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Original Article

Ethanolic, n-hexane and aqueous partitioned extracts of *Xylopia aethiopica* fruit modulated inflammatory responses in turpentine oil induced acute inflammation in male Wistar rats

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ABSTRACT

Background: Folkloric medicine has emphasized the uses of indigenous spices and plants in the prevention of oxidative and inflammatory disorders from time immemorial. Inflammatory responses are patho-physiological immune mediated reactions, which are fundamental in providing protection against tissue injury and opportunistic infections, and have been implicated in the pathology of various degenerative and metabolic disorders, organ function losses and death. **Objective**: Fruit of *Xylopia aethiopica*, a renowned spice in soup/broth brewing in many homes in Africa was investigated for the anti-oxidative and anti-inflammatory potentials of the crude ethanolic extract, aqueous and n-hexane partitioned extracts in turpentine oil-induced acute inflammation in male Wistar rats.
Materials and methods: Sixty three rats, aged 7-8 months old were allotted into nine groups and two groups received 100 and 200 mg/kg body weights doses of either the ethanolic, aqueous or n-hexane extract for 14 days and subsequently, 0.1ml of turpentine oil. The other three groups received respectively distilled water, 0.1ml of turpentine oil and distilled water and 0.1ml of turpentine oil and diclofenac. Various parameters, such as: plasma levels of tumour necrosis factor-alpha, C-reactive proteins, albumin, and peroxidase, gamma-glutamyl transpeptidases, total thiols, superoxide dismutase, catalase and malondialdehyde were determined in the plasma, livers and spleens.
Results: The administration of turpentine oil elevated the levels of oxidative and inflammatory markers, while the activities of antioxidant enzymes were reduced ($p<0.05$). However, the rats administered the various extracts of *X. aethiopica* fruit recorded significant ameliorations ($p<0.05$) in the oxidative and inflammatory markers. The crude ethanolic extract of *X. aethiopica* fruit presented the most promising results with no consistent variations ($p>0.05$) between the doses.
Conclusion: From the foregoing, our study have indicated scientifically the modulation of inflammatory responses by *X. aethiopica* fruit extracts, and thus recommend its regular use as spice and alcoholic tinctures for improved quality life and protection against inflammatory disorders.

Keywords: Ethanolic extract, inflammatory responses, modulation, oxidative and inflammatory markers, improved quality life, *Xylopia aethiopica*.

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INTRODUCTION

Inflammation is a physiological immune reaction that essentially plays a fundamental role in providing protection against tissue injury and infections. It arises as cellular and molecular response to a pathogenic agent or to a tissue injury in order to get rid of the causative agent and to set off the healing process (Medzhitov, 2008). Inflammation can be acute or chronic depending on the type of reaction that triggered it, the intensity of such reaction and the participants involved in mediating the response. Acute inflammation occurs as an instant and rapid response to pathogen or tissue injury which normally lasts for a short period of time in which the damage may be purely physical and or it may involve the activation of an immune response (Butcher and Picker, 1996). Chronic inflammation develops as a complex long term response to an importunate stimulus that is mediated by the persistence induction of acute phase proteins (Oyewo *et al*., 2013). Cellular damage arising from reactive oxygen species (ROS) has been implicated in the aetiology and patho-physiology of many human diseases associated with inflammatory disorders (Repetto and Llesuy, 2002; Surh and Fergusson, 2003).

Inflammation occurs in different tissues in regulatory manners by the activities of anti-inflammatory mediators such as interleukin-10 (IL-10), tumour growth factor-β (TGF-β) (Opal and DePalo, 2000), pro-inflammatory receptor antagonist such as IL-1RA, tumour necrosis factor (TNF) receptor, apoptosis of pro-inflammatory cells (such as macrophages and granulocytes) etc. However, if the regulatory mechanisms of immune system fail to control the excessive inflammatory response by the counteracting mechanisms or when the mediators of inflammatory reactions are continuously exposed to, various dysfunctional manifestations could occur with disastrous consequences, such as autoimmune disorders, inflammatory pathologies and malignancy. All forms of inflammation beyond the physiological requirement are always kept in check through the activities of various anti-inflammatory agents such as steroids, non-steroidal anti-inflammatory agents, intravenous immunoglobulins, immunosuppressing cells, neutralising monoclonal antibodies, anti-inflammatory cytokines and also various natural compounds like phytotherapeutics (Balkwill and Mantovani, 2010). Thus, modulation of body's integrated response to an antigen through stimulation or suppression has been suggested to be effective in maintaining a disease free state (Bafna and Mishra, 2005; Oyewo *et al*., 2013).

In folkloric medicine, several plant regimens have been alleged to abate or modulate the occurrences and severities of inflammatory responses, giving insight to the prospect of managing various conditions associated with inflammatory disorders and diseases. Scientific researchers have reported that some of these herbal regimens improved healing from various diseases due to their antioxidant and anti-inflammatory properties (Kumar *et al.,* 2005; Spelman *et al.,* 2006; Oyewo *et al.,* 2012). Phytotherapy has been a hopeful therapeutic approach in the management of 'oxido-immuno' pathologies, most especially in targeting and influencing various biological pathways linked to patho-physiological conditions, at the same time leaving the healthy tissues unaffected or less affected (Shoskes, 2002; Efferth and Koch, 2010). As a result, there is growing interest in identifying and characterizing phytochemicals with immunomodulatory activities ever since their possible use in treatment and management of diseases has been in existence from ancient times. Therefore, the objective of this study was to assess the antioxidative and anti-inflammatory capabilities of ethanolic extracts of *Xylopia aethiopica* in turpentine-induced acute inflammation in male Wistar rats.

MATERIALS AND METHODS

Materials

Plant materials: The fruit of *Xylopia aethiopica* was collected in August (2015) from farms in North Central Zone of Kwara State, Nigeria. The fruit was authenticated at the Department of Plant Biology, University of Ilorin with the voucher number UIH001/1089.

Experimental animals: Sixty three adult male Wistar rats with average body weight of 265 ± 11g were used for the study. The rats were housed in an experimental animal holding facility in the Department of Biochemistry, University of Ilorin, Nigeria and allowed free access to food and water.

Chemicals: All the chemicals and reagents used in the study were of analytical grade. The enzyme linked immunosorbent assay (ELISA) kits for the determination of tumour necrosis factor-alpha (TNF-α) and C-reactive proteins (CRP) are products of RayBiotech (USA), bromocresol green (Randox, UK), pyrogallol (May and Baker, England), ethanol and n-hexane (Guangdong Guangnua, China).

Preparation of fruit extracts: Dried fruit of *X. aethiopica* was pulverized and stored in tight-seal containers until needed. The powder fruit of *X. aethiopica* was mixed with ethanol for seventy-two hours and solubilised with the aid of an orbital shaker. Filtration of the mixture was done to remove the solutes and extract from the plant

residue. The crude ethanolic extract (ECE) was dried using evaporation under vacuum (rota-evaporation). The resulting extract was partitioned into aqueous and n-hexane fractions as aqueous partitioned extract (APE) and n-hexane (HPE) partitioned extract, respectively.

Animal handling and experimental design: The sixty three (63) Wistar rats were randomly picked into nine groups of seven animals each and allowed to acclimatize for 10 days. From stock solutions of the each extract of *X. aethiopica* fruits, the 100 mg/kg body weight doses of the extracts were prepared by dissolving a calculated weight of the extract in 5% dimethylsulfoxide (DMSO) and then in distilled water. Six (6) groups out of the nine (9) groups of rats were administered orally 100 and 200 mg/kg body weight doses of each fruit extract of *X. aethiopica* for fourteen days at 4:30 GMT ± 30 minutes. On the fifteenth day of the experiment, 0.1 ml of normal saline was administered to the one (1) group from the three (3) unattended groups, while the other eight (8) groups; (six {6} that received extract and two {2} groups from of the three {3} unattended groups of rats, were injected with 0.1 ml of turpentine oil (TURP) to induce acute inflammation. One (1) group from 0.1 ml of turpentine oil (TURP) only administered rats received diclofenac and the other received distilled water. Seventeen hours after the TURP injection, the animals were sacrificed under mild diethyl ether anaesthesia.

Blood was collected from each rat by cardiac puncture into tubes with anticoagulant (heparin) to obtain plasma. Plasma was separated immediately by centrifugation at 930 × *g* for 5 minutes and stored frozen. The liver and spleen were quickly excised, cleansed of blood and tissues, and were homogenised in 0.25M ice-cold sucrose solution and centrifuged at 930 × *g* for 5 minutes. The supernatant of the homogenates were separated and frozen until when needed.

Determination of the concentrations of some makers of inflammation: Plasma levels of CRP, a positive acute phase proteins and TNF-α, a proinflammatory cytokine were quantitatively measured by *in vitro* enzyme linked immunosorbent assay (ELISA). The plasma albumin level, a negative acute phase protein was determined based on the binding of plasma albumin to 3,3',5,5'-tetrabromo-mcresol sulphonephthalein (bromocresol green) according to Doumas *et al*. (1971).

Determination of some markers of oxidative stress: The activities of the enzymes were determined by standard methods. The peroxidase (POD) activity of the homogenates was measured according to the method described by Reddy *et al.* (1995) based on the oxidation of pyrogallol to a coloured product (purpurgallin) in the presence of hydrogen peroxide and monitored kinetically at 420nm using spectrophotometer. Catalase (CAT) activity was assayed based on the formation of unstable intermediate (perchromic acid) when chromic acetate, a reduction product of dichromate in acetic acid, is heated in the presence of H_2O_2 and monitored kinetically at 620nm using spectrophotometer according to the procedure reported by Sinha (1972). The Superoxide dismutase (SOD) activity was determined based on its ability to inhibit the auto-oxidation of epinephrine determined by the increase in absorbance at 480nm as described by Sun and Zigma (1978). Gamma-glutamyl transpeptidase (γ-GT) activity was estimated by the method of Szasz (1969), where γ-GT enzyme reacts with L-γ - glutamyl-3-carboxy-p-nitroanilide (GLUPA) and glycyl-glycine to give L-γ - glutamyl-glycyl-glycine and 5-amino-2-nitrobenzoate. Other biochemical indices determined were reduced glutathione (GSH) and total thiol according to the method described by Sedlak and Lindsay (1958) and malondialdehyde (MDA), the index of lipid peroxidation as described by Buege and Aust (1978)

Statistical Analysis: The results were reported as mean ± standard error mean of five determinations, unless otherwise stated, and analysed using one way analysis of variance (ANOVA) with a Fisher's Least Significant Difference post hoc test to determine significant differences ($p<0.05$) between groups by using SPSS version 17.

RESULTS

The trends obtained in the plasma TNF-α, C-reactive protein and albumin concentrations following the oral administrations of the fruit extracts in the rats are depicted in Figures 1-3. In this study, it was observed that the single injection of turpentine oil (TURP) increased the plasma TNF-α level and the prior administration of the extracts and diclofenac (with the exceptions of HPE at 100 mg/kg body weight and APE at 200 mg/kg body weight) significantly reversed ($p<0.05$) the prior trends in TNF-α concentrations (Fig. 1). In a similar manner, the initial increases recorded ($p<0.05$) in the plasma CRP levels were markedly decreased ($p<0.05$) following the administration of all doses of the fruit extracts of *X. Aethiopica* and diclofenac (Fig. 2). In Figure 3, the injection of TURP, however, gave decreases ($p<0.05$) in the plasma albumin levels, which were also reversed ($p<0.05$) in the rats received the various doses of the fruit extracts.

The patterns recorded in the concentrations total protein (TP), peroxidase (POD), reduced glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), gamma glutamyl transpeptidases (γ-GT), total thiol (TT), and malondialdehyde (MDA) in plasma, liver and spleen are presented in Tables 1-3. The oxidative stress indices in the plasma depicted decreases ($p<0.05$) in GSH, total thiol and total protein concentrations and increases ($p<0.05$) in the MDA and γ-GT in untreated TURP injected rats, which were ameliorated markedly ($p<0.05$) in rats administered the various doses of the fruit extracts of *X. Aethiopica* (Table1) with variations ($p<0.05$) within doses and extracts. In Table 2 and 3 respectively, the oxidative stress indices in the liver and spleen also presented very close and similar patterns to the trends obtained in the plasma. However, the variations ($p<0.05$) were not consistent between the doses of the extracts.

Table 1. Plasma concentrations of MDA, total protein, reduced glutathione, total thiol and γ-GT in fruit extracts of *X. Aethiopica* TURP injected rats

Group	MDA (µmol/l)	Total Protein (g/l)	GSH (µmol/ g of protein)	Total Thiol (µmol/ g of protein)	γ-GT (U/ g of protein)
Control	9.29±0.77^{a}	58.62±2.53^{a}	1.87±0.18^{a}	4.11±0.20^{ac}	0.04±0.01^{a}
Induced control	16.35±0.64^{b}	36.30±4.17^{b}	1.27±0.21^{b}	3.19±0.71^{a}	0.11±0.01^{b}
HPE100mg/kg	11.60±0.85 ^{c}	45.99±4.37^{ab}	1.42±0.35^{ab}	5.80±0.82^{cb}	0.06±0.01^{ac}
HPE200mg/kg	10.83±0.35^{ac}	44.82±3.15^{ab}	1.29±0.15^{ab}	4.13±0.32^{ac}	0.09±0.01^{bc}
ECE100mg/kg	11.54±0.57^{c}	48.61±6.87^{ab}	1.49±0.19^{ab}	6.83±1.22^{b}	0.08±0.02^{abc}
ECE200mg/kg	11.89±1.06^{cd}	59.24±6.69^{a}	1.69±0.21^{a}	4.90±0.63^{acb}	0.07±0.01^{abc}
APE100mg/kg	13.81±0.56^{d}	47.51±8.45^{ab}	1.24±0.16^{b}	4.15±0.53^{ac}	0.09±0.00^{bc}
APE200mg/kg	11.76±0.29^{cd}	49.58±4.53^{ab}	1.23±0.12^{b}	4.84±0.35^{acb}	0.11±0.01^{b}
Diclofenac	11.79±0.70^{cd}	47.64±4.70^{ab}	1.31±0.20^{b}	3.68±0.67^{ac}	0.17±0.03^{d}

Values are mean ± SEM, n=5, values within the column with different superscript are significantly different at $p<0.05$. HPE= n-hexane partitioned extract, ECE= ethanolic extract, APE= aqueous partitioned extract, MDA= malondialdehyde, GSH= reduced glutathione, γ-GT= γ-glutamyl transpeptidase.

DISCUSSION

The efficacies of botanicals against oxidative stress and inflammatory disorders have been reported severally in various scientific works, which have led to the increased awareness and recent escalation in their patronage and usage as alternative medicine in many developing and develop nations. Several reports demonstrated the roles of oxidative stress in the progression of inflammatory disorders, metabolic diseases, degenerative diseases, vulnerability to opportunity infections, organ failure and death (Spelman *et al*., 2006; Oyewo *et al*., 2012).

In this study, the injection of turpentine oil (TURP) induced tissue lipid peroxidation in the experimental rats by upsetting the oxidant and antioxidant balance, in which the various doses of the fruit extracts of *X. Aethiopica* helped to modulate the oxidant and antioxidant balance to the prior status quo (Tables 1, 2 and 3). This trend can only due to the inherent phytochemicals in the fruit of *X. Aethiopica*. The fruits of *X. Aethiopica* indicated high level of polyphenolic compounds, such as flavonoids, terpenoids etc, which are renowned for the antioxidative properties (Ezekwesili *et al.,* 2010). Although, the patterns in the levels of GSH, total thiol, γ-GT, SOD, CAT and peroxidase were not consistently comparable with the non TURP injected rats, but the marked improvements recorded in the plasma, liver and spleen in rats administered the doses of the fruit extracts can only be due to the antioxidants identified in the fruit extract of *X. Aethiopica*. Paradoxically, the effect of the consumption of antioxidant rich diets on the antioxidant enzymes remains contradictory, with some authors reporting increased enzyme activity (Lin *et al.,* 2008), while some reported decreased activity (Breinholt *et al.,* 1999; Youdim and Deans, 2000). However, there is strong evidence affirming a link between consumption of fruits and vegetables and low disease risk, but there is little or no evidence for a direct relationship between exogenous antioxidant consumption and increased endogenous antioxidative activities.

In addition, various works had shown that consumption of polyphenol-rich diet such as red wine, tea, fruits and vegetables did not elicit any effect on any of the antioxidant biomarkers in humans, but could have acted directly in free radical scavenging, thereby sparing the endogenous antioxidant systems (Van der Gaag *et al*., 2000; Oyewo *et al*., 2012; Adekunle *et al*., 2016). The connections between dietary antioxidant consumption and good health have been proposed to arise from a complex mix of mechanisms and circumstances, which are not yet understood scientifically. Thus, the inconsistent patterns in the endogenous antioxidant systems implied a prior

boost in the antioxidative systems that allowed the rats to handle the TURP challenge favourably. Perhaps, the duration after the TURP challenge and the time of sacrifice, might not have allowed enough time for the recuperation in the endogenous antioxidant systems since we examined acute inflammatory response.

Previous studies had shown that the injection of turpentine oil in rodent induces the release of ceruloplasmin and other acute phase proteins (Neuzil and Graham, 1996). The prior administrations of the extracts of fruits of *X. Aethiopica* and diclofenac were shown to modulate favourably the release of inflammatory biomarkers as presented in Figures 1, 2 and 3. The patterns reported in the plasma TNF-α and C-reactive proteins levels were assessed as favourable due to the implication of these trios in various pathological conditions. Diclofenac, a non-selective inhibitor of cyclooxygenase 2, has also been shown to abolish the elevation of serum TNF-α in various studies (Juni *et al.,* 2002; Garjani *et al.,* 2008).

Acute phase proteins, such as ceruloplasmin, cytokines, prealbumin, fibrinogen, C-reactive proteins, cortisols, chemokines etc., are small peptides whose concentration are known to increase in exponential folds in circulation upon the set off of oxidative and inflammatory responses, and their continuous release are the bed rock of inflammatory and metabolic disorders, degenerative and autoimmune diseases, organ function loss, catechia and death. The ability of the various doses of the fruit extracts *X. aethiopica* to modulate the production of these acute phase proteins could underline the immunomodulatory capabilities of the fruit of *X. Aethiopica*. Thus, our results in the inflammatory markers lend support to the use of *X. aethiopica* in the management of bronchitis, asthma, infertility, wounds, arthritis and rheumatism, post-natal pains and dysenteric conditions (Burkill, 1985; Fall *et al.,* 2003; Ezekwesili *et al.,* 2010).

The prior administration of the fruit extracts improved recorded decreases in the level of plasma albumin following TURP injection (Fig. 3). Decreases in the blood albumin concentration have been correlated with inflammatory process that could lead to metabolic deficiencies (lipid and hormone), oedema, liver injury etc (Oyewo *et al*., 2012). In addition, previous reports on the suggestive anti-inflammatory properties of *X. aethiopica* associated the analgesic and antipyretic effects with the inhibition of synthesis and release action of pro-inflammatory molecules (Woode *et al.,* 2012). The overall trends obtained in the oxidative and inflammatory indices among the various doses of the fruit extracts of *X. Aethiopica*, indicated that the ethanolic crude extract presented the most promising entity in modulating the progression of inflammatory responses to TURP in the rats. Perhaps the other solvents (n-hexane and water) were selective in the extraction of the active principles in the fruit of *X. Aethiopica*.

CONCLUSION

In the light of the foregoing, the consistent capabilities of the ethanolic extract of *X. aethiopica* fruit in modulating inflammatory responses, makes it a good regime for regular consumption in broth or soup brewing and as tincture for improved quality of health, which could be a great candidate in abating and, or management of inflammatory disorders.

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Conflict of interest: The authors declare that no conflict of interests existed in the organization, results, presentation and the finance of the research article.

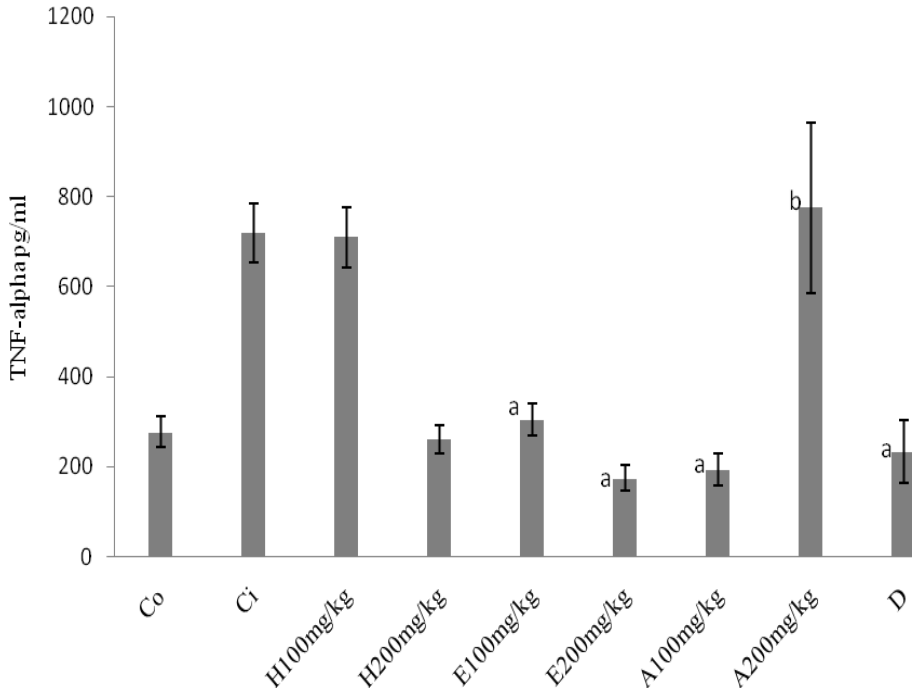

Fig. 1. **Effects of administration of ethanolic extract, aqueous and n-hexane partitioned extracts of *X. aethiopica* on TURP-induced TNF-α secretion in plasma.** Bars represent mean ± SEM, n=5, Bars with different alphabets are significantly different at $p<0.05$.TNF-alpha=Tumour Necrosis Factor-alpha, Co=Control, Ci=Induced Control, H=n-hexane partitioned extract, E=Ethanolic extract, A=Aqueous partitioned extract, D=Diclofenac.

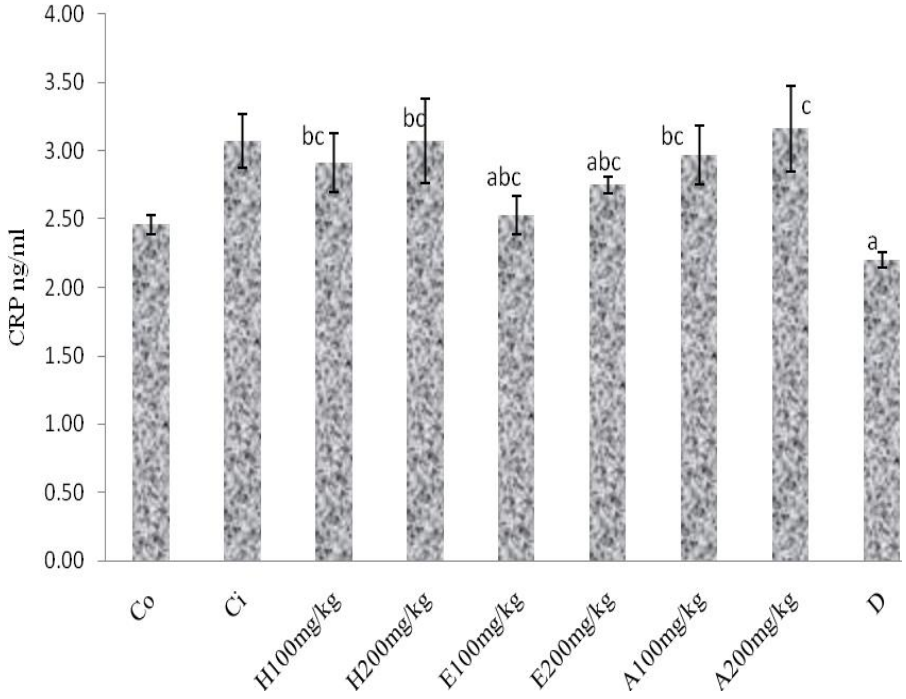

Fig. 2. **Effects of administration of ethanolic extract, aqueous and n-hexane partitioned extracts of *X. aethiopica* on TURP-induced CRP secretion in plasma.**

Bars represent mean ± SEM, n=5, Bars with different alphabets are significantly different at $p<0.05$. CRP=C - reactive protein, Co=Control, Ci=Induced Control, H=n-Hexane partitioned extract, E=Ethanolic extract, A=Aqueous partitioned extract, D=Diclofenac.

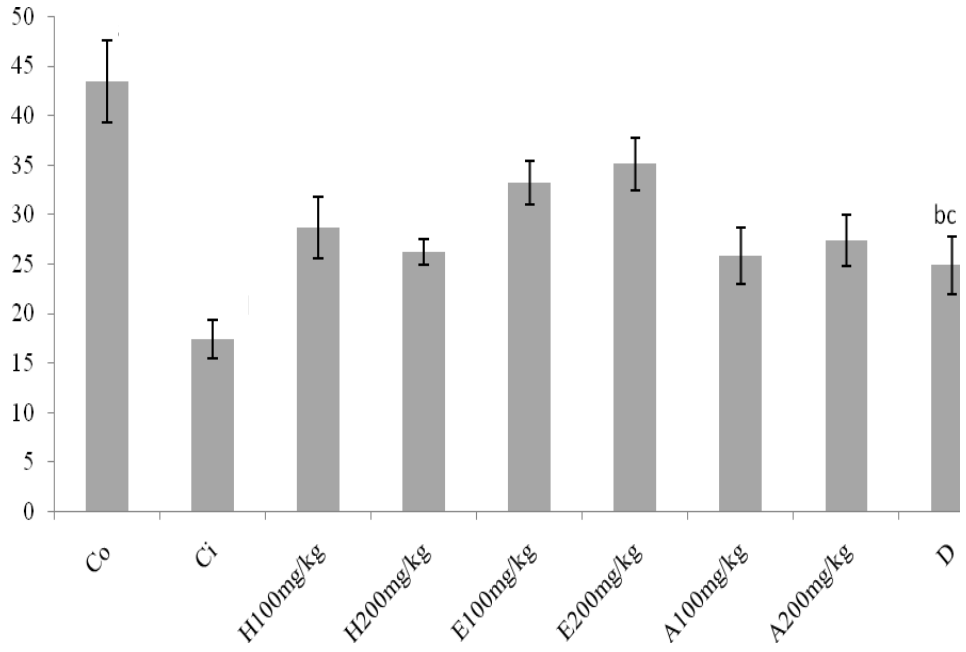

Fig. 3. **Effects of administration of ethanolic extract, aqueous and n-hexane partitioned extracts of *X. aethiopica* on TURP-induced albumin secretion in plasma.** Bars represent mean ± SEM, n=5, Bars with different alphabets are significantly different at $p<0.05$. Co=Control, Ci=Induced Control H=n-hexane partitioned extract, E=Ethanolic extract, A=Aqueous partitioned extract, D=Diclofenac.

Table 2. Patterns in the oxidative stress indices in the liver following fruit extracts of *X. Aethiopica* treatment in TURP injected rats

Group	MDA (µmol/g of tissue)	GSH (µmol/ g of protein)	Total Thiol (µmol/ g of protein)	γ-GT (U/g of protein)	SOD (U/g of protein)	CAT (U/g of protein)	POD (U/g of protein)
Control	57.69±5.54^{a}	2.37±0.25^{a}	8.19±0.88^{ac}	0.32±0.03^{a}	1.46±0.18^{a}	47.62±7.55^{a}	5.05±0.69^{a}
Induced control	84.65±5.05^{bc}	1.38±0.10^{b}	7.74±0.69^{ab}	0.40±0.05^{a}	1.43±0.22^{a}	49.87±12.11^{a}	5.63±0.76^{a}
HPE100mg/kg	61.54±4.37^{a}	1.46±0.16^{b}	8.22±1.05^{ac}	0.34±0.03^{a}	1.94±0.41^{ab}	50.93±9.58^{a}	5.93±1.01^{a}
HPE200mg/kg	56.25±4.70^{a}	1.35±0.15^{b}	8.3±0.58^{ac}	0.25±0.06^{a}	2.37±0.24^{b}	54.29±5.37^{a}	6.38±1.08^{a}
ECE100mg/kg	71.03±9.21^{ab}	1.72±0.30^{c}	8.76±0.41^{ac}	0.38±0.04^{a}	2.19±0.29^{b}	52.56±7.84^{a}	7.81±0.66^{a}
ECE200mg/kg	68.91±4.46^{ab}	1.98±0.33^{ac}	10.49±0.65^{c}	0.42±0.05^{a}	2.19±0.24^{b}	53.1±10.30^{a}	5.44±0.76^{a}
APE100mg/kg	69.07±3.92^{ab}	1.43±0.12^{b}	8.71±0.89^{ac}	0.32±0.02^{a}	2.08±0.29^{b}	63.48±8.13^{b}	7.15±0.88^{a}
APE200mg/kg	69.52±4.66^{ab}	1.91±0.18^{c}	9.22±0.85^{ac}	0.40±0.05^{a}	2.14±0.25^{b}	58.97±6.31^{ab}	7.36±1.17^{a}
Diclofenac	90.29±4.73^{c}	1.41±0.15^{b}	5.61±0.72^{b}	0.40±0.43^{a}	1.77±0.27^{ab}	57.11±8.18^{ab}	4.93±0.82^{a}

Values are mean ± SEM, n=5, values within the column with different superscript are significantly different at $p<0.05$. HPE= n-hexane partitioned extract, ECE= ethanolic extract, APE= aqueous partitioned extract, MDA= malondialdehyde, GSH= reduced glutathione, γ-GT= γ-glutamyl transpeptidase, SOD= superoxide dismutase, CAT= catalase, POD= peroxidase

Table 3. Trends in oxidative stress indices in spleen of TURP injected rats treated with fruit extracts of *X. Aethiopica*

Group	MDA (µmol/g of tissue)	GSH (µmol/ g of protein)	Total Thiol (µmol/ g of protein)	γ-GT (U/g of protein)	SOD (U/g of protein)	CAT (U/g of protein)	POD (U/g of protein)
Control	53.97±3.13^{a}	1.43±0.20^{ab}	6.75±0.34^{a}	0.27±0.06^{ac}	1.19±0.11^{a}	31.86±4.21^{a}	0.48±0.11^{a}
Induced control	70.96±4.76^{b}	1.20±0.15^{ab}	6.55±0.70^{ab}	0.20±0.07^{a}	1.21±0.23^{ab}	42.25±4.28^{ab}	0.67±0.18^{ab}
HPE100mg/kg	60.06±7.79^{ab}	1.22±0.14^{ab}	5.68±0.678^{ab}	0.24±0.04^{ac}	1.54±0.28^{ab}	42.07±7.80^{ab}	0.50±0.09^{a}
HPE200mg/kg	53.91±4.16^{ab}	1.19±0.09^{ab}	6.30±0.82^{ab}	0.34±0.08^{ac}	1.48±0.32^{ab}	51.94±8.54^{ab}	0.65±0.12^{ab}
ECE100mg/kg	58.69±6.14^{ab}	1.62±0.24^{b}	5.69±0.70^{ab}	0.32±0.05^{ac}	1.64±0.23^{ab}	57.48±4.15^{b}	1.45±0.193^{c}
ECE200mg/kg	52.37±5.11^{a}	1.46±0.17^{ab}	6.20±1.04^{ab}	0.40±0.04^{c}	1.65±0.13^{ab}	43.39±9.70^{ab}	0.92±0.32^{abc}
APE100mg/kg	58.43±5.19^{ab}	1.05±0.06^{a}	4.27±0.55^{b}	0.26±0.04^{ac}	1.67±0.29^{ab}	39.40±6.22^{ab}	1.33±0.41^{bc}
APE200mg/kg	51.67±5.03^{a}	1.03±0.04^{a}	4.70±0.83^{ab}	0.36±0.03^{ac}	2.01±0.30^{b}	48.45±10.12^{ab}	0.87±0.25^{abc}
Diclofenac	66.70±5.29^{ab}	1.18±0.21^{ab}	5.00±0.47^{ab}	0.26±0.07^{ac}	1.75±0.24^{ab}	45.04±2.53^{ab}	0.86±0.21^{abc}

Values are mean ± SEM, n=5, values within the column with different superscript are significantly different at $p<0.05$. HPE= n-hexane partitioned extract, ECE= ethanolic extract, APE= aqueous partitioned extract, MDA= malondialdehyde, GSH= reduced glutathione, γ-GT= γ-glutamyl transpeptidase, SOD= superoxide dismutase, CAT= catalase, POD= peroxidase.

REFERENCES

1. Adekunle, A.S., Adelusi, T.O., Oyewo, E.B., Kamdem, J.P. and Akintade, B.B. (2016). Antihypercholesterolemic, Cardioprotective and Vitamins E and C Sparing Properties of *Bryophyllum pinnatum* in Rabbits. European Journal of Medicinal Plants, 11(3): 1-13.
2. Bafna, A. R. and Mishra, S. H. (2005).. Immunomodulatory activity of methanolic extract of roots of *Cissampelos pareira* Linn.Ars. *Pharm*. 46: 253-262.
3. Balkwill, F. and Mantovani, A. (2010). Cancer and inflammation: implications for pharmacology and therapeutics. *Clin Pharmacol Ther* 87: 401-406.
4. Breinholt, V., Lauridsen, S., and Dragsted, L. (1999). Differential effects of dietary flavonoids on drug metabolizing and antioxidant enzymes in female rat. *Xenobiotica*, 29(12): 1227-1240.
5. Buege, J. A. and Aust, S. D. (1978). Microsomal lipid peroxidation. *Meth Enzymol*, 52:302-310.
6. Burkill, H. M. (1985). *The Useful Plants of West Tropical Africa, Families A-D*, Kew, Royal Botanic Gardens.
7. Butcher, E. C. and Picker, L. J. (1996). Lymphocyte homing and homeostasis. *Science* 272, 60-66.
8. Doumas, B. T., Watson, W. A. and Bigsg, H. G. (1971). Albumin standards and measurement of serum-albumin with bromocresol green. *Clin.Chim.Acta*.31: 87- 92.
9. Efferth, T., and Koch, E. (2010). Complex interactions between phytochemicals.The multi-target therapeutic concept of phytotherapy. *Curr Drug Targets* 12: 122-132.
10. Ezekwesili, C. N., Nwodo, O. F. C., Eneh, F. U. and Ogbunugafor, H. A. (2010) Investigation of the chemical composition and biological activity of *Xylopia aethiopica* Dunal (Annonacae). *African Journal of Biotechnology*. 9(43): 7352-7356.
11. Fall, D., Badiane, M., Ba, D., Loiseau, P., Bories, C., Gleye, C., Laurens, A. and Hocquemiller, R. (2003). Antiparasitic activities of Senegalese Annonacae used in traditional medicine. *Dakar Med*. 48(2): 112-116.
12. Garjani, A., Sina, A, Mojtaba, Z and Nazrin, M (2008). Biphasic effects of atorvastatin on inflammation. *Pak J Pharma Sci* 21:125-130
13. Juni, P., Rutjes, A. W. and Dieppe, P. A. (2002.). Are selective COX-2 inhibitors superior to traditional nonsteriodal anti-inflammatory drug? *British Medical Journal*, 324:1287-1288.
14. Kumar, A., Teuber, S. S., and Gershwin, M. E. (2006). "Current perspectives on primary immunodeficiency diseases" .*Clin. Dev. Immunol.* 13 (2–4): 223–59.
15. Lin, Y. L., Cheng, C. Y., Lin, Y. P., Lau, Y. W., Juan, I. M., and Lin, J. K., (1998). Hypolipidemic effect of green tea leaves through induction of antioxidant and phase II enzymes including superoxide dismutase, catalase, and glutathione S-transferase in rats. Journal of Agricultural and Food Chemistry 46(5):1893-1899.
16. Medzhitov, R. (2008). Origin and physiological roles of inflammation. *Nature* 454: 428-435.
17. Neuzil, K. M. and Graham, B. S. (1996). Cytokine release and innate immunity in respiratory viral infection. *Virology*. 7:255 – 264
18. Opal, S. M. and DePalo, V. A. (2000). Anti-inflammatory cytokines.*Chest*. 117:1162-1172.
19. Oyewo, E. B., Akanji, M. A. and Adekunle, A. S. (2012). Immunomodulation Capabilities of Aqueous Leaf Extract of Phyllanthus amarus in male Wistar Rats. *Report and Opinion*, 4(1):22-37.
20. Oyewo, E. B., Adetutu, A., Adebisi, J.A., Olorunnisola, O.S. and Adesokan, A.A. (2013). Sub-chronic administration of Febi super bitters triggered inflammatory responses in male Wistar rats. *J of Med Sci.* 13(8):692–699.
21. Reddy, A. C. and Lokesh, B. R. (1992). Studies on spice principles as antioxidants in the inhibition of lipid peroxidation of rat liver microsomes.*Mol Cell Biochem*, 111 (1-2):117-124
22. Repetto, M. G. and Llesuy, S. F. (2002). Antioxidant properties of natural compounds used in popular medicine for gastric ulcers. *Braz. J. Med. Biol. Res.* 35(35):523-534.
23. Sedlak, J. and Lindsay, R. H. (1958). Estimation of total, protein-bound, and non-protein sulfhydryl groups in tissues with Ellman's reagent. *Anal Biochem* 24(1):192-205.
24. Shoskes, D. A. (2002). Phytotherapy in chronic prostatitis.*Urology* 60: 35-37.
25. Sinha, A. K. (1972). Colorimetric assay of Catalase. *Anal Biochem*, 47:389-394
26. Spelman, K., Burns, J., Nichols, D., Winters, N., Ottersberg, S. and Tenborg, M. (2006). "Modulation of cytokine expression by traditional medicines: a review of herbal immunomodulators". *Alternative Medicine reviews*, 11: 128-150.
27. Sun, M. and Zigma, S. (1978). An improved spectrophotometer assay of superoxide dismutase based on epinephrine autoxidation. *Anal.Biochem*. 90:81-89
28. Surh, Y. Z. and Ferguson, L. R. (2003). *Dietary and medicinal antimutagens and anticarcinogens: molecular mechanisms and chemopreventive potential:* Highlight of a symposium.
29. Szasz G. (1969). A kinetic photometric method for serum γ-glutamyl transpeptidase. *Clin Chem*, 15:124-136.
30. Van der Gaag, M., Van den Berg, R., Van den Berg, H., Schaafsma, G., and Hendriks. H., (2000). Moderate consumption of beer, red wine and spirits has counteracting effects on plasma antioxidants in middle-aged men. *Eur J of Clin Nutr*. 54(7):586-591.
31. Woode, E., Ameyaw, E. O., Boakye-Gyasi, E., and Wonder, K. M. (2012). AbotsiAnalgesic effects of an ethanolic extract of the fruits of *Xylopia aethiopica* (Dunal) A. Rich (Annonaceae) and the major constituent, xylopic acid in murine models. *J Pharm Bioallied Sci.* 4(4): 291–301.
32. Youdim, K. A. and Deans, S. G. (2000). Effect of thyme oil and thymol dietary supplementation on the antioxidant status and fatty acid composition of the ageing rat brain. *British J of Nutr*, 83(1): 87-93.