

Antioxidant properties and effects of *Justicia carnea* leaf ethanolic extract on organs and atherogenic factors in healthy albino rats.

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Abstract:

Medicinal plants are good source of antioxidant that prevents body cells against oxidative assaults. They can also occasionally act as pro-oxidant to alter biochemical pathways and organ functions. This study examines the *in-vitro* antioxidant properties and effects of ethanolic extract of *Justicia carnea* (EtJC) in normal rats' biochemical parameters. Antioxidant evaluation was done by assessing the bioactive contents, ferric reducing antioxidant power (FRAP), 1, 1-diphenyl-2-picrylhydrazyl (DPPH) scavenging ability and nitric oxide (NO) inhibition using standard procedures. Additionally, the effects oral administration of EtJC for 14 days on organs of experimental animals and possible risk of atherogenesis were assessed using standard biochemical protocols. Ethanolic extract of *Justicia carnea* leaf showed the presence of biologically active constituents. The extract demonstrated weak FRAP activity but appreciably inhibited DPPH and NO in concentration dependent manner with IC₅₀ value of 818.24 µg/mL and 578.77 mg/mL respectively. Administration of extract caused significant (p<0.05) decrease in alanine aminotransferases (ALT), aspartate aminotransferases (AST) and creatinine levels in experimental rats. Varying concentration of extract significantly (p<0.05) decrease triglyceride concentration while other lipid parameters were not adversely affected. Only dose at 500 mg EtJC/kg caused significant reduction in atherogenic factors. Ethanolic extract of *Justicia carnea* leaf could serve as an important source of antioxidant, reduce the risk of cardiovascular related disease with no deleterious effect on liver and kidney functions.

Keywords: Antioxidant, Atherogenic, FRAP, *Justicia carnea*, Lipid, Pro-oxidant.

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Introduction

Several reactive species and free radicals are continuously encountered exogenously through anthropogenic activities or endogenously produced in humans as by-products of normal metabolism (Omoriege et al. 2014). Depending on the nature of reactive species, they are rapidly detoxified to non-toxic substances by various cellular enzymic and non-enzymic mechanisms. When reactive oxygen species (ROS) production in the cell supersedes the body detoxifying mechanisms, oxidative stress occurs resulting in perturbation of biological pathways, cell structures and subsequently tissue functions (Erhunse et al. 2016).

Imbalance of pro-oxidants/antioxidants ratio in biological systems has provided the impetus for scientists to search for naturally occurring antioxidants from plants sources to augment the body antioxidant defence system. Dietary vegetables have been investigated to contain significant amounts of antioxidants through evaluation of their total flavonoid, phenol content and other antioxidant activities such as reducing power and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging ability (Dontha, 2016; Awad et al. 2018). Besides dietary plants, medicinal plants that are rich in bioactive constituents with novel antioxidant properties are also currently receiving momentous attention and being identified (Nirmalraj and Perinbam, 2015; Erhunse et al. 2016). Regardless of the health benefits attached to medicinal plants, several studies have also convincingly demonstrated pro-oxidative influence of plant bioactive compounds in living cells. These pro-oxidant activities often occur under certain conditions where they modifying biological pathways and cellular functions (Al Joudi, 2013; Sotler *et al.* 2019). Common factors such as the presence of metal ions, concentration of antioxidant in matrix environments and their redox potential that have been identified to commonly modify antioxidants by transforming them to pro-oxidant (Sotler *et al.* 2019). Important biochemical studies for assessing the possible effects of pro-oxidants after administration of medicinal plant include experimental analysis on lipid profiling, liver and kidney function indices (Chanda et al. 2015).

Justicia carnea (Acanthaceae family), commonly known as Pink jacobinia, flamingo flower or pine-bur begonia in English and indigenously called “Ewe eje” in Yoruba is a tender perennial shrub that is widely cultivated in tropics and subtropics. *Justicia* species have been reported to perform diverse health benefit such as treatment of arthritis, gastrointestinal disturbances, manage diabetes and cancer (Corrêa et al. 2012; Onyeabo et al. 2017). The

health benefits of *J. carnea* leaf have been linked to the presence of active phytochemicals, nutrients and important micronutrients such as iron, copper, zinc folic acid and selenium (Asogwa et al. 2020). Owing to possibilities that biological active constituents from plant origin could sometimes incite prooxidative activities (Al Joudi, 2013), this study was designed to assess the *in-vitro* antioxidant potential of *Justicia Carnea* leaf ethanolic extract and examine its possible effects on organ functions as well as atherogenic indices in healthy albino rats.

Materials and methods

Chemical Reagent

Chemicals reagents used in this study were of analytical grade. Sulfanilic acid, naphthylethylene diamine dihydrochloride, potassium hydroxide (KOH), 2,4,6-tripyridylstriaizine (TPTZ), acetic acid, sodium nitrite (NaNO_2), ferrous sulphate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$), ferric chloride (FeCl_3), aluminum chloride ($\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$) sodium carbonate (Na_2CO_3), trichloroacetic acid (TCA), sodium phosphate, ammonium molybdate and Folin-Ciocalteu's reagent (Folin C) were all purchased from Sigma Chemical Co. (St. Louis, MO). Randox diagnostic kits, purchased from USA was used to perform organ function analysis and lipid profiling.

Equipments

UV-Spectrophotometer (T70+ UV/VIS, PG Instrument), Mettler balance (PA213 Ohaus), water bath (HH-S6), Refrigerator (Scanfrost), Micropipettes (0-100 μL , 100-1000 μL), Centrifuge (80-2 windfield medical, UK), Test-tube racks, Oral gavage, pH meter (PHS-550).

Plant material and extract preparation

Leaves of *J. carnea* were harvested between August and September from a local farmland in Okitipupa Local Government, Ondo State, Nigeria. Sample was deposited at the University herbarium unit and identity of plant was authenticated with the voucher number OAUSTECH/HB/044. The fresh leave samples were washed under running tap to remove dirt and air-dried at ambient temperature for 14 days. Dried samples were crushed into powdered form using an electric blender and stored hermetically in a container prior extraction. One thousand grams (1000 g) of powdered sample was soaked in 3 L of absolute ethanol with frequent stirring. After 72 h of maceration, it was filtered with a double cheese

cloth to obtain a filtrate that was concentrated using rotary evaporator at 40 °C to obtain a paste of crude ethanolic extract of *Justicia carnea* (EtJC).

Antioxidant analysis

Total phenol content (TPC) estimation

Total phenolics in EtJC was estimated spectrophotometrically (Kim et al. 2003). One milliliter (1 mL) of Folin–C mixture (1:15 v/v) was added to 1 mL of sample (1 mg/mL) and allowed stand for 5 min. Added to the mixture was five milliliter of 7% Na₂CO₃ followed by of 6.5 mL of distilled water to make appropriate volume and mixed thoroughly. The reaction medium was allowed to incubate (at 25 °C) for 90 min, after which the absorbance was taken at 750 nm using spectrophotometer. Concentration of total phenol content (mg/mL) was extracted from a calibrated Gallic acid standard curve. Samples were analyzed in triplicates and TPC concentration (mg gallic acid equivalents/ gram of extract) in extract was estimated.

Total flavonoids content (TFC) estimation

Flavonoid content was measured using Park et al. (2008) method. In a reaction medium containing 1 mL of sample (1 mg/mL) were added 3.4 mL of methanol (30%), 0.15 mL of 0.5 M NaNO₂ and 0.15 mL of AlCl₃.6H₂O (0.3 M) solutions. After 5 min of incubation at room temperature, 1 mL of 1 M NaOH was introduced into the mixture and mixed gently. Absorbance of reaction medium was read against the reagent blank at 506nm using spectrophotometer. Total flavonoid content was extrapolated from quercetin standard curve. The standard calibration curve was prepared using graded concentration of quercetin.

Total antioxidant capacity (TAC) estimation

The total antioxidant capacity of *Justicia Carnea* leaf ethanolic extract was determined using phosphomolybdenum complex formation method (Prieto et al. 1999): Briefly, combination of extract (0.1 mL) and 1.0 mL of working reagent (600 mM sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) in test tubes were boiled at 95 °C in a boiling water bath (HH-S6 China). After 90 min of incubation, mixtures were allowed to cool to ambient temperature and the absorbance was taken at 765 nm against blank. Assay was performed in triplicate and the total antioxidant capacity of extract was expressed as mg of ascorbic acid equivalent.

Estimation of ferric reducing antioxidant power (FRAP)

FRAP was evaluated following Benzie and Strain, (1996) method. One milliliter (1 mL) of extracts at various concentrations (200 – 1000 µg/mL) was mixed with 1.5 mL of freshly prepared FRAP solution [containing 1:1:10 of 10mM 2,4,6-tripyridylstriaizine (TPTZ) in 40mM HCl, 20 mM ferric chloride (FeCl₃.6H₂O) and 300 mM acetate buffer (pH 3.6)]. The mixture was allowed to incubate for 30 min at 37 °C followed by absorbance reading at 593 nm. Various concentration of FeSO₄ was used for calibration curve and ascorbic acid was used as the reference control. The assays were carried out in replicate of three times.

Estimation of DPPH inhibition

Ability to reduce free DPPH radical was estimated following McCune and Johns (2002) method. Concisely, 1.0 mL of 0.3 mM DPPH in methanol was introduced into medium containing 1.0 mL of the extract (200 – 1000 µg/mL) and 1.0 mL of methanol making final volume of 3.0 mL. The mixture was allowed to incubate in the darkroom for 10 min and absorbance (517 nm) was recorded. Ascorbic acid prepared in methanol was used as standard using similar concentrations (200 – 1000 µg/mL) as the extract. Control blank containing 1 mL of 0.3 mM DPPH and 2 mL methanol was also prepared and treated as the extract.

Estimation of nitric oxide (NO) scavenging potential

The reaction mixture (2 mL) containing 1.0 mL of sodium nitroprusside (10 mM) in phosphate buffer (pH 7.4) and 1.0 mL of the extract at varying concentrations were placed in water bath (37°C) for 150 min. Thereafter, 1.0 mL of freshly prepared Griess reagent (1% sulfanilic acid, 0.1% naphthylethylene diamine dihydrochloride in 2% phosphoric acid) was added to the reaction medium and absorbance of the chromophore formed was measured at spectrophotometrically at 546 nm. Similar assay without extract was used as control (Marcocci et al. 1994).

Animal study

Experimental animals

Male albino rats (weighing 120 – 200 g) were purchased from the animal house, Biochemistry Department, University of Benin, Benin city, Nigeria. Animals were

acclimatized in an aerated cage for two (2) weeks under ambient temperature and given access to rat feed and water uninterrupted. The experimental protocol, animal handling and management was in accordance to the Institutional Ethical Committee guidelines on the use of experimental Animal with approved number OAUSTECH/ETHC–BCH/2021/02.

Experimental Protocol

Total of twenty-four (24) male experimental rats were distributed into four (4) groups of six rats each ($n = 6$). Animals in group I (control) were given 1.0 mL of distilled water while animals in other groups (II, III, IV) were given varying dose of 50, 100 and 500 mg EtJC/kg body weight for 14 days in a single oral dose using gavage. Initial weight of animals was taken before the start of extract administration and on the last day before sacrifice. Upon completion of treatment, the experimental animals were allowed to fast overnight. On the 15th day, all rats in each group were sequentially sacrificed and blood samples withdrawn through cardiac puncture for biochemical analysis. The blood samples were dispensed in a plain sterile bottle, allowed to clot and further centrifuged to obtain the sera. After collecting the samples, remains on animals were cremated.

Liver and kidney function assay

Organ function was performed using Randox diagnostic kits following manufacturer's instructions. Final reaction mixtures were quantified by taking absorbance at specified wavelength using Spectrophotometer. Analysis of total protein was based on the principle Tietz (1995), alanine aminotransferases (ALT) and aspartate aminotransferases (AST) were respectively estimated following Reitman and Frankel (1957). Urea and creatinine levels were measured by Fawcett and Scott (1960) and Bartels and Bohmer (1972) respectively.

Lipid profile assay and atherogenic indices

Lipid profile were also performed using Randox diagnostic kits. Final reaction mixtures were quantified by taking absorbance at specified wavelength using UV-Spectrophotometer. Total triglycerides (TRIG) and cholesterol (CHOL) were determined by the methods of Jacobs and Van Denmark (1960) and Trinder (1969) respectively. Estimation of low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) were respectively according to Friedewald et al. (1972) and Lopes-Virella et al. (1977) methods. Atherogenic indices such as cardiac risk ratio (CRR), atherogenic coefficient (AC) and atherogenic index

(AI) were calculated using the equation below:

Cardiac Risk Ratio (CRR) = CHOL / HDLC (Martirosyan et al., 2007)

Atherogenic Coefficient (AC) = (CHOL – HDLC) / HDLC (Brehm et al., 2004)

Atherogenic Index (AI) = (LDL – cholesterol) / HDL – cholesterol (Beyegue et al., 2012)

Statistical Analysis

The results of analysis were presented as mean \pm standard error of mean (SEM) of three experimental determinations. The statistical data were analyzed by SPSS version 17.0 (SPSS Inc., Armonk, NY, USA). One-way analysis of variance (ANOVA) was used followed by post hoc (using LSD) test with significant level obtained at $p < 0.05$. The IC_{50} was determined by non-linear regression curve fit using Microsoft excel package.

Results and Discussion

Results

Antioxidants

Total phenolics in the extract of *J. carnea* was (15.06 ± 0.50) mg GAE/g while the total flavonoid content and total antioxidant capacity were (15.69 ± 0.03) mg QE/g and (4.85 ± 1.14) mg AAE.100/g EtJC respectively (Table 1). The ferric reducing ability (expressed in $\mu\text{mole Fe}^{2+}/\text{g}$) of EtJC is presented in Figure 1. Varying concentration of *J. carnea* extract demonstrated low ferric reducing ability compared to the standard ascorbic acid. The extract of *J. carnea* leaf inhibited DPPH and nitric oxide (NO) radical in concentration dependent manner above 50 % (Figure 2). The IC_{50} values obtained for the inhibition study on both DPPH and NO is presented in Table 2. The respective IC_{50} values of 818.24 ± 29.64 and 578.77 ± 20.00 $\mu\text{g/mL}$ obtained from percentage inhibition of DPPH and NO were significantly ($p < 0.05$) high compared to standard ascorbic acid.

Organ function result

The differences in body weight of animals orally administered with varying concentration of EtJC for 14 days is presented in Table 3. Relative to control, animals treated with different doses of EtJC demonstrated significant ($p < 0.05$) increase in body after 14 days of treatment. The results of total protein, liver and kidney markers are presented in Table 4. Total protein

Table 1

Figure 1

Figure 2

Table 2

Figure 3

Table 3

in groups treated with graded dose of EtJC showed no significant difference ($p > 0.05$) compared to control. The level of ALT and AST in rat that were administered with doses of EtJC reduced significantly ($p < 0.05$) relative to untreated group (control). Concentration of urea in group II and III significantly ($p < 0.05$) increased while urea level of rats administered with the highest dose (500 mg/kg) was not affected. Contrary to urea, creatinine concentration in treated rats significantly ($p < 0.05$) reduced in dose dependent manner.

Lipid profile and atherogenic indices results

The lipid and atherogenic profile of rats in control and groups orally administered with EtJC is presented in Table 5. Animals that were administered with graded dose of extracts demonstrated no significant difference in cholesterol concentration while their triglyceride reduced significantly ($p < 0.05$) relative to control. Only group that received the lowest dose (50 mg EtJC/kg) demonstrated significantly ($p < 0.05$) rise in LDL concentration while values obtained for other treated groups were statistically not affected compared to control. Additionally, varying concentration of extract caused no significant difference in HDL level. Animals that were administered with high dose (500 mg/kg) of *J. carnea* demonstrated significant decrease in cardiac risk ratio (CRR) and atherogenic coefficient (AC). Groups that received the lowest dose of extract showed significant rise in atherogenic index while values obtained for other groups (above 50 mg EtJC/kg) were indistinguishable relative to control.

Table 4

Discussion

The concentration of polyphenolics and total antioxidant capacity obtained from this study is low compared to other *Justicia* species. For instance, Nirmalraj and Perinbam, (2015) obtained total phenolic content to be 34.10 mg GAE/100 mg and total flavonoid to be 15.01 mg QE/100 mg in *Justicia gendarussa* while Awad et al. (2018) obtained total phenolic content of 26.54 ± 0.58 mg GAE/g ethanol extracts from *Justicia spicigera*. Besides the health importance of *J. carnea* as blood booster and anti-anaemic plant, availability of these bioactive compounds in the plant extract could also suggest protective effect on the blood cells against oxidative damage (Onyeabo et al. 2017).

The level of ferric reducing ability obtained in this study is similar to values obtained for *P. minarum* and *F. delavayi* plant (Maharjan and Baral, 2013). The low FRAP activity observed

in this study could suggest hydrogen donating mechanism rather than single electron transfer by the bioactive compounds that are domicile in the plant extract (Alvarez-Suarez et al. 2009). From this findings, the highest concentration of *J. carnea* ethanolic extract slightly inhibited DPPH radical above 50 % with IC₅₀ value of 818.24 µg/mL ($y = 0.052x + 7.92$, $R^2 = 0.9631$) compared to IC₅₀ value of 216.50 µg/mL ($y = 0.047x + 39.78$, $R^2 = 0.9609$) obtained for ascorbic acid. The inhibitory concentration of DPPH above 50 % observed in this study is similar to value obtained by other researchers on *Justicia species* (Nirmalraj and Perinbam, 2015; Asogwa et al. 2020). The ability of *J. carnea* ethanolic extract to inhibit DPPH may be connected to hydrogen donation potential of polyphenolic compounds in the extract. The number and position of –OH groups in polyphenolic compounds represent an important factor that determines the antioxidant nature of plants. Among the various types of secondary metabolites in plant, flavonoids and its classes represent important phytochemicals that have shown to possess notable health benefit as well as free radical alleviation (Dontha, 2016). In living cells, NO engage in diverse physiological processes such as neurotransmission, blood clotting, and posttranslational modification of proteins through different mechanisms. However, the redox microenvironment where NO is produced, presence of reactive radicals and disruption in quantity that is synthesized could result to pathological consequences hence the need for NO inhibition through external sources (Barouch et al. 2002). In agreement with the anti-inflammatory role of *Justicia spp*, one possible mechanism through which EtJC perform it anti-inflammatory role could probably be inhibition of intracellular nitric oxide synthase (iNOS) expression (Barouch et al. 2002).

The significant increase in body weights of rats administered with ethanolic extract of *J. carnea* could suggest that the animals tolerated the plant extract hence not harmful in subacute administration. The insignificant difference observed in the concentration of total protein could suggest that active compounds in EtJC did not disrupt protein metabolism in organs and possibly its function in the blood. Blood proteins are frequently used to perform diverse physiological functions such as oxygen and lipids transport, immune function, regulation of hormones and other cellular activities. However, decrease in protein concentration have been reported to be associated with massive liver necrosis and alterations in hepatocellular function (Olaniyan et al. 2016).

In healthy state, hepatic markers (AST and ALT) are frequently found in low amount in the blood. However, slight surge in the blood concentration could portray hepatic injury (Chanda

et al. 2015): The reduction in serum ALT and AST level across tested doses could suggest that the administration of EtJC within tested concentration for 14 days had no detrimental effects on the liver. Additionally, the statistical ($p < 0.05$) decline in the hepatic markers could suggest that bioactive constituents in the extract might be hepatoprotective (Chanda et al. 2015). Urea and creatinine that are produced from normal metabolic processes of proteins are usually excreted from the blood by nephron (Chanda et al. 2015): Significant increase in urea level that was noticed in selected groups might not necessarily point to kidney damage since the creatinine level in those groups significantly reduced (Table 4). Owing to specificity of creatinine as important renal biomarker, studies have shown that level of creatinine are usually maintain unless significant renal damage has occurred (Price and Finney, 2000).

From this study, the insignificant difference observed in cholesterol concentration of treated animals could suggest that bioactive constituents in EtCJ did not pose any threat of hypercholesterolemia. In addition, the steady decrease in cholesterol level at the highest dose might be an indication that slight increase in the dose of extract (above 500 mg/kg) or duration of administration above 14 days might presumably cause significant reduction in blood cholesterol. The significant decrease observed in triglyceride concentration of tested animals could be linked to decrease in absorption of dietary lipid as well as alteration of β -oxidation of fatty acids, mediated by phytochemical compounds in *J. carnea* extract. Previous studies have demonstrated that fibre and phytochemicals constituents such as saponin in plant could cause reduction in dietary lipids (Omonkhua et al. 2013; Onyeabo et al. 2017). The significant rise in LDL-C of rats administered with 50 mg EtJC/kg could be due to the non-significant increase observed in the cholesterol concentration of this group. Studies has revealed that approximately 7% of circulatory body cholesterol in plasma form low-density lipoproteins (Arun et al. 2013). HDL-C perform crucial function in the mobilization of excess cholesterol from the blood to the liver and other hormone producing sites for utilization and excretion (Arun et al. 2013).

Atherogenic indices are important toxicological tools for monitoring the risk of cardiovascular diseases, atherosclerosis and other risk factors associated with abnormal metabolism of lipids (Omonkhua et al. 2013). The results of atherogenic parameters from this study could suggest that only dose at 500 mg/kg could possibly lower the risk of cardiovascular diseases and atherogenesis. Possible means by which the extract of *J. carnea* leaf exerted this anti-atherogenic effect could be linked to the slight increase of HDL-C that

was observed in this study. Several medicinal plants that are rich in fibre and antioxidants have been reported to demonstrate antihyperlipidemic activity using different mechanisms (Arun et al. 2013; Chanda et al. 2015).

Conclusion

The result of this study has confirmed the presence and activities of biologically active constituents in the ethanolic extract of *J. carnea* leaf. These antioxidants could be beneficial in the attenuation of ROS activities thereby delaying the progression of diseases like erectile dysfunction, degenerative disorder and diseases associated with oxidative stress. In addition, this study has also demonstrated that administration of ethanolic extract of *J. carnea* leaf did not pose any risk of atherogenesis neither do the extract have noticeable adverse effects on the tissues (liver and kidney) of experimental animals.

Acknowledgements

The authors acknowledge the assistance rendered by Mr. Ojo Samuel of Biological Sciences Department, Olusegun Agagu University of Science and Technology, Okitipupa, Ondo State during plant sample collection. The authors are also grateful for the technical support offered by the laboratory technologist of Department of Chemical Sciences, Olusegun Agagu University of Science and Technology, Okitipupa, Ondo State during animal handling.

Conflict of Interests

The authors declare no conflict of interest.

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Table 1. Result of bioactive constituents and total antioxidant capacity in *J. carnea* ethanolic leaf extract

Extract	<i>J. carnea</i> ethanol extract
Total phenol content (mg GAE/g)	15.06 ± 0.50
Total flavonoid content (mg QE/g)	15.69 ± 0.03
Total antioxidant capacity (mg AAE 100/g)	4.85 ± 1.14

Data represents the mean ± SEM of three separate measurements of total phenol, flavonoid and antioxidant capacity of EtJC. Where GAE – Gallic acid equivalent; QE – Quercetin equivalent; AAE – Ascorbic acid equivalent

Table 2. The IC₅₀ (µg/ml) values of *J. carnea* ethanolic leaf extract against inhibition of DPPH and NO

Extract/Standard	IC ₅₀ (µg/mL)	
	DPPH	NO
<i>J. carnea</i>	818.24 ± 29.64 ^a	578.77 ± 20.00 ^a
Ascorbic acid	216.50 ± 3.65	336.12 ± 0.82

Values are IC₅₀ (µg/ml) obtained from inhibition study of DPPH and NO. Data are mean ± SEM of three separate measurements and superscript letters (a) indicate significant ($p < 0.05$) difference compared to the standard (ascorbic acid).

Table 3. Effects of oral administration of EtJC on total protein concentration, liver and kidney function indices

Group	Total protein (g/dL)	ALT (U/L)	AST (U/L)	Urea (mmol/L)	Creatinine (µmol/L)
Group I	2.51 ± 0.36	68.53 ± 4.66	346.67 ± 13.19	2.02 ± 0.00	190.31 ± 6.10
Group II	2.51 ± 0.17	54.07 ± 3.12 ^a	323.67 ± 21.86	4.54 ± 0.41 ^a	112.48 ± 22.08 ^a
Group III	2.04 ± 0.46	45.07 ± 1.27 ^a	267.00 ± 15.70 ^a	4.09 ± 0.63 ^a	70.75 ± 13.02 ^a
Group IV	2.49 ± 0.46	57.67 ± 0.33 ^a	230.67 ± 15.60 ^a	2.92 ± 0.00	52.69 ± 6.10 ^a

Values represent the means ± standard error of mean (SEM); *n* = 6. Superscript with letters (a) indicate significant (*p* < 0.05) difference compared to group I (control). Where ALT- alanine aminotransferase; AST - aspartate aminotransferase; Group I = Control; Group II = 50 mg EtJC /kg; Group III = 100 mg EtJC /kg and Group IV = 500 mg EtJC/kg.

Table 4. Serum lipid and atherogenic indices of rats administered with EtJC

Parameters	Group I	Group II	Group III	Group IV
CHOL (mg/dL)	205.33 ± 16.33	226.33 ± 10.17	231.00 ± 12.12	175.00 ± 7.00
TRIG (mg/dL)	130.58 ± 5.28	104.16 ± 9.87 ^a	104.92 ± 7.20 ^a	101.14 ± 3.29 ^a
HDL-C (mg/dL)	130.20 ± 11.40	109.20 ± 7.00	135.80 ± 10.60	140.00 ± 5.60
LDL-C (mg/dL)	49.02 ± 19.50	96.30 ± 3.30 ^a	74.22 ± 13.61	14.77 ± 2.02
CRR	1.60 ± 0.16	2.08 ± 0.06 ^a	1.72 ± 0.12	1.25 ± 0.00 ^a
AC	0.60 ± 0.16	1.08 ± 0.06 ^a	0.72 ± 0.12	0.25 ± 0.00 ^a
AI	0.40 ± 0.16	0.89 ± 0.04 ^a	0.56 ± 0.12	0.10 ± 0.01

Values depicts means ± standard error of mean (SEM); *n* = 6. Mean values with letters (a) indicate significant (*p* < 0.05) difference compared to group I (control). TRIG: triglycerides; CHOL: cholesterol; HDL-C: high-density lipoprotein-cholesterol; LDL-C: low-density lipoprotein-cholesterol; CRR: cardiac risk ratio; AC: atherogenic coefficient; AI: atherogenic index. Group I = Control; Group II = 50 mg EtJC /kg; Group III = 100 mg EtJC /kg and Group IV = 500 mg EtJC/kg.

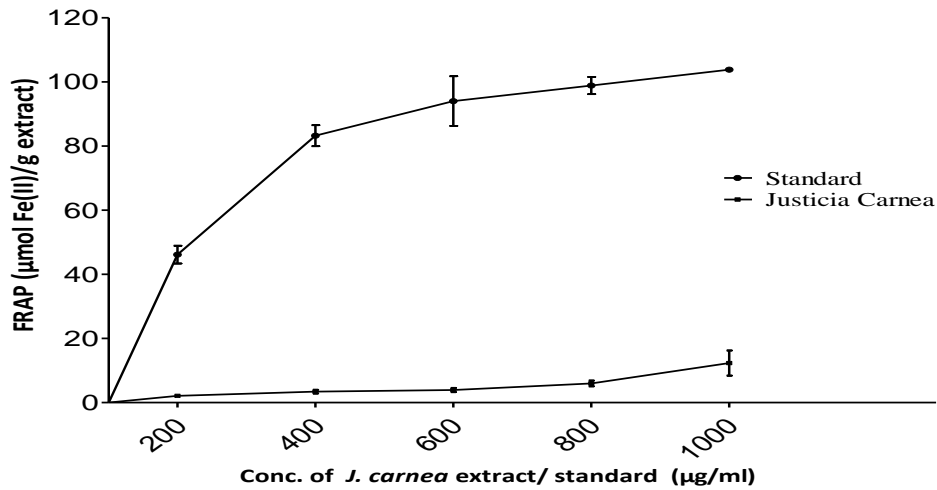
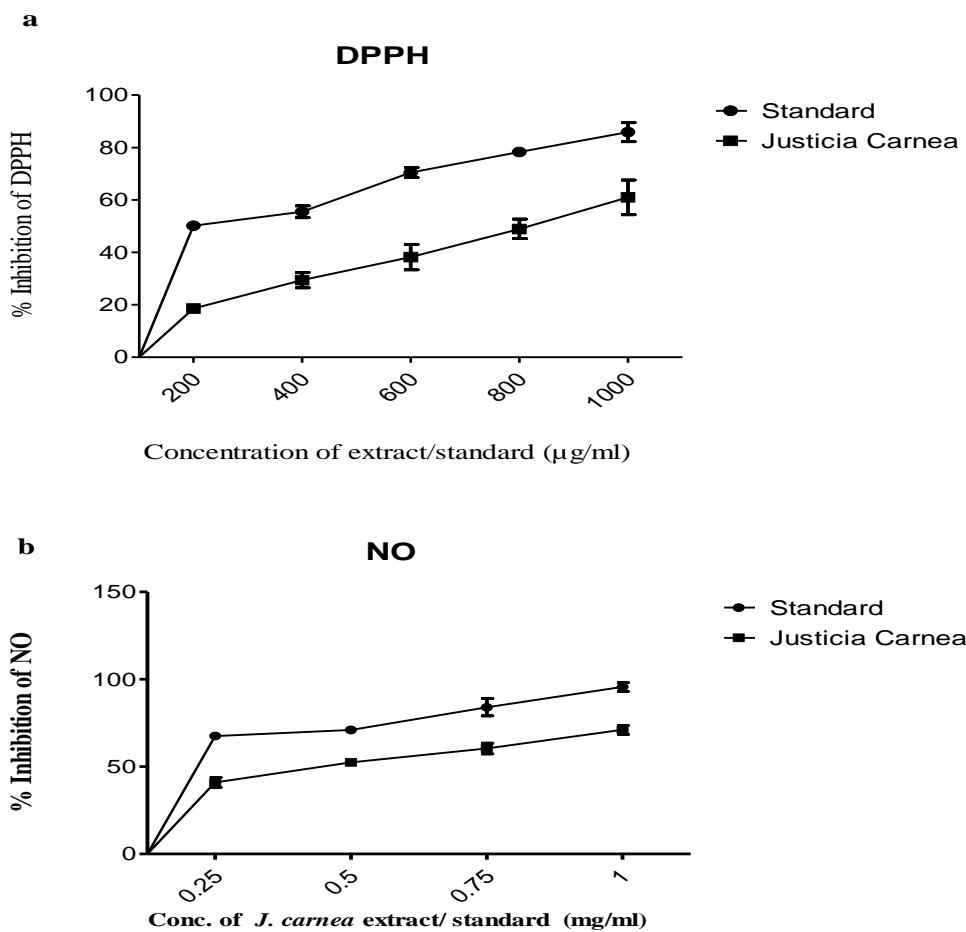


Figure 1. Ferric reducing antioxidant power of



EtJC

Figure 2. Percentage inhibition (a) DPPH radical and (b) Nitric oxide by ethanolic extract of *J. carnea* leaf. Values are mean \pm SEM of three independent experiments.

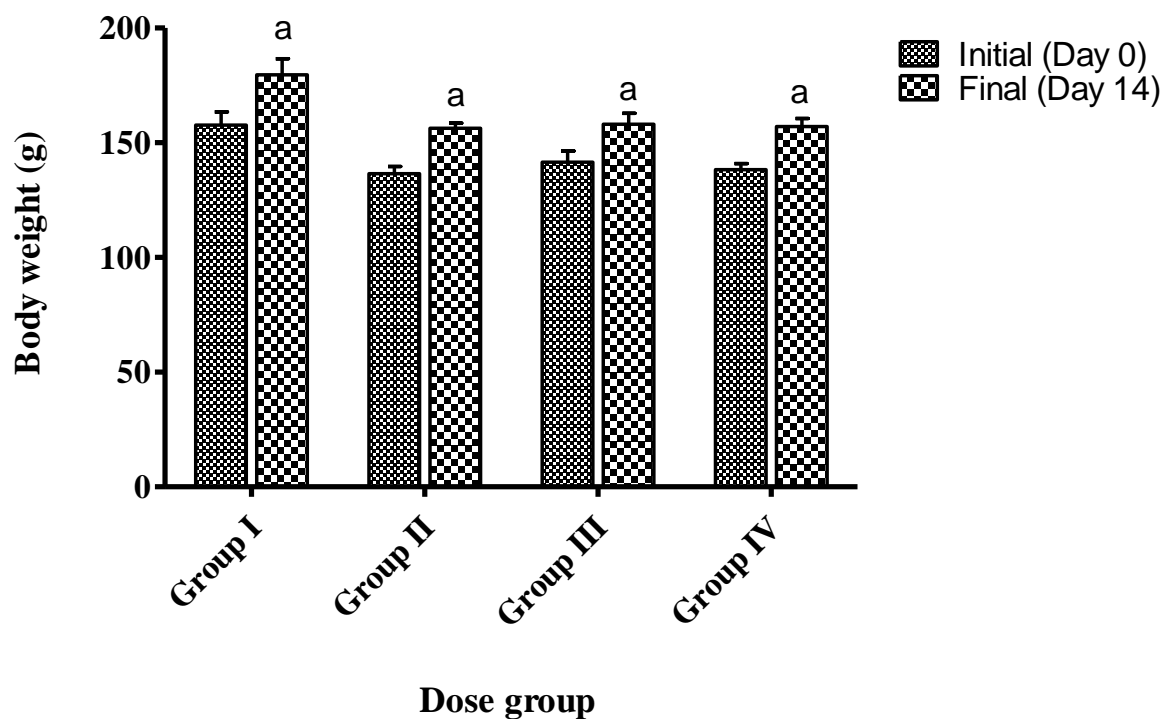


Figure 3. Effect of oral administration of *J. carnea* ethanolic leaf extract on rats' body weight. Values are means \pm standard error of mean ($n = 6$). Bars with letters (a) are significant ($p < 0.05$) compared to the initial weight. Where Group I = Control; Group II = 50 mg EtJC /kg; Group III = 100 mg EtJC /kg and Group IV = 500 mg EtJC/kg.