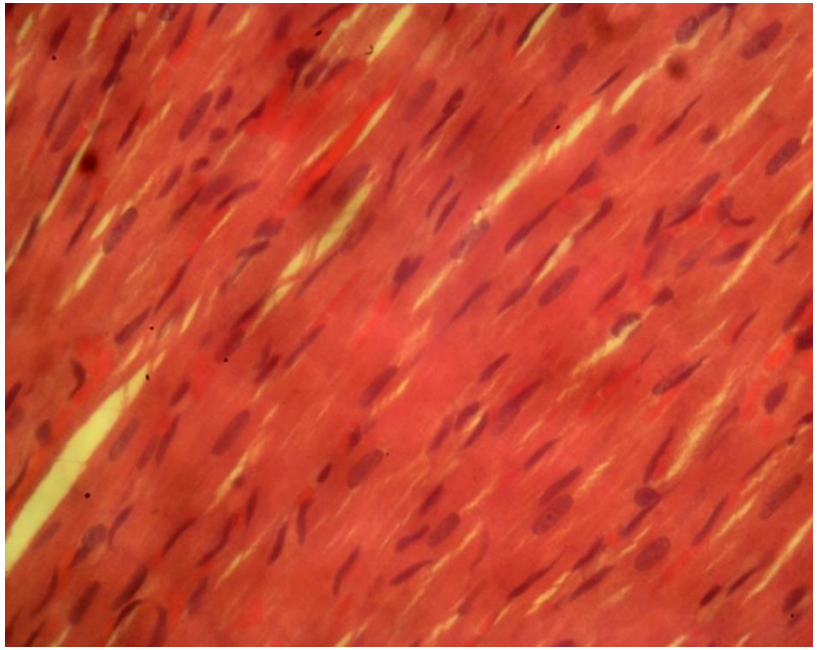


Fig 2: diagram of the myocardial histomorphology of the standard control group (group 3) with H&E x 400

A normal myocardial histomorphology was shown in groups 1 and 3, with a rich network of blood cells surrounding elongated myocytes

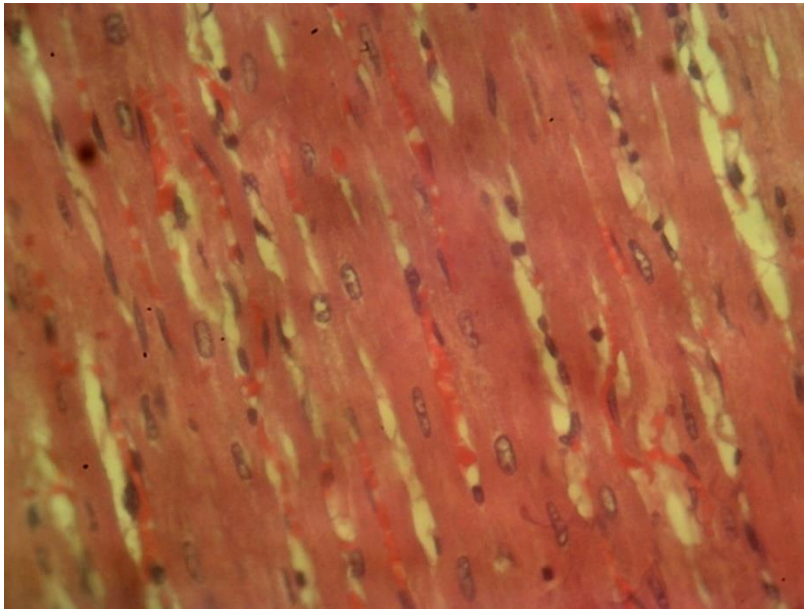


Fig 3: diagram of the myocardial histomorphology of the positive control group (group 2) with H&E x 400

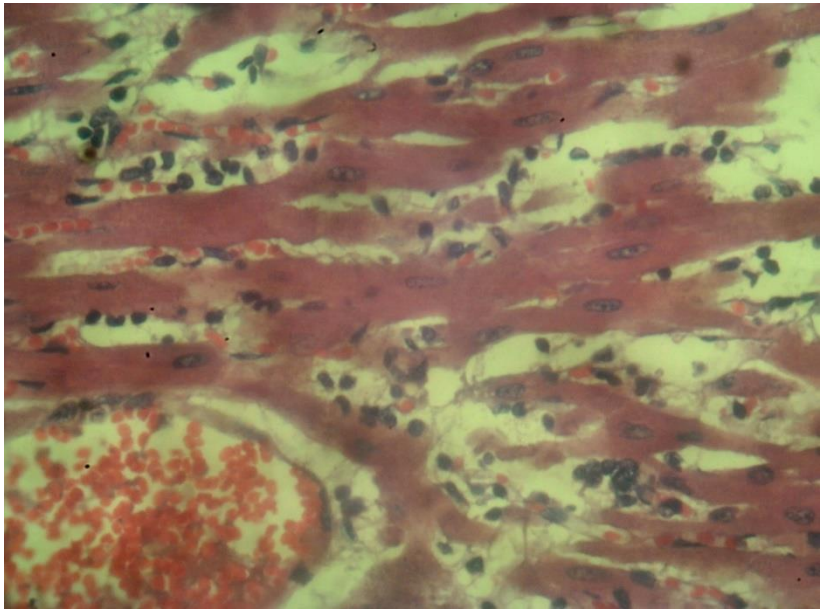


Fig 4: diagram of the myocardial histomorphology of group 4 experimental rats (200 mg/kg bw DOX) with H&E x 400

In groups 2 and 4, myocardial degeneration was observed

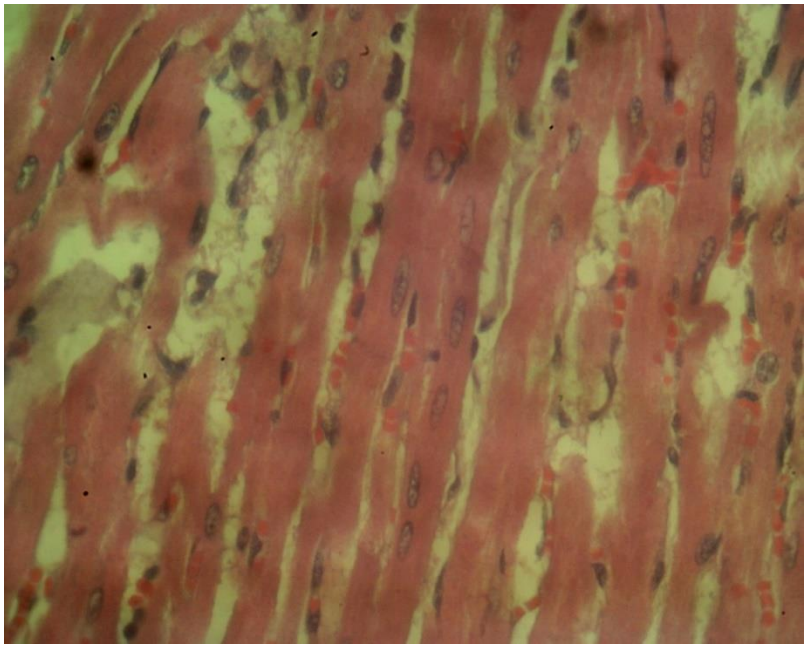


Fig 5: diagram of the myocardial histomorphology of group 5 experimental rats (400 mg/kg bw DOX) with H&E x 400

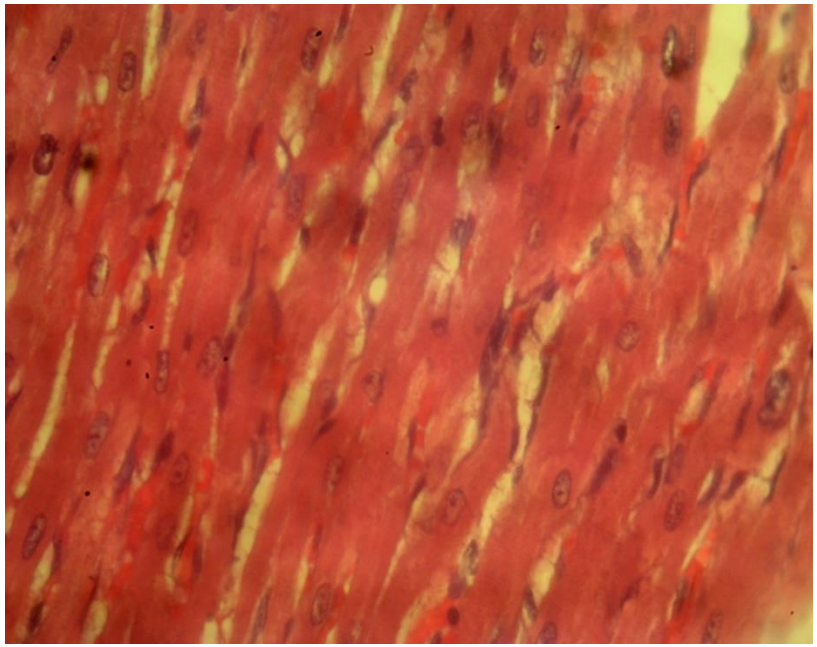


Fig 6: diagram of the myocardial histomorphology of group 6 experimental rats (400 mg/kg bw DOX) with H&E x 400

While group 5 showed myocardial degeneration, a normal myocardial histomorphology was observed in group 6, with little or no changes to the cardiac cells

- Doxorubicin causes an excessive production of reactive oxygen species (ROS).
- The ROS is generated through a reduction of the quinone moiety of DOX by reductases present in the mitochondria.
- A semiquinone is formed, and is subsequently oxidized by molecular oxygen to regenerate the parent quinone. This exposes the cell to higher than normal levels of reactive oxygen species (ROS) (Menna *et al.*, 2012).
- ROS induces the alteration of the structural and functional integrity of cellular and mitochondrial membranes, sometimes, through lipid peroxidation, proteolysis, or other mechanisms.
- This results in the necrosis of the cardiomyocyte and consequent a release of Cardiac Troponin I (cTnI) and Aspartate Aminotransferase (AST). cTnI acts as a biomarker for detecting injuries to the heart.
- Results showed a significant increase ($p < 0.05$) in the level of cTnI in the DOX control group when compared to the normal group.
- This may be indicative of a cardiac injury.

- Doxorubicin also brought about significant reduction ($p < 0.05$) in the activities of the antioxidant enzymes, superoxide dismutase (SOD) and catalase (CAT), and the non-antioxidant enzyme, reduced glutathione (GSH).
- This was also observed in the DOX control group.
- In effect, the ROS scavenging capacity of the cardiac cells is greatly reduced.
- Significantly high ($p < 0.05$) levels of malondialdehyde (MDA) was noticed in the DOX control group as well. This may be an indication of lipid peroxidation.
- All alterations were however, restored to normalcy by the extract. This is alluded, as a result of the significant differences ($p < 0.05$) in the biochemical indices measured, as can be seen when the treated groups are compared with the DOX control group.
- These results are consistent with the findings of Mohit *et al.,* 2016.
- Also, high concentrations of terpenoids, phenols and flavonoids in the extract may be a contributing factor to the results observed in the treated groups, as terpenoids, phenols and flavonoids have been shown to have high antioxidant properties.

Conclusions

The biochemical findings obtained from this study indicates that the ethanol root bark extract of *Cleistopholis patens* offers protection to the myocardium against DOX-induced myocardial infarction and may serve as a useful adjuvant, along with doxorubicin to possibly reduce the cardiotoxicity in DOX treated cancer patients.
Further studies are thus required, to decipher the exact cardioprotective mechanism of the ethanol root bark extract of *Cleistopholis patens.*

Acknowledgments

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