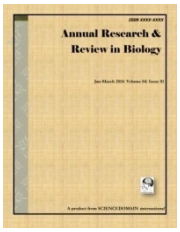

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Tumour Suppressive and Organ Protective Effects of Aqueous *Andrographis paniculata* Leaves Extract on Benzene Induced Leukaemia Bearing Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author AEO designed the study, wrote the protocol and the first draft of the manuscript. Author ORF managed the analyses of the study and wrote a revised manuscript and author CI managed the literature searches, performed statistical analysis and provided other logistics. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: To investigate and compare the protective and ameliorative role of aqueous leaf extract of Andrographis paniculata and standard drug 5-Fluorouracil on select organs of male Wistar rats exposed to benzene carcinogen.
Study Design: Histological assessment of protective and ameliorative activity of the aqueous leaf extract of Andrographis paniculata and standard drug 5-Fluorouracil in some organs of experimental animals exposed to benzene carcinogen.
Place and Duration of Study: Department of Biomedical Sciences Ladoke Akintola University of Technology, College of Health Sciences, Osogbo. Nigeria between May and August 2012.
Methodology: 72 adult male Wistar rats weighing 150-180 g were grouped into six (A-E), each group comprised of 6 rats in replicate of two (n=12).
Group A (control) received distilled water and normal saline (0.5 ml/kg each), group B received benzene chromasolv (0.2 ml at 1:10 dilution (water/2-propanol), group C received aqueous Andrographis paniculata (10 mg/kg bodyweight), group D was administered 5 -

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fluorouracil only (5 mg/kg body weight) for four weeks, group E was pretreated with Andrographis paniculata for 4 weeks prior to the administration of benzene chromasolv and group F was post treated with Andrographis paniculata for 4 weeks after pre-exposure to benzene chromasolv for 4 weeks. Leukaemia burden was assessed using haematological parameters such as Packed cell volume, Haemoglobin concentration, Red blood cells count and Total leukocyte count in the control and treatment groups.

Results: Results showed that leukemia was induced within 4 weeks as a significantly elevated WBC (leukocyte) counts and anaemia over the group not exposed to benzene which served as baseline were noted ($p< 0.05$).Organ histology showed varying lesions of the heart (mild to marked) coronary congestion, severe vacuolar degeneration and necrosis of the hepatocytes with cellular infiltration by mononuclear cells, diffuse tubular degeneration and necrosis with renal interstitial hemorrhage observed in the groups exposed to benzene carcinogen, 5-Fluorouracil and co-treatments of these two agents. However, hepato-renal and heart histio-architectures were intact in the extract pre-treatment and post-treatment groups.

Conclusion: These results suggest that Andrographis paniculata might be more effective than some other drugs currently in use as cancer suppressive chemotherapeutic agents and also may be a novel bioagent for the treatment of acute or chronic injuries induced on the liver, kidneys and heart by toxicants and carcinogens.

Keywords: Andrographis paniculata; 5- fluorouracil; benzene; leukaemia; carcinogen.

1. INTRODUCTION

Since time immemorial, plants have been used for maintaining health and curing diseases. It is a widely recognized fact that many pharmacologically active drugs are derived from natural resources such as medicinal plants [1,2]. Therefore, it is reasonable to search for novel drug molecules in herbs. With cancer being a widespread threat to humanity, plants play an important role in cancer prevention, as well as in therapy.

Medicinal plants provide new active chemopreventive molecules. In addition; treatment with plants can ease side-effects as well as provide support to the fears and anxieties of the sick. Some of the plants with reported anticancer properties are *Crocus sativus, Vitex, agnus-cactus, Withania somnifera, Curcuma longa, Scutellaria baicalensis, Gleditsia sinensis, Magnolia officinalis, Acanthopanax gracilistylus.*

Andrographis paniculata is an herbaceous plant of the family Acanthaceae that is found in evergreen, pine and deciduous forests and along road sides. It grows luxuriously in mild humid locations with tropical temperature and high rainfall [3]. It is cultivated extensively in China and Thailand.

The Chemical composition of *Andrographis paniculata* showed that it is a rich source of diterpenoids and flavonoids including other metabolites [4]. “Andrographolide” a diterpene lactone (SCHRI,1973), which is the major bioactive component isolated from *Andrographis paniculata,* has a colourless crystalline appearance with a bitter taste and is responsible for pharmacological activities observed from this plant [5-9]. The underlying molecular mechanisms have also been investigated and has been attributed to the nuclear transcription factor kappa B (NF-kB) which is the molecular target for the anti-inflammatory activity of *Andrographis paniculata* [10,11].

Andrographis paniculata is reputed for treating upper respiratory tract infections and typhoid fever [12] and has been documented to possess anti-inflammatory [13,10] antiviral [14] hepatoprotective and anti-hypertensive activities [15,12].

Benzene chromasolv is an environmental carcinogen, a haematoxin that is linked to increased incidence of cancer in humans and laboratory animals (Sigma-Aldrich Co. LLC.). Several types of neoplasm's have been reported to be associated with benzene exposure in rats and mice after oral dosing or inhalation exposures for example it causes chromosome aberrations in humans and laboratory animals and such chromosomal rearrangements are relevant steps in the carcinogenic process [16].

Fluorouracil or 5-FU is a drug that is a pyrimidine analog which is used in the treatment of cancer [17]. The chemotherapy agent has been in use against cancer for about 40 years, it acts in several ways, but principally as a thymidylate synthase inhibitor. Interrupting the action of this enzyme blocks synthesis of the pyrimidine thymidine which is a nucleoside required for DNA replication so rapidly dividing cancerous cells undergo cell death [17].

Fluorouracil effects are felt system wide but fall most heavily upon rapidly dividing cells that make heavy use of their nucleotide synthesis machinery, such as cancer cells, but also other cells in parts of the body that are rapidly dividing, may also feel its effect after prolong usage. Adverse effects of this drug include myelosuppression, mucositis, dermatitis, diarrhea [18] and both acute central nervous system (CNS) damage and progressively worsening delayed degeneration of the CNS in mice [19].

1.1 Aims of the Present Study

Despite the popular use and numerous therapeutic benefits of *Andrographis paniculata*, little is known about its anti cancer property especially on in vivo studies. This work aimed at investigating and comparing the possible protective and ameliorative roles of aqueous extract of the leaves and standard drug 5-fluorouracil which possess cancer chemopreventive activity on some organs (liver, heart and kidney) of wistar rats exposed to benzene carcinogen.

2. MATERIALS AND METHODS

2.1 Reagents

Benzene chromasolv (Sigma-Aldrich Co. LLC.), 5 Fluorouracil (5-FU), 10% buffered formalin, distilled water.

2.2 Plant Material

Andrographis paniculata leaves were harvested from Osogbo Local Government Area, Osun State and authenticated at Department of Botany and Phamacogonosis Obafemi Awolowo University, Ile Ife. The leaves were air dried at room temperature (24-27°C) for fifteen days; the air dried leaves were then milled into powder using electric blender (Phillips).

2.3 Preparation of Extract

Milled leaves weighing 150 grams was soaked in 1,500 mLs of distilled water for extraction and was kept at room temperature for 48 hours after which it was filtered through Whatman

filter paper (No.1), concentrated using a rotary evaporator at low temperature (40-50°C). The extracts were preserved in airtight containers and kept at 4°C until further use.

2.4 Experimental Animals

Seventy-two adult male Wistar rats weighing 150-180 g, purchased from the Experimental Animal house of Ladoke Akintola University of Technology, Ogbomoso Nigeria, were used for this study. These animals were kept in cages made with wood and galvanized wire mesh, at the College of Health Science Animal House, Mercyland campus in two replicates of six per group of 12 rats each. They were exposed to half daily cycle of light and dark. The diet and drinking water were provided *ad libitum*.

2.5 Experimental Design

72 adult male Wistar rats weighing 150-180 g were randomly allocated into six groups (A-E), each group comprised of 6 rats in replicate of two(n=12).

Group A: received distilled water (0.5 ml/kg) + normal saline (0.5ml/kg) orally for four weeks.
Group B: was given benzene chromasolv (0.2 ml at 1:10 dilution (water/2-propanol) 50/50 v/v in water) 48 hourly for 4 weeks prior to the administration of 5-Fluorouracil (5 mg/kg body wt) daily via tail vein for 4 weeks.
Group C: received *Andrographis paniculata* (10 mg/kg body wt) via gastric gavage daily using oral cannula for 4 weeks.
Group D: was administered 5-Fluorouracil only (5 mg/kg body wt) daily via tail vein for four weeks.
Group E: was pretreated with *Andrographis paniculata* (10 mg/kg body wt) for 4 weeks prior to the administration of benzene chromasolv (0.2ml at 1:10 dilution (water/2-propanol) 50/50 v/v in water) for 4 weeks.
Group F: was post treated with *Andrographis paniculata* (10 mg/kg body wt) for 4 weeks after pre-exposure to benzene chromasolv (0.2 ml at 1:10 dilution (water/2-propanol). The rats were sacrificed by cervical dislocation at the end of the treatment period.

2.6 Sample Collection

Each animal was weighed before sacrifice. The liver, kidney and heart were harvested and fixed in 10% buffered formalin in labeled bottles.

3. RESULTS AND DISCUSSION

Our previous study showed leukaemia induction in experimental rats exposed to benzene chromasolv within four weeks [20]. In this study leukaemia induction was evaluated using the estimated haematological parameters (blood counts) as in the Table 1. Results reflects a significant difference ($p<0.05$) between leukocyte counts of the rat group induced with leukaemia (marked leukocytosis) with anaemia (reduced haemoglobin concentration, red blood cells count and packed cell volume) when compared with rats that treated with water and saline. Therapeutic and chemo preventive properties of the extract are also evidenced by reversal of leukocytosis and anaemia in the various treated groups.

Table 1. Mean±SD haematological parameters in the treatment groups and leukaemia control group

Treatment group	Leukocyte Count cmm	Red cells count X10^9/mm	Haemoglobin Concentration (g/dl)	Packed cells volume PCV (%)
A	5.84 ±0.92	8.21±0.50	13.38±0.81	48.93 ±4.03
B	6.64 ±0.68	7.79±0.64	13.23±1.18	50.20±5.68
C	7.80 ±1.40	8.40±0.49	14.52±0.71	50.22±2.95
D	8.22±1.35	7.85±0.32	13.82±0.62	48.18±2.88
E	5.76±1.97	7.54±0.69	12.90±0.66	42.98±3.70
F	6.12±1.40	7.51±0.17	13.13±0.41	44.65±1.39
G	12.03±0.49*	3.77±0.36*	7.50±0.62*	25.60±1.21*

** Significant difference between the leukaemia control group and other treatment groups ($p<0.05$).*

A, Water and saline plus feed for 4 weeks; B, Leukaemia induction for 4 weeks prior to treatment with 5 FU; C, Aqueous *Andrographis paniculata* leaves extract with feed only; D, 5 FU only; E, Aqueous *Andrographis paniculata* leaves extract administration prior to leukaemia induction; F, Leukaemia induction followed by treatment with Aqueous *Andrographis paniculata* leaves extract, G, Leukaemia positive control.

3.1 Histopathology

The histio-architecture of the heart was retained in the control (Plate 1A), *Andrographis paniculata* (Plate 1C), *Andrographis paniculata* pre-treated + benzene chromasolv (Fig1E) and benzene chromasolv pre-treated + *Andrographis paniculata* (Plate 1F) rats. However, mild to marked coronary congestion were observed in the heart of 5-Fluorouracil only (Plate 1D) and benzene chromasolv + 5-Fluorouracil treated rats (Plate 1B).

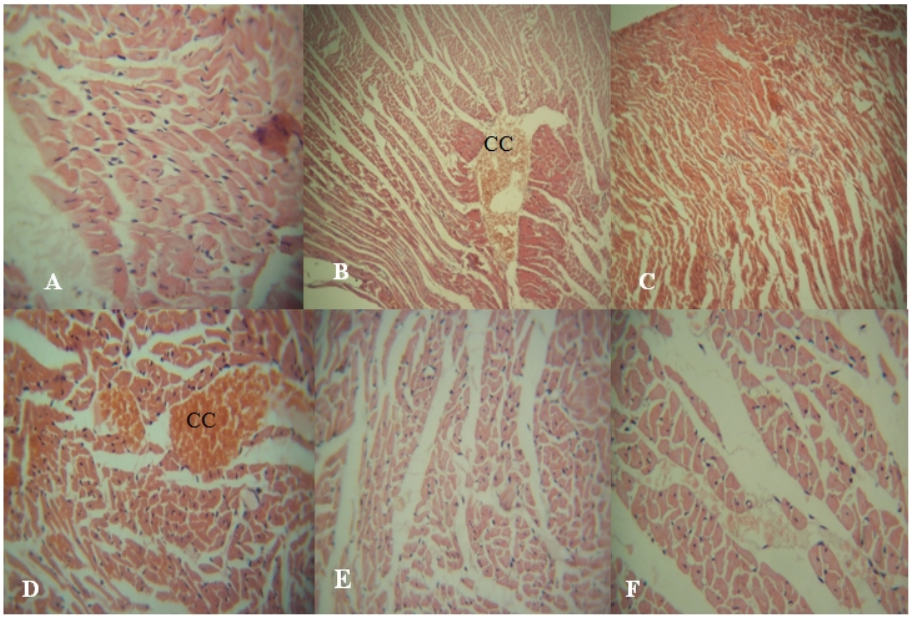

Plate 1. Shows effect of aqueous extract of *Andographis paniculata* and 5-fluorouracil on the heart of male wistar rats exposed to benzene carcinogen

A. No visible lesions seen, B. There is mild coronary congestion (CC), C. no visible lesions D. there is marked coronary congestion (CC) in the heart, E. no visible lesions F. no visible lesions. Magnification: X400; Stain: H&E

The kidneys of the control, *Andrographis paniculata*, *Andrographis paniculata* pre-treated + benzene chromasolv and benzene chromasolv pre-treated + *Andrographis paniculata* rats showed no visible lesion. The kidneys of benzene chromasolv + 5-Fluorouracil treated rats' demonstrated diffuse tubular necrosis and interstitial cellular infiltration. Similarly, there was marked tubular degeneration and necrosis coupled with some areas of hemorrhage into the interstitial in the kidney of 5-Fluorouracil treated- rats (Plate 2).

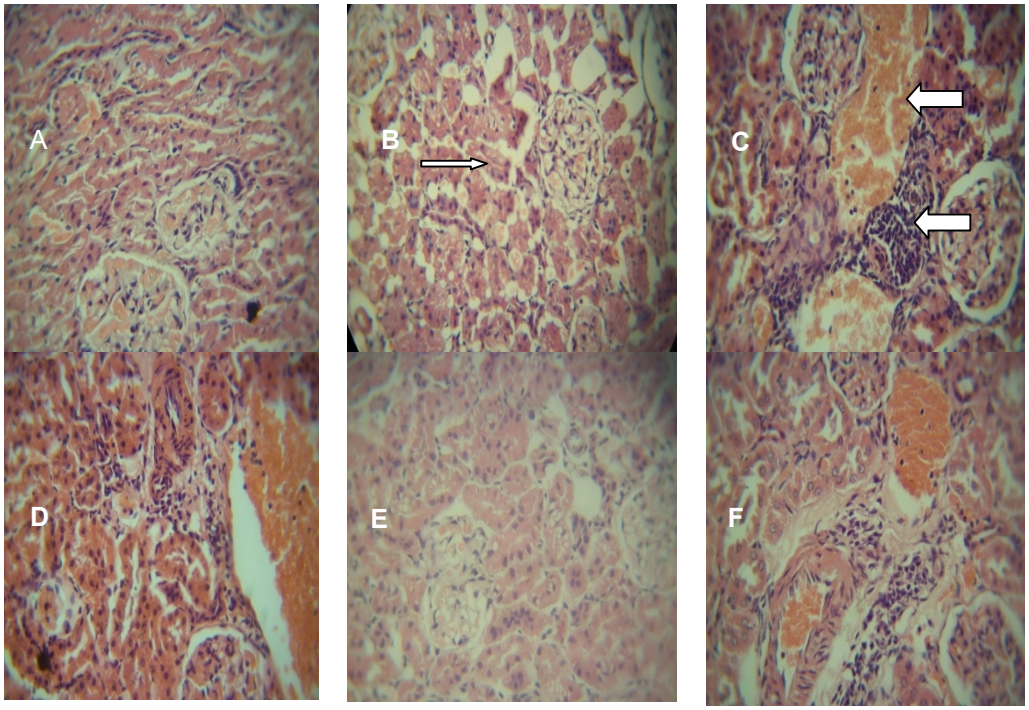

Plate 2. Shows effect of aqueous extract of *Andographis paniculata* and 5-fluorouracil on the kidney of male wistar rats exposed to benzene carcinogen

A. No visible lesion B. Diffuse tubular necrosis (white arrow) and interstitial cellular infiltration in the kidney, C. mild congestion with perivascular cellular infiltration (white arrow), both closely associated with the glomeruli in the kidney, D. there is marked tubular degeneration and necrosis and there are also areas of haemorrhage into the interstitial in the kidney, E. no visible lesions F. no visible lesions. Magnification: X400; Stain: H&E

The liver architecture of the control, *Andrographis paniculata*, *Andrographis paniculata* pre-treated + benzene chromasolv and benzene chromasolv pre-treated + *Andrographis paniculata* rats showed no visible lesion. On the contrary, the liver of benzene chromasolv + 5-Fluorouracil and 5-Fluorouracil only treated rats' showed varying degrees of severe vacuolar degeneration and necrosis with mild to moderate periportal cellular infiltration by mononuclear cells (Plate 3)

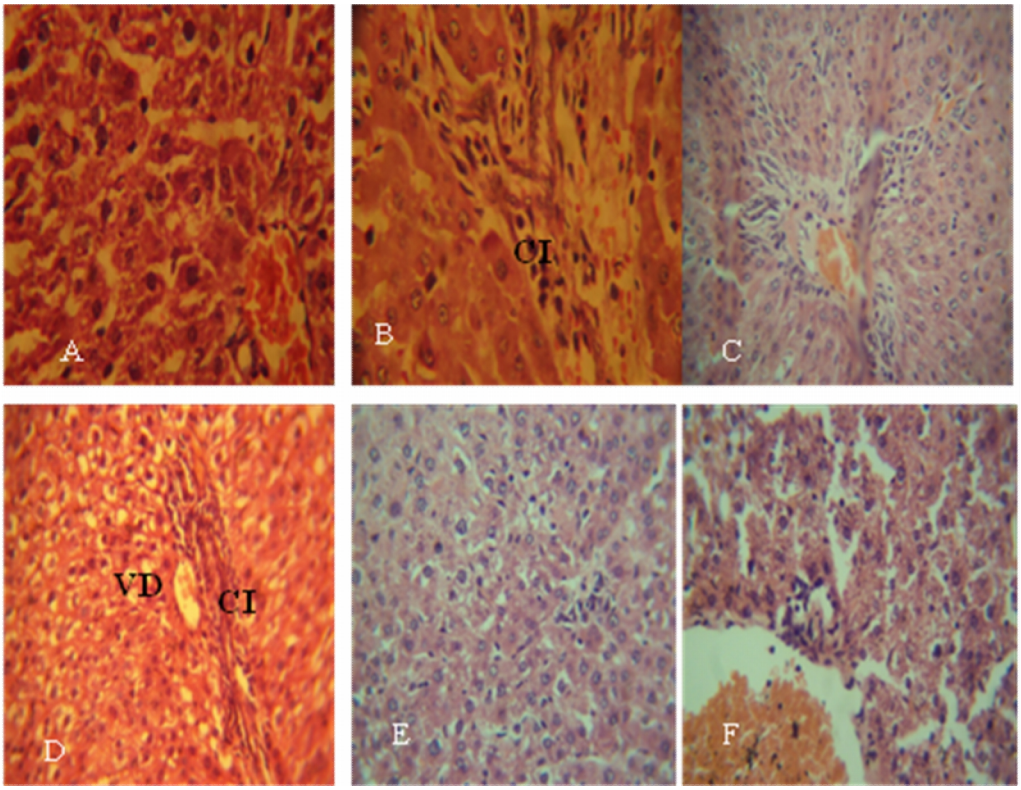

Plate 3. Shows effect of aqueous extract of *Andographis paniculata* and 5-fluorouracil on the liver of male wistar rats exposed to benzene carcinogen

A. No visible lesions B. Severe vacuolar degeneration and necrosis with mild periportal cellular infiltration (CI) by mononuclear cells in the liver. C. No visible lesions D. mild to moderate portal cellular infiltration (CI), with mild diffuse and severe vacuolar degeneration (VD) of hepatocytes in the liver. E. no visible lesions seen lesions F. no visible lesions. Magnification: X400; Stain: H&E.

The various lesions of the heart (mild to marked coronary congestion), the liver (severe vacuolar degeneration and necrosis with mild to moderate periportal cellular infiltration by mononuclear cells) and the kidneys (diffuse tubular necrosis, tubular degeneration, renal interstitial hemorrhage and cellular infiltration) observed in this study are typical of histopathological lesions associated with 5-Fluorouracil and benzene chromasolv damage (Andrew and Janet, 1997). This has been attributed to DNA damaging ability of the agents (Andrew and Janet, 1997). In the same vein, the aggravated damage to the organs of rats co-treated with 5- Fluorouracil and benzene chromasolv is suggestive of the deleterious effects of Fluorouracil (anticancer drug) on normal cells after prolong usage.

The morphological appearance of the heart, kidneys and liver of the rats treated with Andrographis *paniculata* only was not disrupted in this study. This may indicate the safety of this herb as a natural and non-toxic traditional nutritional supplement for the management of liver, kidneys and cardiovascular disorders, as was previously reported [21,22,23].

Similarly, rats pre-treated with *Andrographis paniculata* and later exposed to benzene carcinogen or post-treated with *Andrographis paniculata* after exposure to benzene chromasolv pre-treatment had no distortion in their histio-architectures. This indicates that the plant extract could offer either protective or ameliorative properties accordingly. The possible mechanism underlying the hepato-renal and cardio-protections of *A. paniculata* may be attributed to its antioxidant and free radical-scavenging properties [24].

This study also revealed that *Andrographis paniculata* could be more effective as a cancer suppressive chemotherapeutic agent than the standard drug 5-fluorouracil. Due to mild congestion with perivascular infiltration, both closely associated with renal glomeruli, of the group exposed to 5-fluorouracil only. It suggested that further research be carried out to determine the dosage and duration of usage of the drug.

4. CONCLUSION

In conclusion, the observed protective and ameliorative effects of *A. paniculata* could enhance the use of its aqueous leaf extract of as a novel bioagents for the treatment of acute or chronic injuries induced on the liver, kidneys and heart by toxicants and carcinogens.

CONSENT

Not applicable.

ETHICAL APPROVAL

Experimental protocol was approved by Institutional Ethics Committee of Ladoke Akintola University of Technology, College of Health Sciences, Osogbo, Osun - State, Nigeria. Animals were handled in accordance with international principles guiding the, use and handling of experimental animals (United States National Institutes for Health Publication, 1985).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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