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# AMELIORATIVE EFFECT OF *HYDROCOTYLE VERTICILLATA* AND *LAPORTEA AESTUANS* LEAVE EXTRACTS ON HEMATOLOGICAL, BIOCHEMICAL AND HISTOPATHOLOGICAL INDICES ON 7, 12-DIMETHYLBENZ (A) ANTHRACENE (DMBA) INDUCED-LEUKEMIA IN RATS

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**ABSTRACTS**: *This study evaluated the ameliorative effect of Hydrocotyle verticillata (H.v) and Laportea aestuans (L.a) leaves extracts on hematological, biochemical and histopathological indices on 7,12-dimethylbenz(α) anthracene (DMBA) induced-leukemia in wistar rats. Leukemia was induced in rats using standard method. Both extracts were evaluated for their effects on hematological, biochemical and histopathological indices using standard methods. The hematological indices showed a decrease in packed cell volume (21.83±0.85%) and hemoglobin concentrations (7.28±0.28g/dl) with an increase in Total white blood cells (30.08±0.51x10$^9$/L) in the negative control group. Treatment with the extracts significantly ($p<0.05$) increased the Hb, and PCV with a decrease in the TWBC levels in all treated groups. Liver enzymes of the standard drug and treated groups showed a significant ($p<0.05$) decrease when compared to the negative control (group 3) for both extracts. Urea (10.17±0.25mmol/l), creatinine (1.98±0.77mg/dl) hepatic enzymes levels reduced significantly ($p<0.05$) while Oxidative stress biomarker levels significantly ($p<0.05$) increased in the standard drug and treated groups when compared with the negative control. Histopathological examination showed strong hepato-renal protective effects. Hydrocotyle verticillata and Laportea aestuans have shown to possess hepato-renal protective effect and modulated hematological indices in induced-leukemia.*

**KEYWORDS:** DMBA, Leukemia, hepatic enzymes, renal biomarkers, hematological indices.



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## INTRODUCTION

Chemical compounds of herbal origin have since been known for their healing prowess, easier to source, low toxic effects on the mammalian body and these has placed them in high demand across cultural and religious divide. This awareness about herbal medicine has been linked to their known mechanism of action, as well as their usefulness in treating many ailments of note [1-4].

The abnormal cell proliferation with the propensity to spill to other cellular compartment has been associated with cancerous condition whose presentation differs based on different types that exists. This metabolic derangement has been noted as most prevailing health challenge afflicting man in recent years [5]. World Health Organization (WHO) has reported that there are more than 10 million fatalities in 2020 alone as a result of neoplastic diseases with cancer of the breast and leukemia being at the forefront [6]. It has been established that these data are attributed to life style habits, chronic diseases and exposure to noxious chemicals and radiations respectively.

Medical experts in Rivers State, Nigeria are raising alarm on the growing incidence of leukemia in the Niger Delta and are advocating for the establishment of oncology reference centers across the oil rich region. This could be related to the report issued by researchers at University of Port Harcourt Teaching Hospital that out of over 4000 diagnosed case of cancer, about 313 of them are of leukemia origin [1,7]. On summation, the etiology of leukemia has been strongly linked to chemical exposure and environmental indices. Researchers are at present cubing this ailment by employing synthetic agents which are designed to target concisely, the rapidly proliferating cells of different tumor origin. These chemically synthetic drugs do cause major damage in addition to affecting the growing cancerous cells.

The plant family known as Apiaceae contains more than 100 species of which the genus Hydrocotyle is a notable member and located mainly in Tropical and Temperate region of the world including the Americas and Western Africa [8]. The genus Hydrocotyle is mainly called whorled pennywort and the possession of a half dollar leaf like structure is a great presenting feature of the plant. It is mainly a creeping plant with unusual leaves where the name originates [9, 10].

*Hydrocotyle verticillata* as reported by Odo *et al*., [9] has been mistaken to Gotu kola which is Centella asiatica by the local populace within the Niger Delta area of Nigeria. There has been much reported ethno pharmacological action of plant including its role in the treatment of inflammatory conditions, anemia, blood disorders, discharges from urinary tract and enlargement of the spleen [9, 10].

Odo *et al*., [9] posited that in folk medicine practice, *H. verticillata* is utilized in the treatment of abscesses, cold, cough, viral hepatitis, respiratory ailment, and healing of wounds respectively. It is reported that in district of India, the plant is used in the treatment of skin diseases, muscular dystrophy, and Typhoid fever [11].

The plant *Laporta aestuans* is an annual herb of the Indian wood nettle origin of the family Urticaceae and comprised of about 22 other species [9, 12]. Odo *et al*., [9] and Olufunke *et al*., [13] reported exclusively on the ethno-medical role of Laportea aestuans ranging from its use as an abortifacient, anti-malaria, laxative, pain killer as well as in the treatment of urinary


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and respiratory impairment. It finds usefulness in ulcer treatment as reported by Okereke *et al*., [3], as an anti-oxidant and anti-inflammatory agent respectively [14]. Epidemiological analysis shows that it inhibits the damaging actions of free radicals in humans [15-16].

There were tremendous progress in the past years on the management and treatment protocol for leukemia mainly by utilizing factual manipulation of a rather minor chemotherapeutic procedures through a well-thought out clinical trials. It is important from the foregoing to note that existing management protocols for leukemia such as use of bone marrow transplant and chemotherapy; may be very much reliable but they pose serious adverse side effect on the individual, in addition to the treatment cost which are not readily available for those from the developing countries. It is imperative that new means need to be sought for which could be readily available, cheap and with minimal toxicity which can help to battle this disease and molecules derived from plants are now studied as they possesses various disease fighting properties [1]. Odo *et al.,* [1], noted that these phytochemicals exert their action by targeting various signaling molecules that are involved in the process of leukemia development.

Recent studies have reported that *Hydrocotyle verticillata* and *Laportea aestuans* are being used by Trado-medical practitioners in the management of hematological disorders in our local community without any supporting scientific studies to justify their use, organ toxicity studies, and dose relationship [17]. Few literature abound on the role of these plants extracts in ameliorating toxic effects on hematological and biochemical parameter as a result of leukemia induction but based on the available knowledge that these plants extract possess anti-oxidant, anti-inflammatory, cytotoxic effects in addition to their traditional role, no studies have investigated their organ ameliorating potentials in-vivo in experimental induced leukemia in rats.

**Plate 1: *Hydrocotyle Verticillata* Plant and Laportea aestuans (Source:** Odo *et al*., [ 9]**)**

This study investigated the hematological and biochemical effects of oral administration of ethanol extract of *Hydrocotyle verticillata* and *Laportea aestuans* on DMBA-induced leukemia in rats.


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## MATERIALS AND PROTOCOLS

### Collection and identification of the plants

Odo *et al.*, [1] had written extensively on how the plants used in this was procured and identified in a similar published article on leukemia and S1PR-1.

### Preparation of the Plants extracts for analysis

The plants used in this study were prepared based on the method devised by Odo *et al.*, [9].

### Animal Study

Male Wistar rats weighing between 30-33g were used for this study and the preparations were outlined by Odo *et al.*, [1]. The experimental animals were handled based on the protocol for the use of laboratory animals.

### Acute Toxicity Testing of the plants extracts

The modified methods of Lorkes, [18] was used for this study by assigning graded doses of the plants extract as outlined in the previous work by Odo *et al*., [1].

### Groupings and induction of weanling Wistar rats

Experimental leukemia was induced in the animals at the age of 26 days using a pulse dose of 25% DMBA except the DMSO and the control group for an interval of 6 weeks duration. Leukemia was induced by loss of weight, and abnormality in hematological disorders as well as other parameters outlined by Odo *et al*., [1]. DMSO was used as a conveyance vehicle.

### Biochemical and Hematological Studies

An automated hematology analyzer from Mindray Company was used for to determine the hematology parameters; automated chemistry analyzer was used to assess renal function tests, Liver function tests as well as anti-oxidant enzymes according to standard protocol.

### Histopathological examinations

The kidney and liver were harvested from the laboratory animals and the various weights taken. The organs were then fixed in Neutral buffered formalin, processed using automatic tissue processor, and thin sections were made and stained using Heamatoxylin and Eosin staining technique. It was then mounted on DPX and examined using x100 oil immersion lens.

### Statistical Analysis

Data obtained from this study were statistically analyzed using SPSS version 25.0. Statistical significance was established using one-way ANOVA and Post-Hoc multiple comparison test. p-value less than 0.05 ($p<0.05$) was taken to be statistically significant.


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## RESULTS

### Acute Toxicity Testing of leaves extract of *Hydrocotyle verticillata* and *Laportea aestuans*

The result of the acute toxicity testing ($LD_{50}$) on the extract of *Hydrocotyle verticillata* and *Laportea aestuans* are as shown in Table 1. The result shows that there was no mortality recorded across various animal groupings up to a concentration of 4000mg/kgbw of the administered extracts after 48hours of observation.

**Table 1: Acute toxicity testing of extract of *Hydrocotyle verticillata* and *Laportea aestuans***

| Groupings | Duration extract administration | | |
|---|---|---|---|
| | 30 Minutes | 4 Hours | 48 Hours |
| Group 1 (Normal Control) | No mortality | No mortality | No mortality |
| Group 2 (500mg/kw bw) | No mortality | No mortality | No mortality |
| Group 3 (1000 mg/kg bw) | No mortality | No mortality | No mortality |
| Group 4 (2000 mg/kg bw) | No mortality | No mortality | No mortality |
| Group 5 (4000 mg/kg bw) | No mortality | No mortality | No mortality |

### Effects of *H. verticillata* and *L. aestuans* on hematological indices of DMBA induced leukemia in wistar albino rats

Tables 2 and 3 shows the effects of ethanol extracts of *H. verticillata* and *L. aestuans* on hematological indices of DMBA induced leukemia in wistar albino rats in the various test groups.

**Table 2: Effects of ethanol extract of *Hydrocotyle verticillata* and *Laportea aestuans* in hematological indices in Wistar rats.**

| Groups | PCV(%) | HB(g/Dl) | T.WBC($10^9$/L) | Neut. (%) | Lymph. (%) |
|---|---|---|---|---|---|
| Group 1(Control) | 42.43 ± 0.20[a] | 14.14 ± 0.07[a] | 4.41 ± 0.07[a] | 41.83 ± 0.09[a] | 53.56 ± 0.21[a] |
| Group 2 (1000mg/kg *H. verticillata*) | 42.50 ± 0.19[a] | 14.17 ± 0.06[a] | 4.55 ± 0.03[a] | 46.99 ± 0.25[b] | 49.51 ± 0.26[b] |
| Group 3(1000mg/kg *L. aestuans*) | 42.30 ± 0.21[a] | 14.10 ± 0.07[a] | 4.51 ± 0.17[a] | 48.88 ± 0.74[b] | 47.09 ± 0.18[b] |
| Group 4(2000mg/kg *H. verticillata*) | 42.54 ± 0.18[a] | 14.18 ± 0.06[a] | 4.57 ± 0.22[a] | 55.83 ± 0.36[b] | 40.77 ± 0.15[b] |
| Group 5(2000mg/kg L. aestuans) | 42.40 ± 0.18[a] | 14.13 ± 0.06[a] | 4.88 ± 0.09[a] | 57.10 ± 0.58[b] | 39.21 ± 1.03[b] |


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**Data expressed as mean ± SEM. Values in the same column bearing different superscript are significant at p<0.05**

**Table 3: Effects of ethanol extract of *Hydrocotyle verticillata* and *Laportea aestuans* on hematological indices in wistar rats**

| Groups | Monocytes% | Eosinophils% | Basophils% | Platelet Count |
|---|---|---|---|---|
| Group 1(Control) | $2.59 \pm 0.24^{a}$ | $2.02 \pm 0.03^{a}$ | $0.008 \pm 0.005^{a}$ | $229.60 \pm 1.58^{a}$ |
| Group 2 (1000mg/kg H. verticillata) | $2.15 \pm 0.07^{a}$ | $1.35 \pm 0.20^{a}$ | $0.005 \pm 0.003^{a}$ | $221.75 \pm 2.32^{a}$ |
| Group 3(1000mg/kg L. aestuans) | $2.35 \pm 0.05^{a}$ | $2.31 \pm 0.14^{a}$ | $0.005 \pm 0.005^{a}$ | $227.08 \pm 1.80^{a}$ |
| Group 4(2000mg/kg H. verticillata) | $2.06 \pm 0.07^{b}$ | $1.34 \pm 0.22^{a}$ | $0.003 \pm 0.003^{a}$ | $229.63 \pm 2.25^{a}$ |
| Group 5(2000mg/kg L. aestuans) | $2.99 \pm 0.04^{a}$ | $2.21 \pm 0.11^{a}$ | $0.005 \pm 0.003^{a}$ | $234.50 \pm 2.10^{a}$ |

**Data expressed as mean ± SEM. Values in the same column bearing different superscript are significant at p<0.05**

**Blood film report of DMBA induced leukemia in rats treated with extracts of *H. verticillata* and *L. aestuans***

Plate 2 shows a photomicrograph of blood film depicting normal lymphocytes as well as other hematological indices as seen in field stain film from group 1 normal control. Plate 3 shows a photomicrograph of a blood picture from group 2 with normal blood film characteristics as seen in a field stain slide. Plate 4 depicts a photomicrograph from groups induced with DMBA showing atypical lymphocytes and basophilic cell in a field stain slide. Plate 5 is a photomicrograph of field stain thin blood film from groups induced with DMBA but treated with standard anti-leukemic drugs showing reduced lymphocyte abnormality together with absence of basophils. Plate 6 shows the photomicrograph of blood picture from group induced with DMBA but treated with 200mg/kg of ethanol extract of *H. verticillata* leave showing moderate atypical lymphocytes. Plate 7 depicts a photomicrograph of blood film from group induced with DMBA and treated with 400mg/kg of ethanol extract of *H. verticillata* leaf with absence of basophils but atypical lymphocytes. Plates 8 is a photomicrograph of blood picture from group induced with DMBA and treated using 200mg/kw of ethanol extract of *L. aestuans* leaf depicting atypical lymphocytes and almost absence of basophilic granules. Plate 9 is a photomicrograph of blood film from group induced with DMBA and treated with 400mg/kg body weight of ethanol leaf extract of *L. aestuans* with no basophils but reduced atypical lymphocytes. Plates 10 shows a photomicrograph from group induced with DMBA but treated with combined leaves extract of 400mg/kg body weight of H. verticillata and L. aestuans with no basophils as well as atypical lymphocytes.


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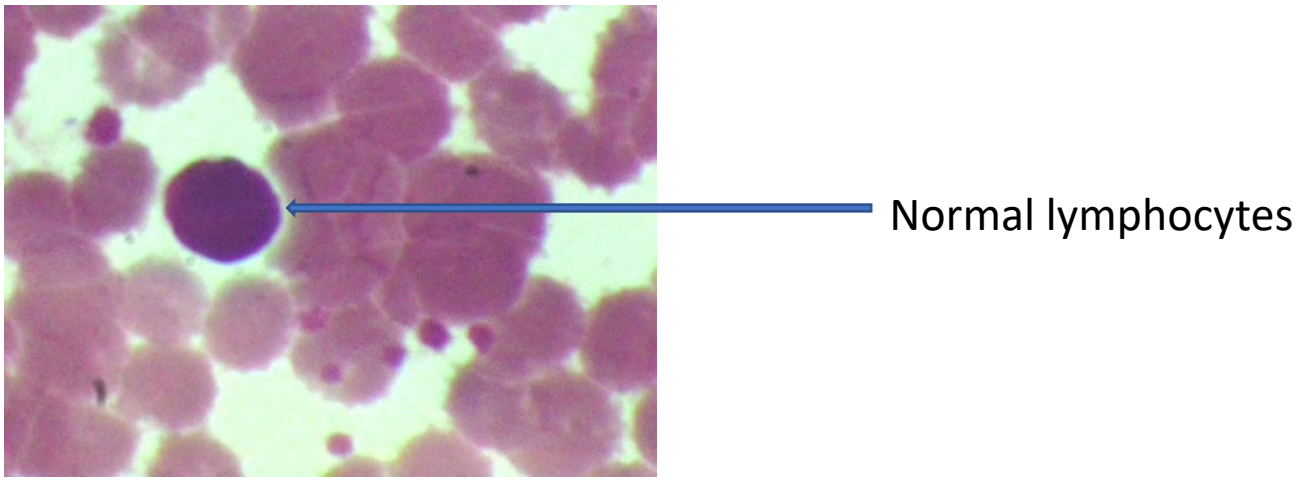


Plate 2: Photomicrograph of blood film from normal control group. X100 stained with field stain.

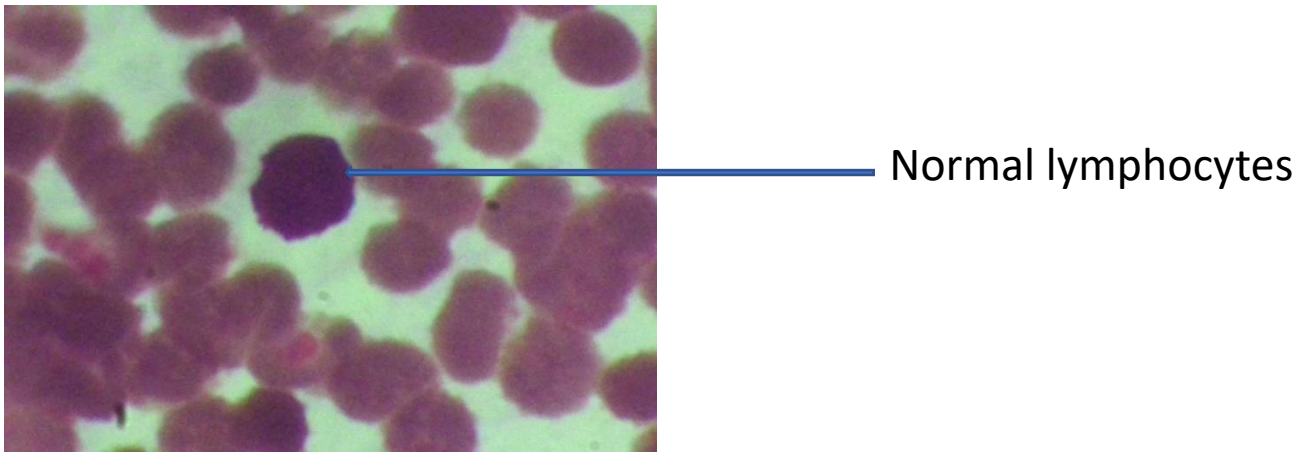


**Plate 3: Photomicrograph of blood film from group 2. X100 stained with field stain.**


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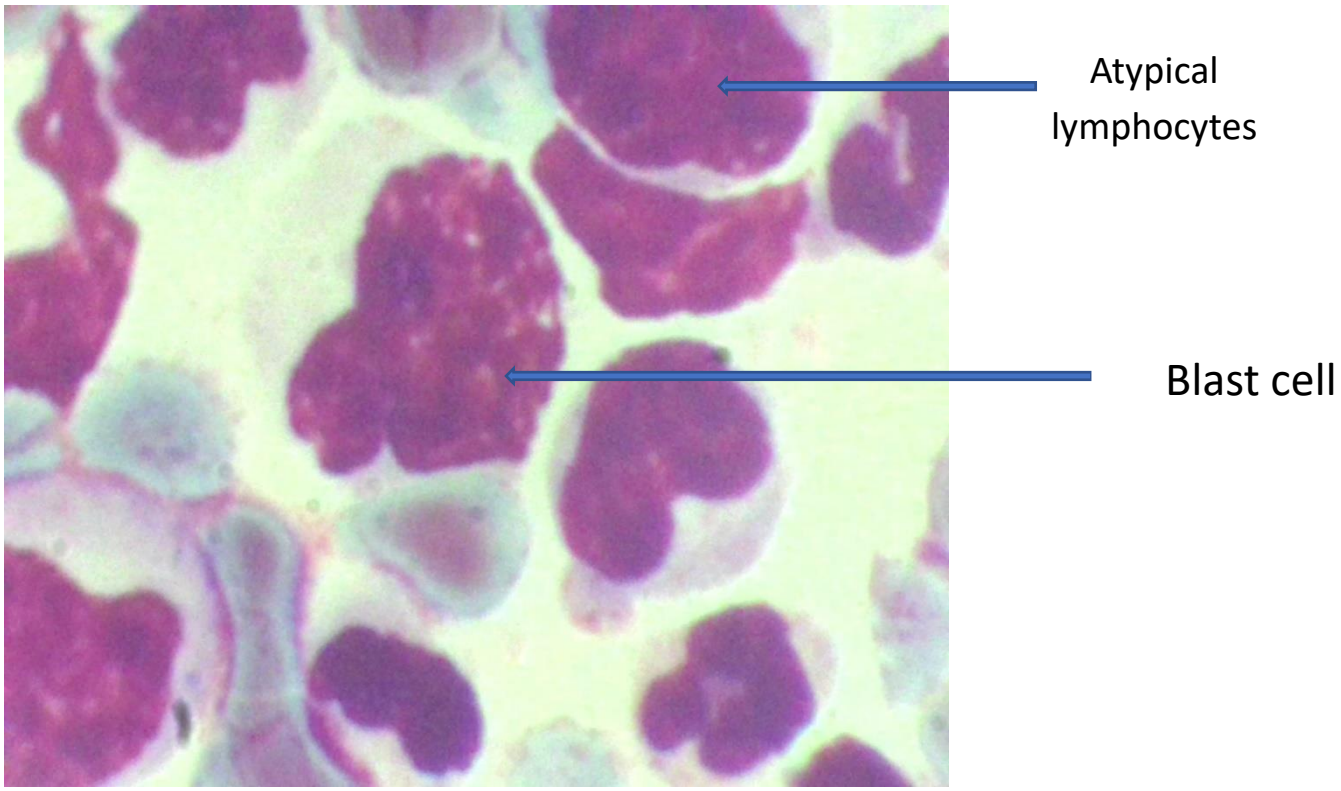


Plate 4: Photomicrograph of blood picture of group 3 induced with DMBA. X100 stained with field stain.

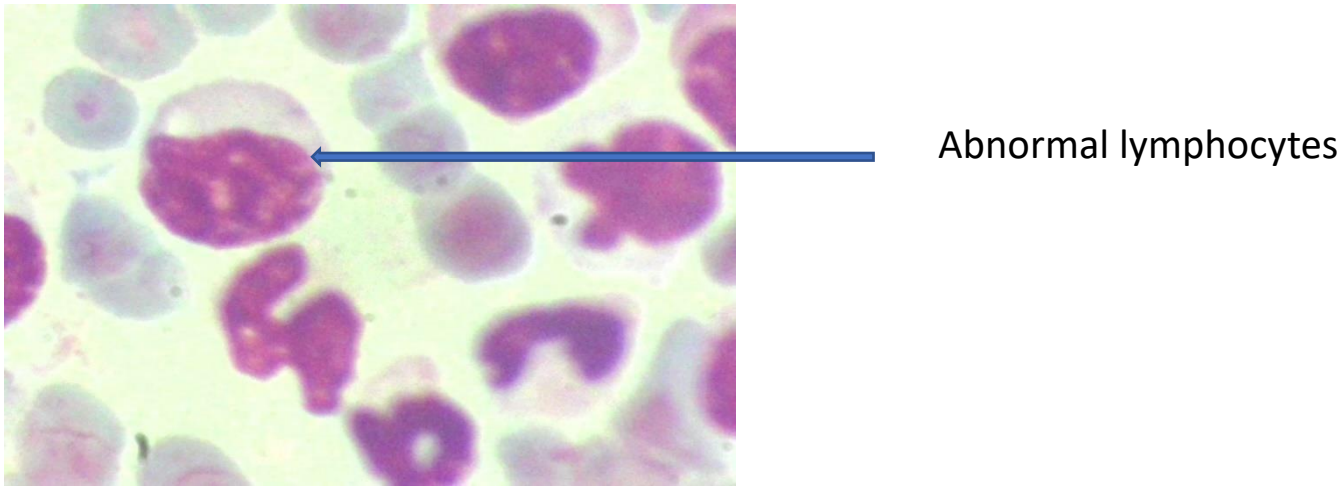


**Plate 5: Photomicrograph of blood film from group treated with standard drug. X100 stained with field stain.**


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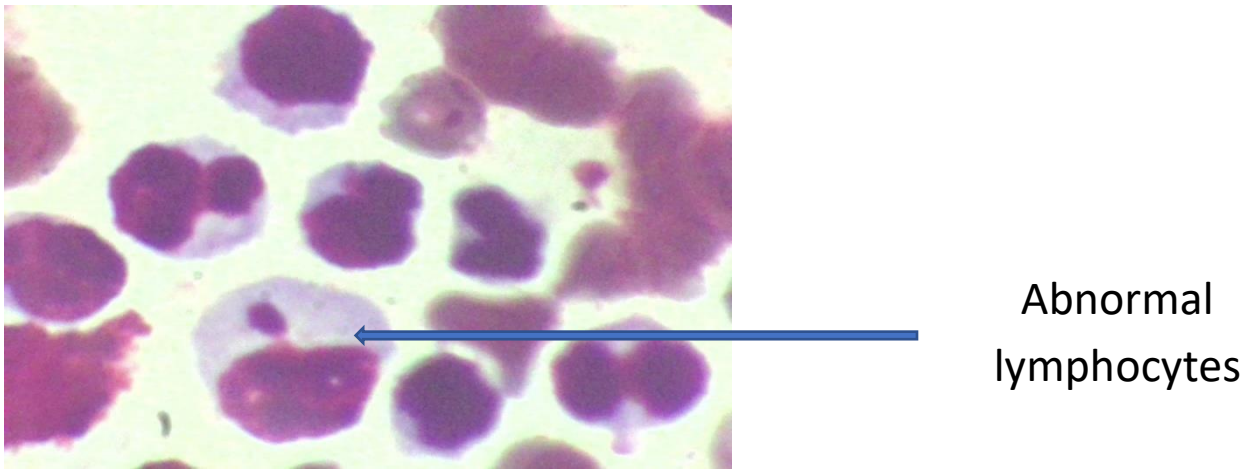


**Plate 6: Photomicrograph of blood picture from group treated with 200mg/kg of *Hydrocotyle verticillata*. X100 stained with field stain.**

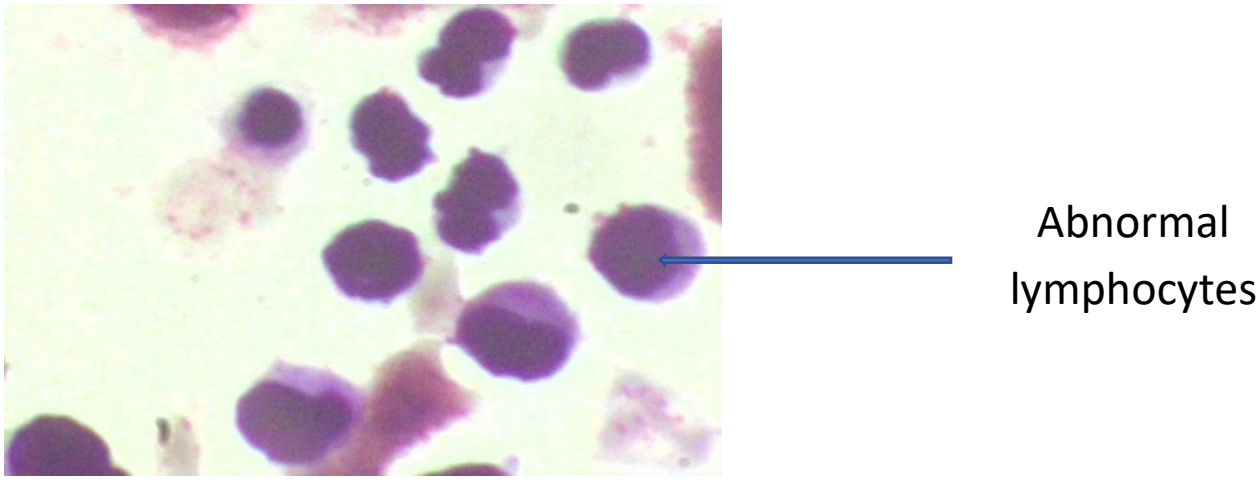


**Plate 7: Photomicrograph of blood film from group treated with 400mg/kg of *Hydrocotyle verticillata*. X100 stained with field stain.**

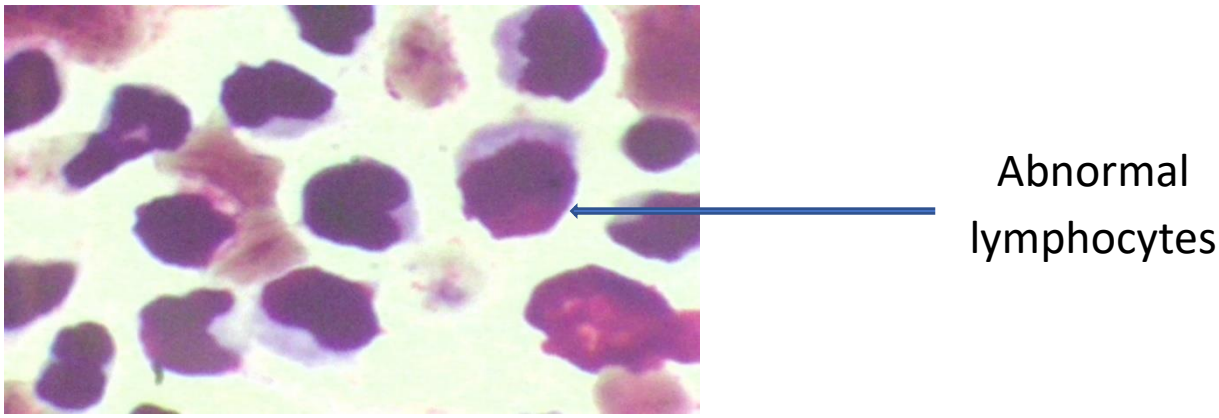


**Plate 8: Photomicrograph of blood film from group treated with 200mg/kg of *Laportea aestuans*. X100 stained with field stain.**


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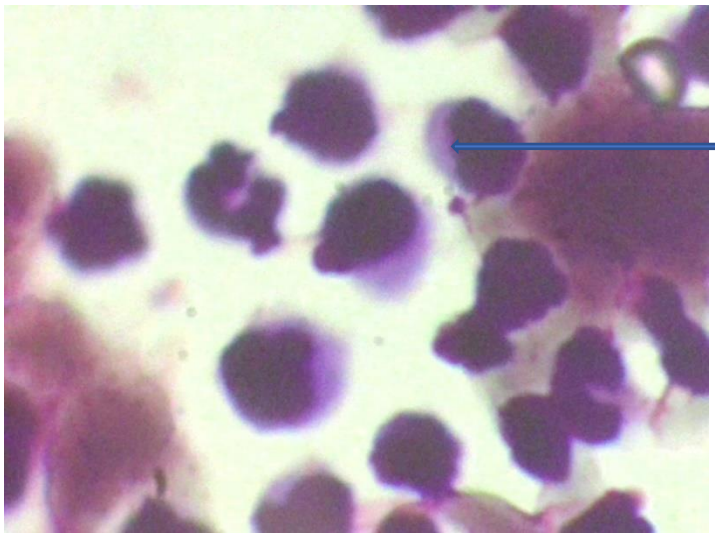


**Plate 9: Photomicrograph of blood picture from group treated with 400mg/kg of *Laportea aestuans*. X100 stained with field stain.**

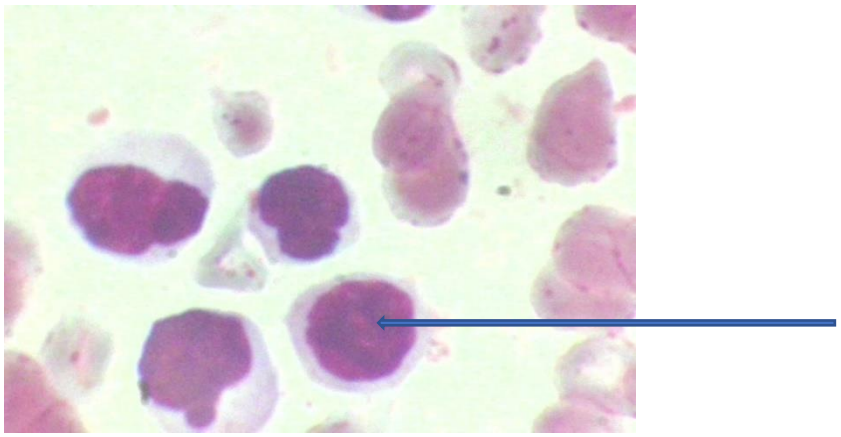


**Plate 10: Photomicrograph of blood film from group treated with combined extract of *H. verticillata* and *L. aestuans.* X100 stained with field stain.**

**Effects of ethanol extracts of *H. verticillata* and *L. aestuans* on renal function indices of DMBA induced leukemia in wistar albino rats**

Table 4a and 4b displays the effects of ethanol extracts of *H. verticillata* and *L. aestuans* on renal function of DMBA induced leukemia in wistar albino rats. The induction of leukemia produced statistically significant ($p<0.05$) increases in the levels of sodium and chloride but ($p<0.05$) decreases in calcium and potassium levels in induced group 3-9 relative to non-induced groups 1 and 2. It equally showed that there was no significant ($p<0.05$) difference in the electrolyte level between group 1 and 2. Among the induced groups, there were significant ($p<0.05$) reduction in the levels of sodium, and chloride but a significant ($p<0.05$) increase in calcium and potassium levels across the various treated groups.

Again, compared to the induced and treated groups, there were significant ($p<0.05$) reductions in the sodium and chloride levels in the group given standard anti-leukemic drug followed by the group given combined extract of 400mg of *H. verticillata* and *L. aestuans*,


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then 400mg of *Laportea aestuans*, 400mg of *Hydrocotyle verticilata*, 200mg of *Hydrocotyle verticilata* and 200mg of *Laportea aestuans* in that order.

There were statistically significance ($p<0.05$) increases in the levels of urea and creatinine after induction of leukemia in groups 3 to 9 when compared to groups 1 and 2. Although there exists slight variations between the urea and creatinine levels of groups 1 and 2, this changes were not statistically significant at ($p<0.05$). However, after the treatment, these increases were significantly ($p<0.05$) reversed upon administration of the standard anti-leukemic drug, and the extract treated groups when compared to the negative control group. Thus, the treatment with the extracts positively impacted on the Renal function of DMBA induced leukemia with tendency towards restoration to normal.

**Table 4a: Effects of ethanol extracts of *H. verticillata* and *L. aestuans* on renal function**

**Indices (electrolytes) of DMBA induced leukemia in wistar albino rats**

| Groups | Sodium(mmol/L) | Potassium(mmol/L) | Chloride(mmol/L) | Calcium(mg/dl) |
|---|---|---|---|---|
| Normal control | $129.39\pm0.85^{a}$ | $4.65\pm0.07^{a}$ | $91.40\pm0.57^{a}$ | $8.82\pm0.09^{a}$ |
| DMSO control | $130.48\pm1.06^{a,d}$ | $4.48\pm0.11^{a,d}$ | $92.88\pm0.66^{a,d}$ | $8.50\pm0.04^{a,d}$ |
| Negative control | $156.70\pm0.69^{b,e,g}$ | $2.63\pm0.10^{b,e,g}$ | $117.37\pm0.35^{b,e,g}$ | $4.82\pm0.02^{b,e,g}$ |
| Positive control | $142.12\pm1.90^{b,e,h}$ | $3.43\pm0.15^{b,e,h}$ | $109.75\pm0.63^{b,e,h}$ | $7.61\pm0.01^{b,e,h}$ |
| DMSO +DMBA +200 H.V | $149.00\pm0.62^{b,e,h}$ | $3.05\pm0.06^{b,e,h}$ | $115.41\pm0.27^{b,e,g}$ | $5.81\pm0.03^{b,e,h}$ |
| DMSO +DMBA +400 H.V | $147.33\pm1.11^{b,e,h}$ | $3.64\pm0.13^{b,e,h}$ | $111.83\pm0.44^{b,e,h}$ | $6.77\pm0.01^{b,e,h}$ |
| DMSO +DMBA +200 L.A | $150.47\pm0.31^{b,e,h}$ | $3.00\pm0.04^{b,e,h}$ | $114.14\pm0.43^{b,e,h}$ | $5.91\pm0.01^{b,e,h}$ |
| DMSO +DMBA +400 L.A | $142.50\pm0.65^{b,e,h}$ | $3.69\pm0.08^{b,e,h}$ | $111.00\pm0.40^{b,e,h}$ | $6.84\pm0.07^{b,e,h}$ |
| DMSO +DMBA +400 H.V +400L.A | $140.00\pm1.47^{b,e,h}$ | $3.57\pm0.06^{b,e,h}$ | $109.50\pm0.65^{b,e,h}$ | $7.16\pm0.06^{b,e,h}$ |

**Data expressed as mean ± SEM. Values in the same column bearing different superscript are significant at $p<0.05$**

**Table 4b: Effects of ethanol extracts of *H. verticillata* and *L. aestuans* on renal function indices of DMBA induced leukemia in wistar albino rats**

| Groups | Urea(mmol/L) | Creatinine(mg/dl) |
|---|---|---|
| Normal control | $2.54\pm0.09^{a}$ | $0.73\pm0.05^{a}$ |
| DMSO control | $2.80\pm0.14^{a,d}$ | $0.87\pm0.02^{a,d}$ |
| Negative control | $10.17\pm0.25^{b,e,g}$ | $1.98\pm0.02^{b,e,g}$ |
| Positive control | $7.29\pm0.02^{b,e,h}$ | $1.61\pm0.01^{b,e,h}$ |
| DMSO +DMBA +200 H.V | $8.77\pm0.09^{b,e,h}$ | $1.79\pm0.00^{b,e,h}$ |
| DMSO +DMBA +400 H.V | $8.01\pm0.07^{b,e,h}$ | $1.71\pm0.01^{b,e,h}$ |
| DMSO +DMBA +200 L.A | $8.83\pm0.03^{b,e,h}$ | $1.77\pm0.01^{b,e,h}$ |
| DMSO +DMBA +400 L.A | $7.59\pm0.02^{b,e,h}$ | $1.67\pm0.01^{b,e,h}$ |
| DMSO +DMBA +400 H.V +400L.A | $7.60\pm0.02^{b,e,h}$ | $1.65\pm0.00^{b,e,h}$ |

**Data expressed as mean ± SEM. Values in the same column bearing different superscript are significant at $p<0.05$**


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**Effects of ethanol extracts of *H. verticillata* and *L. aestuans* on hepatic function indices of DMBA induced leukemia in wistar albino rats**

Tables 5a and 5b showed the effects of ethanol extracts of *H. verticillata* and *L. aestuans* on hepatic function of DMBA induced leukemia in wistar albino rats in the leukemia induced, non-induced and induced /treated groups. There were significant ($p<0.05$) elevations in the direct and total bilirubin and total protein levels upon induction of experimental leukemia when compared to the non-induced groups (1 and 2), whereas the values of albumin dropped ($p<0.05$).

The elevations became significant ($p<0.05$) decreased after the treatment with the extracts and standard drugs. These decreases were higher in groups 4 and 9 when compared to groups 4-8. The serum enzyme activity of AST, ALT, and ALP Biomarkers of Liver tissue wellbeing were raised upon leukemia induction but the treatment with the standard drug and the extracts at both doses produced a significant ($p<0.05$) reduction in the levels of these enzymes relative to the negative control pointing to restorative ability of the extracts and recovery from the cancer of the blood.

**Table 5a: Effects of ethanol extracts of *H. verticillata* and *L. aestuans* on hepatic function indices of DMBA induced leukemia in wistar albino rats**

| Groups | Total Bilirubin (µmol/L) | Direct Bilirubin (µmol/L) | Total Protein (g/dL) | Albumin (g/dL) |
|---|---|---|---|---|
| Normal control | $11.29\pm0.01^{a}$ | $2.19\pm0.07^{a}$ | $7.78\pm0.11^{a}$ | $3.81\pm0.02^{a}$ |
| DMSO control | $11.71\pm0.21^{a,d}$ | $2.63\pm0.02^{a,d}$ | $7.93\pm0.03^{a,d}$ | $3.82\pm0.04^{a,d}$ |
| Negative control | $37.05\pm0.44^{b,e,g}$ | $7.76\pm0.03^{b,e,h}$ | $10.52\pm0.22^{b,e,g}$ | $2.77\pm0.02^{b,e,g}$ |
| Positive control | $20.02\pm0.27^{b,e,h}$ | $4.89\pm0.02^{b,e,h}$ | $8.44\pm0.02^{b,d,h}$ | $3.42\pm0.04^{b,d,h}$ |
| DMSO +DMBA +200 H.V | $24.85\pm0.12^{b,e,h}$ | $6.20\pm0.01^{b,e,h}$ | $9.20\pm0.10^{b,e,h}$ | $3.00\pm0.01^{b,e,g}$ |
| DMSO +DMBA +400 H.V | $22.93\pm0.03^{b,e,h}$ | $5.31\pm0.02^{b,e,h}$ | $8.77\pm0.04^{b,e,h}$ | $3.25\pm0.02^{b,e,h}$ |
| DMSO +DMBA +200 L.A | $24.09\pm0.09^{b,e,h}$ | $6.15\pm0.02^{b,e,h}$ | $9.01\pm0.04^{b,e,h}$ | $2.95\pm0.02^{b,e,g}$ |
| DMSO +DMBA +400 L.A | $21.03\pm0.21^{b,e,h}$ | $5.10\pm0.01^{b,e,h}$ | $8.69\pm0.02^{b,e,h}$ | $3.25\pm0.02^{b,e,h}$ |
| DMSO +DMBA +400 H.V + 400 L.A | $20.24\pm0.16^{b,e,h}$ | $4.98\pm0.01^{b,e,h}$ | $8.57\pm0.03^{b,e,h}$ | $3.36\pm0.02^{b,e,h}$ |

**Data expressed as mean ± SEM. Values in the same column bearing different superscript are significant at $p<0.05$**

**Table 5b: Effects of ethanol extracts of *H. verticillata* and *L. aestuans* on hepatic function Indices (hepatic enzymes) of DMBA induced leukemia in wistar albino rats**

| Groups | AST (IU/L) | ALT(IU/L) | ALP(IU/L) |
|---|---|---|---|
| Normal control | $23.92\pm0.09^{a}$ | $21.78\pm1.28^{a}$ | $92.39\pm1.01^{a}$ |
| DMSO control | $26.65\pm0.26^{a,d}$ | $26.59\pm0.61^{a,d}$ | $97.67\pm1.03^{a,d}$ |
| Negative control | $166.26\pm0.88^{b,e,g}$ | $171.25\pm1.89^{b,e,g}$ | $331.10\pm3.44^{b,e,g}$ |
| Positive control | $70.18\pm0.31^{b,e,h}$ | $78.19\pm0.91^{b,e,h}$ | $147.81\pm1.77^{b,e,h}$ |
| DMSO +DMBA +200 H.V | $108.17\pm0.96^{b,e,h}$ | $117.39\pm1.41^{b,e,h}$ | $205.75\pm2.78^{b,e,h}$ |
| DMSO +DMBA +400 H.V | $84.28\pm0.75^{b,e,h}$ | $94.42\pm1.13^{b,e,h}$ | $179.27\pm3.20^{b,e,h}$ |


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| | | | |
|---|---|---|---|
| DMSO +DMBA +200 L.A | 110.96±0.62$^{b,e,h}$ | 110.52±0.91$^{b,e,h}$ | 194.77±2.06$^{b,e,h}$ |
| DMSO +DMBA +400 L.A | 76.01±0.43$^{b,e,h}$ | 86.94±1.23$^{b,e,h}$ | 165.51±2.00$^{b,e,h}$ |
| DMSO +DMBA +400 H.V +400L.A | 71.64±1.15$^{b,e,h}$ | 81.82±0.85$^{b,e,h}$ | 149.24±3.66$^{b,e,h}$ |

**Data expressed as mean ± SEM. Values in the same column bearing different superscript are significant at p<0.05**

### Effects of ethanol extracts of *H. verticillata* and *L. aestuans* on oxidative stress markers of DMBA induced leukemia in wistar albino rats

Table 6 compares the effects of ethanol extracts of *H. verticillata* and *L. aestuans* on oxidative stress markers of DMBA induced leukemia in wistar albino rats. Upon the induction of experimental leukemia, CAT, SOD and GSH levels significantly (p<0.05) decreased when compared to the non-induced groups. Conversely, the decreased levels of CAT, SOD and GSH just after induction of leukemia were significantly (p<0.05) elevated after administration of the plants extracts and standard drug across groups 4 to 9 when compared to the negative control group.

**Table 6: Effects of ethanol extracts of *H. verticillata* and *L. aestuans* on oxidative stress markers of DMBA induced leukemia in wistar albino rats**

| Groups | CAT (U/ml) | SOD (µg/ml) | GSH (mU/ml) |
|---|---|---|---|
| Normal control | 0.40±0.03$^{a}$ | 0.59±0.06$^{a}$ | 4.93±0.03$^{a}$ |
| DMSO control | 0.38±0.05$^{a,d}$ | 0.45±0.03$^{a,d}$ | 3.54±0.08$^{a,d}$ |
| Negative control | 0.13±0.05$^{b,e}$ | 0.16±0.01$^{b,e,g}$ | 0.76±0.28$^{b,e,g}$ |
| Positive control | 0.30±0.04$^{b,e,h}$ | 0.28±0.00$^{b,e,g}$ | 3.03±0.16$^{b,e,h}$ |
| DMSO +DMBA +200 H.V | 0.25±0.05$^{b,e,h}$ | 0.22±0.00$^{b,e,g}$ | 2.03±0.06$^{b,e,h}$ |
| DMSO +DMBA +400 H.V | 0.28±0.07$^{b,e,h}$ | 0.25±0.00$^{b,e,g}$ | 2.74±0.24$^{b,e,h}$ |
| DMSO +DMBA +200 L.A | 0.26±0.03$^{b,e,h}$ | 0.22±0.01$^{b,e,g}$ | 2.24±0.07$^{b,e,h}$ |
| DMSO +DMBA +400 L.A | 0.29±0.14$^{b,e,h}$ | 0.26±0.00$^{b,e,g}$ | 2.85±0.09$^{b,e,h}$ |
| DMSO +DMBA +400 H.V +400L.A | 0.30±0.02$^{b,e,h}$ | 0.28±0.00$^{b,e,g}$ | 2.86±0.23$^{b,e,h}$ |

**Data expressed as mean ± SEM. Values in the same column bearing different superscript are significant at p<0.05**

### Effects of ethanol extracts of *H. verticillata* and *L. aestuans* on percentage body weight changes of DMBA induced leukemia in wistar albino rats

Table 7 displays effects of administration of ethanol extracts of *H. verticillata* and *L. aestuans* on percentage body weight changes of DMBA induced leukemia in wistar albino rats.

The result indicated significant (p<0.05) decreases in the percentage change in body weights of the rats in days 7, 14 and 21 when compared to groups 1 and 2 respectively. However, at the 28$^{th}$ day of treatment, apart from group 3 with significantly (p<0.05) decreased percentage change in their body weights, groups 4 to 9 indicated significantly (p<0.05) increased percentage change in their body weights when compared to groups 1 and 2 respectively.


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Although, there was also an increase in body weights of rats of group 2 when compared to those of group 1 but this increase was not significant ($p<0.05$).

**Table 7: Effects of oral administration of ethanol extracts of *H. verticillata* and *L. aestuans* on percentage body weight changes of DMBA induced leukemia in wistar albino rats**

| Group | Day 1 Value (g) | Day 7 Weight (g) | % increase | Day 14 Weight (g) | % increase | Day 21 Weight(g) | % increase | Day 28 Weight (g) | % increase |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 32.29 | 62.50 | 93.56 | 86.25 | 167.11 | 108.75 | 26.09 | 129.25 | 49.86 |
| 2 | 32.43 | 56.00 | 72.68 | 79.00 | 143.6 | 96.00 | 21.52 | 117.00 | 48.10 |
| 3 | 30.76 | 35.75 | 16.22 | 39.25 | 27.6 | 42.50 | 8.28 | 46.50 | 18.47 |
| 4 | 30.83 | 35.25 | 14.34 | 39.50 | 28.12 | 59.00 | 49.37 | 79.50 | 101.27 |
| 5 | 30.69 | 36.00 | 17.3 | 39.50 | 28.71 | 52.00 | 31.65 | 63.50 | 60.76 |
| 6 | 31.78 | 36.75 | 15.54 | 41.25 | 29.8 | 53.75 | 30.30 | 70.75 | 71.52 |
| 7 | 31.37 | 36.25 | 15.56 | 40.50 | 29.1 | 50.75 | 25.31 | 66.50 | 64.20 |
| 8 | 31.23 | 36.75 | 17.68 | 40.75 | 30.48 | 53.50 | 31.29 | 69.00 | 69.33 |
| 9 | 31.55 | 36.75 | 16.48 | 41.25 | 30.74 | 55.00 | 33.33 | 74.25 | 80.00 |

**Histopathological report of the effect of ethanol leaf extract of *H verticillata and L. aestuans* in DMBA induced leukemia in wistar-rats**

Plate 11-22 shows the photomicrograph of liver and kidney tissues as seen in X100 H&E stain depicting normal hepatocytes in the normal, induced without treatment, induced and treated with various percentage of the 2 extracts as well as the standard drug respectively.

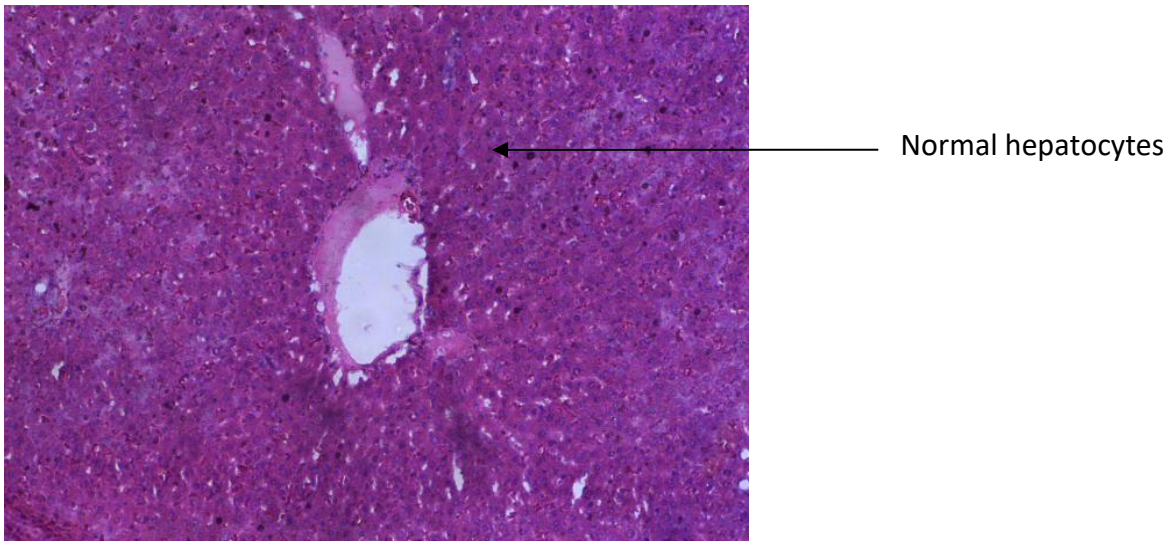


Plate 11: Photomicrograph of Normal liver tissue 100 X H &E staining


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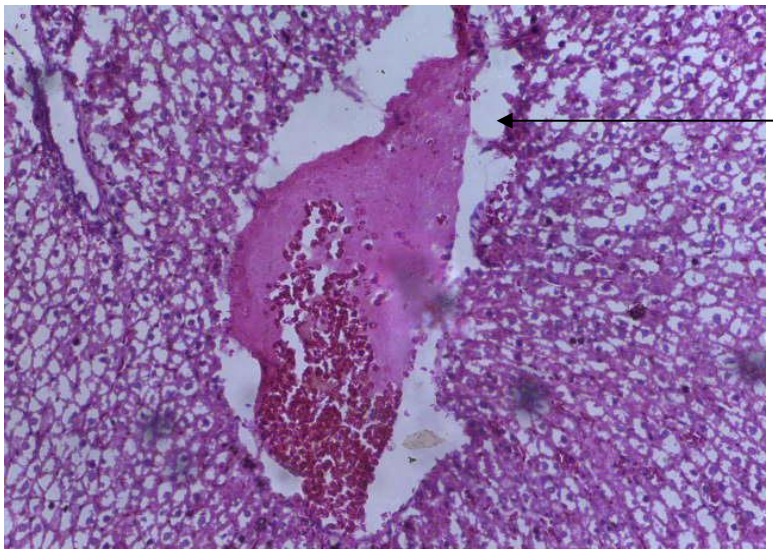


Plate 12: Photomicrograph of DMBA induced group. Magnification ×100. H&E stain.

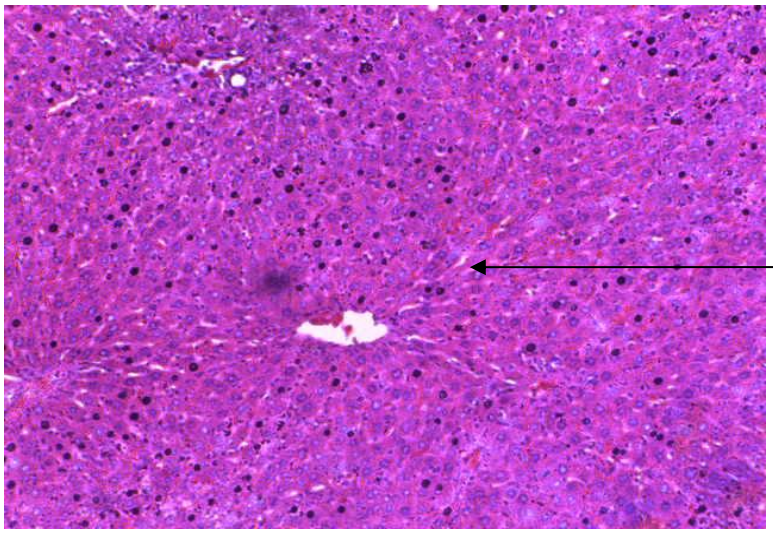


**Plate 13: Photomicrograph of liver tissue of group treated with standard anti-leukemic drug. Magnification ×100. H&E stain**.


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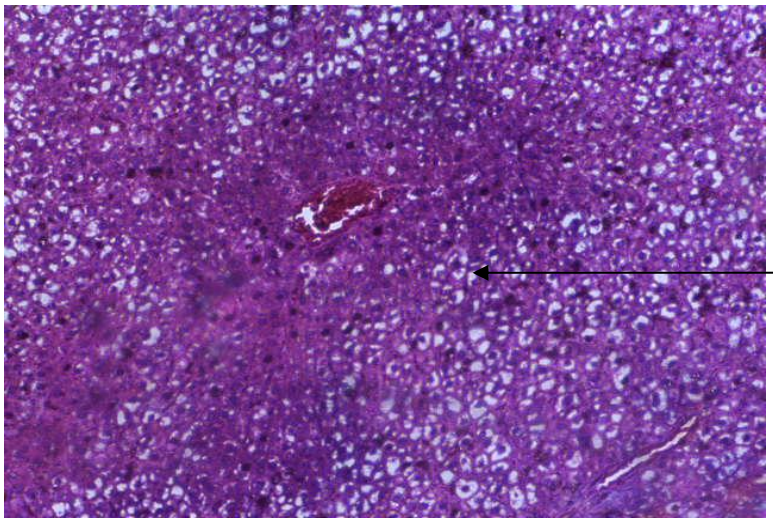


**Plate 14: Photomicrograph of liver tissue of group treated with 400mg of *L. aestuans*. Magnification ×100 H&E stained.**

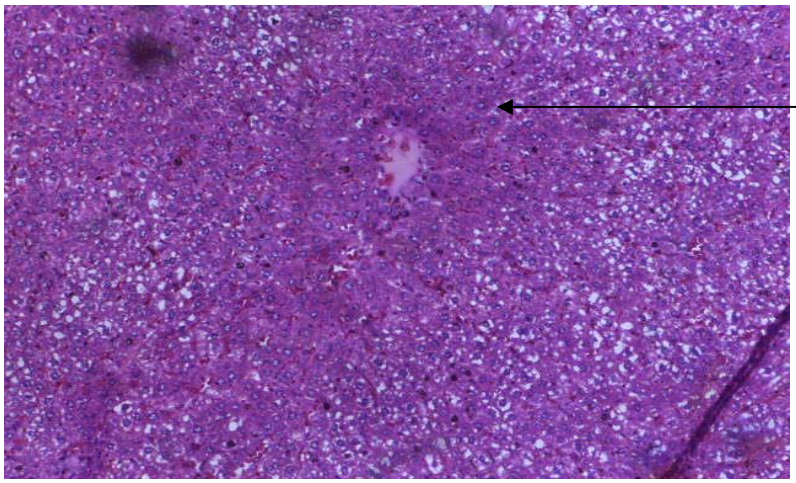


**Plate 15: Photomicrograph of DMBA induced and treated 400mg of *H. verticillata*. 100 X H &E stain.**


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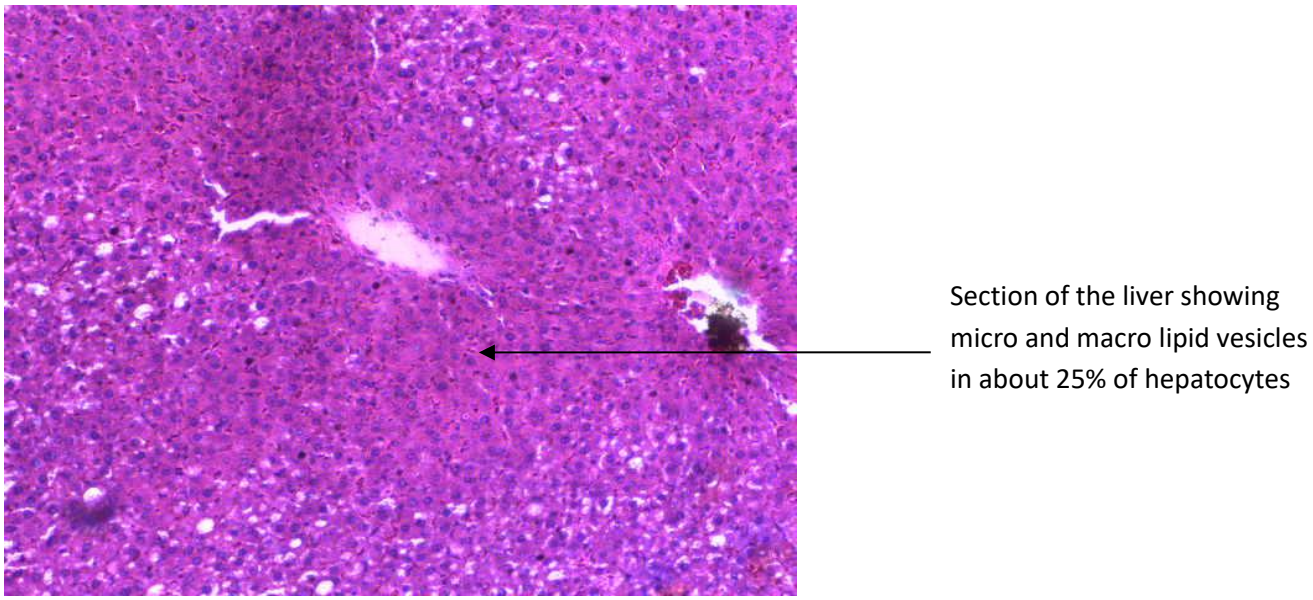


**Plate 16: Photomicrograph of DMBA induced and treated with combined 400mg of *H. verticillata* and *L. aestuans*. 100 X H &E stain.**

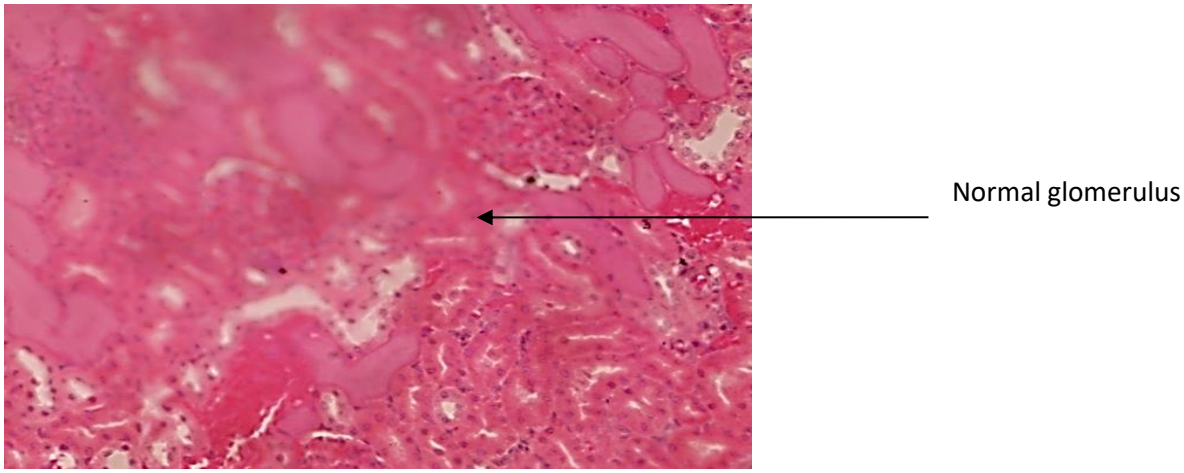


**Plate 17: Photomicrograph of a normal kidney (Group 1: Control Group), 100 X H &E stain.**



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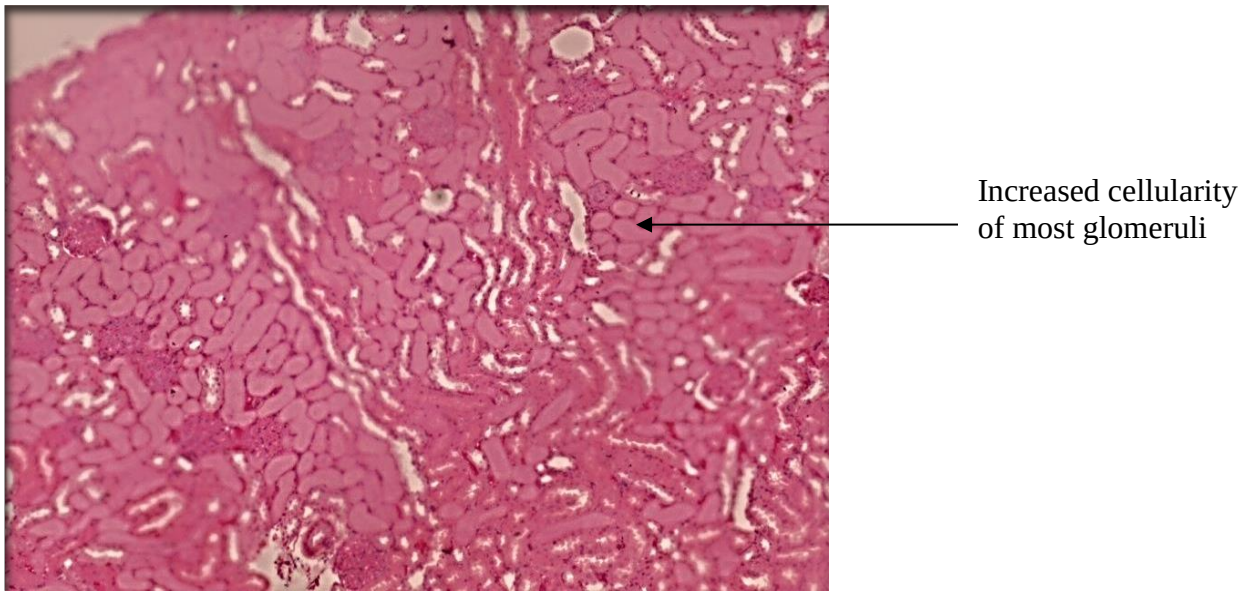


**Plate 18: Photomicrograph of Renal tissue of DMBA induced leukemia. 100X outlined by H&E stain**.

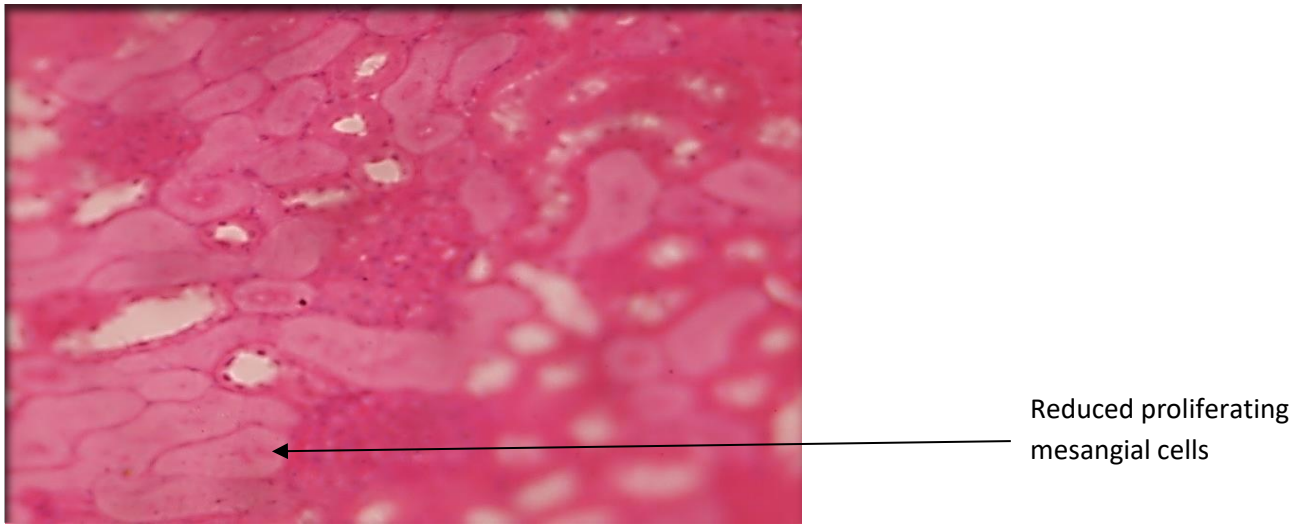


**Plate 19: Photomicrograph of kidney tissues of rat induced with DMBA but treated with standard drug, 100X outlined by H&E stain.**



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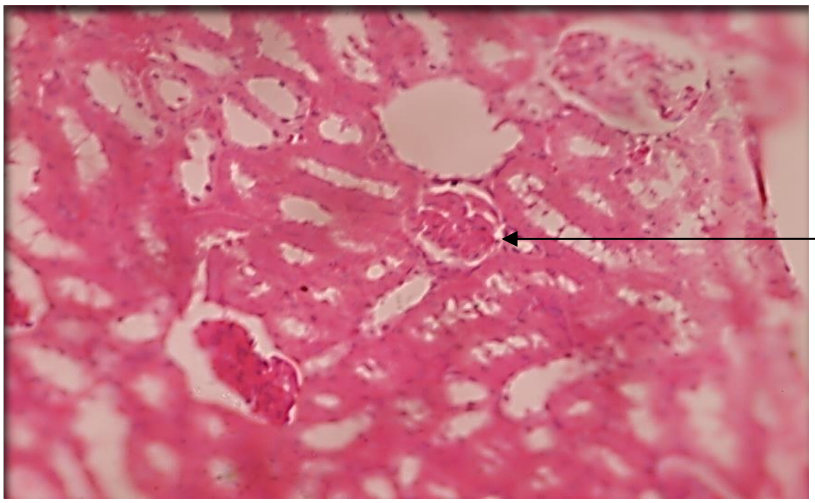


**Plate 20: Photomicrograph of kidney tissues of rat induced with DMBA treated with 400mg of *H. verticillata*, 100X outlined by H&E stain.**

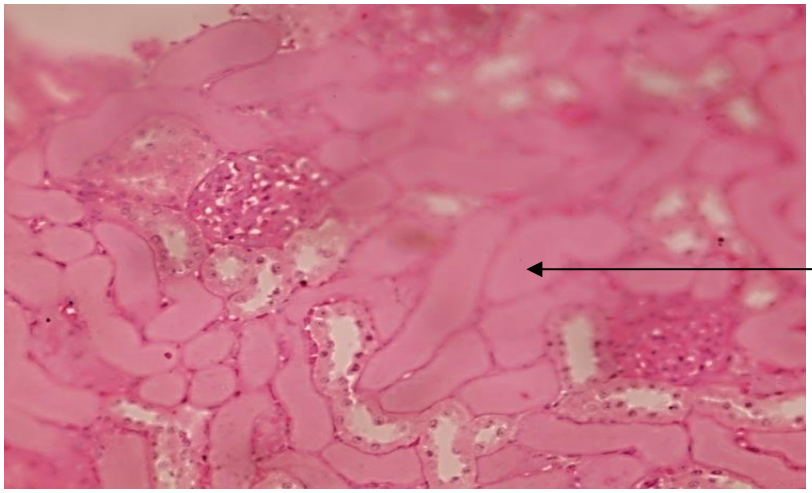


**Plate 21: Photomicrograph of kidney tissues of rat induced with DMBA treated with 400mg of *L. aestuans*, 100X outlined by H&E stain.**



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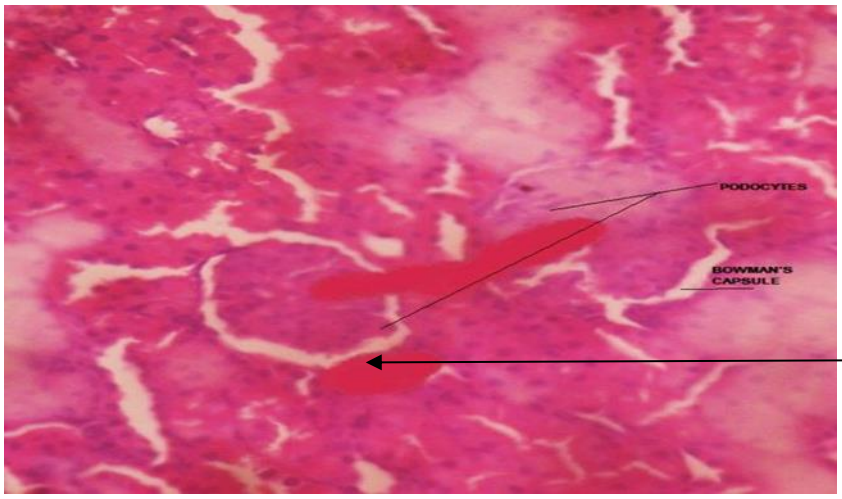


**Plate 22: Photomicrograph of kidney tissues of rat induced with DMBA and treated with 400mg of *H. verticillata* and *L. aestuans*, 100X outlined by H&E stain.**

## DISCUSSION

The abnormal cell proliferation with the propensity to spill to other cellular compartment has been associated with cancerous condition whose presentation differs based on different types that exists. Cancer has been reported as the leading global cause of childhood and adult mortality with an estimated 429000 in recent study with breast cancer and leukemia taking the lead. This has equally led to the derangement in hematological and biochemical indices together with histopathological abnormalities. Complementary medicine having known curative potentials, easy to procure and with reduced side effects has led to high demand on them. These demands could be linked to their proven pharmaceutical potentials in many health conditions [2-4, 9]. It is then hopeful that the comprehensive study of the hepato-renal and hematological ameliorative potentials of the extracts of *H. verticillata* and *L. aestuans* that abound in our local environment may be of utmost importance. Of course, this discovery may present these plants as a possible remedy to a wide myriad of hematological and organ abnormalities.

The acute toxicity test from this study shows that the ethanol extract of *H. verticillata* and *L. aestuans* is more than 4000mg/kg body weight of the rats. The inference is that the animals administered with the extracts showed no incidence of death after observation for a period of 48 hours. It is an established fact in toxicology that any chemical agent with oral $LD_{50}$ of 4000mg/kg or more are taken as practically innocuous [18-19]. From the foregoing, it shows that the plants extracts may be safe and contain useful important agents.

The findings on some haematological parameters revealed that a significant decrease in the levels of PCV, HB, neutrophils, eosinophils and platelets, raised levels of lymphocytes, basophil, and total white blood cell count following experimental induction of leukemia were significantly and respectively reversed. Lymphocytosis, Basophilia and Neutropenia are common presentation in leukemias and cancer-related lymphocytosis and neutropenia have a high mortality rate due to susceptibility to infectious diseases, and combined with fever is considered an oncological emergency [20]. The regulation of neutrophil production by granulocyte colony stimulating factors (G-CSF) is in part known to be controlled by


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interleukins (IL) -17 and -23. This is in agreement with Blériot *et al.*, [20]; Suzuki & Hirano, [21] and To & Villatoro, [22]. Therefore, therapeutic strategies focused on inducing apoptosis and cellular arrests are of clear significance. Phytochemicals (Tannins, Alkaloids, steroids) and essential oils have shown to induce both intrinsic and extrinsic apoptotic pathways [23]. Gupta *et al.*, [23], tested the anti-tumor properties of a camphene isolated from essential oils of *Piper cernuum* in melanoma cells and demonstrated that this compound was able to induce apoptosis through the caspase-3 pathway activation as well as activating the endoplasmic reticulum. Juxtaposing this established fact with the forgoing result of the present study, ethanol extracts of *L. aestuans and H. verticillata* possess beneficial phytonutrients as reported by Odo *et al.*, [9] that could be said to either regulate these granulocyte colony stimulating factors, induce apoptotic pathway, interleukins (IL-17 and IL-23) productions or may be eliciting exogenous IL-17 and IL-23 effects. Thus, these extracts may be helpful in ameliorating neutrophils and lymphocytes deranged conditions, like that of leukemia. The peripheral blood film from the current study on DMBA induced leukemia showed the presence of anisocytosis, poikilocytosis and appearance of basophils compared to the control group. Upon treatment with the extract of *Hydrocotyle verticillata* and *Laportea aestuans*, these alterations were subsequently reversed. Routine hematological assessment has become a diagnostic index for monitoring the medical and clinical status of blood borne conditions including leukemia. This agrees with Kabeel *et al*. [4], which stated that leukemogenic effects of DMBA have been linked to hematological imbalance in human and animal models where reduced red blood cells, hematocrit contents, hemoglobin and platelets counts are routine occurrence. The abnormal incidence of anisocytosis and poikilocytosis as seen in the induced groups could be an indication of a hematological abnormality. This is in agreement with Kabeel *et al.*, [4]. The occurrence of blasts cells in the peripheral blood film in the induced groups in the present study is an indication of undifferentiated blood forming cells in the bone-marrow which were subsequently reduced upon the administration of *Hydrocotyle verticillata* and *Laportea aestuans* leaves extract. Our finding here has not only scientifically proven that the ethanol leaf extracts of *L. aestuans and H. verticillata* are potent anti-leukemic agents but also validates an earlier report which stated that some herbs can be strong moderators of hematological indices particularly, leukocyte count [24]. This reveals that the phytochemical and essential oils in *Hydrocotyle verticillata* and *Laportea aestuans* extracts may have possibly acted to moderate leucopoiesis.

The findings of the present study on renal function indices in the experimentally induced leukemia rats, reveal changes in a no specific manner; while urea, creatinine, sodium and chloride levels showed significant ($p<0.05$) increase, potassium and calcium levels showed a significantly reduced levels upon induction of leukemia. These alterations in renal function indices were significantly ($p<0.05$) reversed after treatments with the standard anti-leukemic drug, ethanol extracts of *L. aestuans and H. verticillata* leaves. This alteration could be that the leaves extracts possess properties that induce the alteration of the electrolytes levels that induces the depressant or sedative effects, which may have affected glomerular filtrations and thereby decreasing the excretion of substances in the urine including the electrolytes. The increase in potassium ion and decrease in sodium ion in the present study supports the report of Ogunka-Nnoka *et al.*, [25], on the renal protective effect of administration of root bark of *vitex doniana*. The polycyclic aromatic hydrocarbon, 7, 12-dimethylbenz[α]anthracene (DMBA), is a known immunosuppressor and potent organ-specific carcinogen [26]. Apart from mainly causing immunotoxicity through the microsomal epoxide hydrolase (mEH) in the spleen or related lymphoid organs, expectedly DMBA may have also elicited varying


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degrees of multi-organ toxicity [26], hence the abnormalities in the renal function. The ameliorative effect of *L. aestuans and H. verticillata* leaves extract on the dys-regulated renal function indicates that the extracts may possess promising therapeutic agents with vast beneficial potentials. This agrees with the submission of Ogunka-Nnoka *et al.,* [25]. Of course, the serum levels of urea and creatinine indicates the glomerular filtration rate (GFR) of the kidneys [27]. It has been observed that a wide variety of renal disease with different permutation of glomerular, tubular, interstitial, or vascular damage can cause an increase in serum urea and creatinine [25]. Thus, a possible prognosis of raised levels of these substances is a dysfunctional state of the kidneys following the induction of the experimental leukemia. Therefore, the ameliorative effect of the extracts shows that the extracts possess a reasonable level of renal protective properties.

The present study on liver enzymes biomarkers showed that AST, ALT, and ALP levels were considerably elevated upon induction of leukemia in rats, of course, this is indicative of severe liver toxicity by the DMBA [1]. The liver is arguably the most vital body organs, due to its multi-biochemical roles in the metabolic pathways. This is in line with the position of Kabeel *et al*., [4]. Acute and chronic liver abnormalities are a global challenge, and therapeutic interventions for these diseases do pose serious issue and may have limited efficiency [25, 28]. Liver diseases may be fatal posing a grave challenge to the populace. It has thus been reported that developing therapeutically effective agents from natural products may reduce the risk of toxicity when these drugs are used in a clinical setting [28, 29]. It is evidence from the finding of this study particularly the significantly ($p<0.05$) reversal of the raised levels of the liver enzymes by the plants, it is suggestive to say that the extract of these plant possess hepato-protective properties. This implies that the leave extracts of the plants may be a natural therapeutical active agent capable of reducing the risk of hepatotoxicity and much so, these effects exhibited by H. verticillata and L. aestuans extracts could as well be attributed to the various medical roles of phytonutrients in the leaves.

In the present study, treatment with extract of *Hydrocotyle verticillata* and *Laportea aestuans* significantly ($p<0.05$) increased GSH, CAT, SOD levels and facilitated the rapid and efficient consumption of ROS generation in DMBA induced leukemia in rats. GST, CAT, and SOD are soluble enzyme located in the cytosol, which plays a significant function in the detoxification of xenobiotics [30]. It increases the solubility of hydrophobic substances and metabolizes toxic compounds to non-toxic ones, which means they have an increasing protective activity of the liver [30]. The thiol group located on protein in oxidant enzymes constitutes the major in vivo antioxidant and reducing group in the body fluid. This is in agreement with Sukalingam *et al*., [30]. Therefore, antioxidant status is indicative of the concentration of total thiol where the low protein thiol ratio correlates with a rise in peroxides and advanced oxidation protein products [30]. The decrease in CAT, SOD, and GSH levels in this study suggests the induction of oxidative stress due to DMBA induced reactive metabolites that subsequently gave rise to oxidant-linked protein damage. However, supplementation by ethanol extracts of *Hydrocotyle verticillata* and *Laportea aestuans* as well as the standard drug in the present study significantly ($p<0.05$) abolished the reduced levels of these oxidant markers and restored their status when compared to negative control group. These bioactive constituents (essential oils constituents and phytochemicals) found in leaves of *Hydrocotyle verticillata* and *Laportea aestuans* may have acted to modulate the molecular targets of leukemia and induce cytoprotective enzymes that act in a coordinated fashion to detoxify and remove dangerous reactive substances formed by leukemic cells


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causing agents. The phytochemicals and other bioactive compounds present in the leave extracts may have acted as exogenous antioxidant enzyme synthesis stimulatory agents; this finding validates an earlier claim by Altemimi *et al*., [31] that submitted that plant rich in beneficial phytochemicals can be a good source of antioxidants.

The percentage change in body weights of the rats in the present study showed a marked increase in the test animals (groups 4 to 9) compared to groups 1 and 2 respectively upon administration of *H. verticillata* and *L. aestuans* extracts after induction of leukemia. This implies that, the active ingredients in *H. verticillata* and *L. aestuans* extracts may also possess body weight enhancing properties. According to Gupta *et al.,* [32], some uncontrollable factors affecting body weight changes may include growth determinants, genetic makeup, gender, and age and that other factor that influence body weight over which the individual can potentially control include level of physical activity, diet, and some environmental and social factors. Thus, *H. verticillata* and *L. aestuans* leave extracts as possible dietary and medicinal source, may have increasingly stimulated adiposity in the leukemic rats.

In the present study, hepatic steatosis and renal necrosis was induced by a series of subcutaneous injection of DMBA. It is important to note that DMBA is normally metabolized by CYP2E1 releasing ROS which attacks cells DNA, lipids, and proteins [33]. This is turn may lead to centrilobular necrosis, hepatic fibrosis, collagen and fibronectin deposition, Bowman's capsule dilation, deterioration and detachment of epithelial renal lining. The metabolism of DMBA have been noted to induce tumor formation, through a primary step carried out by hepatocytes to give a detoxification and bioactive products [33-35]. Upon treatment with the extracts and the standard drug, these distortions were moderately reversed with the group administered with combined dose of 400mg/kgbw and the standard drug showed much effects compared with the negative control group. Afsar *et al.*, [36], had reported the excellent hepato-renal protective effects of some selected phytochemicals against chemical and drug induced kidney and liver alterations and posited that they are potential candidates in therapy by functioning as a potent quencher of radical species in the kidney and liver thus, reducing the toxic effects at Histological levels.

The findings from this study have thus further shown that Histological alterations orchestrated by DMBA treatment may be ameliorated by the extracts of *Hydrocortyle verticillate* and *Laportea aestuans* which may be due to the abundance of polyphenols. Polyphenolic compounds have been shown to boost cellular antioxidants enzymes, enhance GSH concentrations, and free radical quenching [37]. This findings from this current study revealed that treatments with ethanol leaves extracts of *Hydrocortyle verticillate* and *Laportea aestuans* have a strong hepato-renal protective effects on DMBA induced oxidative damage and necrosis in rats.

## CONCLUSION

The active ingredients present in these plants have demonstrated hepato-renal protective properties against DMBA induced-leukemia. The plant extracts have proven to reverse lowered levels of packed cell volume, hemoglobin, oxidative stress and high lymphocyte, total white blood cell and basophils.


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## ETHICAL APPROVAL

The experiment was conducted in accordance with international standard protocol while ethical approval was granted by the ethical clearance unit of the University.

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## CONFLICTS OF INTEREST

We hereby declare that there is no conflicts interest

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