

## Full length Research Article

# Antioxidant Activity Enhancement and Oxidative Damage Inhibition by *Lagenaria breviflora* fruit and *Xanthosoma sagittifolium* corm in Hypertensive Wistar Rats

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**Summary:** Cardiovascular diseases are the leading causes of mortality in the world today with hypertension being the major clinical presentation of these diseases. This study assessed anti-hypertensive effects of *Lagenaria breviflora* whole fruit and *Xanthosoma sagittifolium* corms in experimentally-induced hypertensive Wistar rats. The ability of the plants to ameliorate oxidative damage accompanying hypertension was evaluated using changes in oxidative stress markers as well as monitoring of cardiovascular parameters. Hypertension was induced by intraperitoneal injection of DOCA salt twice weekly and daily inclusion of NaCl (1%) in drinking water. Methanol extracts of *L. breviflora* or *X. sagittifolium* was administered to hypertensive rats for 35 days and the outcome was compared to hypertensive rats administered with lisinopril or hydrochlorothiazide and a group of normotensive rats (control). Systolic, diastolic and mean arterial pressures were determined on day 34 and blood sample collected on day 35. The rats were thereafter humanely sacrificed and organs were harvested. This study showed the extracts lowered blood pressure, free protein thiols but increased total proteins, glutathione peroxidase, reduced glutathione, glutathione S-transferase, catalase and nitric oxide in the heart, kidney and liver compared to untreated hypertensive rats. However, malondialdehyde levels and hydrogen peroxide activities were reduced. *L. breviflora* fruit and *X. sagittifolium* corm exhibited antihypertensive properties and ameliorated oxidative damage associated with hypertension by enhancing the antioxidant defense system and inhibiting generation of free radicals.

**Keywords:** Hypertension, Oxidative stress, Antioxidant, *Lagenaria breviflora*, *Xanthosoma sagittifolium*

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Manuscript received- August 2022; Accepted- February 2023

DOI: <https://doi.org/10.54548/njps.v38i1.14>

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## INTRODUCTION

Hypertension is a medical condition clinically determined as systolic values  $\geq 140$  mmHg and diastolic  $\geq 90$  mmHg, with normotensive values averagely at 120/80 mmHg (WHO, 2011). Arterial hypertension is characterized by increased oxidative stress and inflammation, which are associated with further cardiovascular risk. An increase in oxidative stress favors endothelial dysfunction by reducing nitric oxide (NO) availability and subsequent beneficial effects on vascular function (Ghosh *et al.*, 2004; Pierini and Bryan, 2015). Oxidative stress is sequel to a shift in the normal equilibrium of oxidant molecules in the body to the anti-oxidants which results in generation of excessive reactive oxygen species (ROS) clinically presented as oxidative stress (Puddu *et al.*, 2009; Pohanka *et al.*, 2017). Amelioration of oxidative stress with pharmacological doses of different antioxidants has proven to reduce blood pressure in hypertensive animals, but not in the

normotensive subjects (Ding *et al.*, 2001; Koo and Vaziri, 2003; Rodriguez-Iturbe *et al.*, 2003).

Important occurrence in the hypertension pathogenesis include increased cardiac output and total peripheral resistance. The kidneys control blood pressure through regulation of blood volume (Berl and Henrich, 2006). A close association has been established between hypertension and progressive kidney dysfunction, manifested as glomerulosclerosis, interstitial fibrosis, proteinuria, and eventual glomerular filtration decline (Tedla *et al.*, 2011). Thus, treatment of hypertension has been geared towards enhancement of renal function and reduction of oxidative stress induction. Antioxidants play a key role in removal of free radical intermediates and inhibition of oxidation reactions caused by reactive oxygen species generated from oxidative stress. Medicinal plants with antioxidant properties are proving to be good alternative antihypertensives with lesser adverse effects compared to orthodox synthetic drugs (Tabassum and Ahmad, 2011; Eghianruwa *et al.*, 2016).

*Lagenaria breviflora*, a perennial climbing plant belonging to the family Cucurbitaceae is used traditionally in the treatment of diseases of inflammatory origin such as diabetes, ulcer, jaundice, piles, colitis, congestive cardiac failure and skin disease (Oridupa and Saba, 2012). *L. breviflora* has antibacterial activity (Tomori et al., 2007), anti-inflammatory, anti-nociceptive and antioxidant properties (Oridupa and Saba, 2012; Onasanwo et al., 2011). *Xanthosoma sagittifolium*, also known as arrow leaf elephant's ear is an edible starchy corm in the family Araceae (Jennings, 1987). It is reported to exhibit potent antihyperglycemic effect (Folasire et al., 2016). The use of herbal remedies is effective with chronic conditions such as hypertension and are cheaper with less side effects when compared to orthodox drugs. There is currently a dearth of information on the antihypertensive effects of these medicinal plants. This study was therefore designed to investigate the antihypertensive properties of *L. breviflora* and *X. sagittifolium* on experimentally-induced hypertension in Wistar rats and the effect of these plants on oxidative stress which accompanies hypertension.

## MATERIALS AND METHODS

**Preparation of methanol fruit extract:** Fresh fruit of *Lagenaria breviflora* Roberty and corms of *Xanthosoma sagittifolium* Schott were obtained from the Agbowo and Oje Markets in Ibadan, Oyo State, Nigeria. The plant samples were identified by a botanist in Botany Department, University of Ibadan. A total of 5kg of fresh fruit were washed and cut into small pieces and dried with a hot air oven at a temperature of 25°C. The bark of the corms was peeled and cut into smaller pieces and were air dried. A total of 525g was obtained from the corms.

**Extraction of the plant samples:** The whole fruit or corms were separately tied up in small portions in sieves and extracted by cold maceration in methanol (96%) in well labelled containers. Each batch of solvent were harvested and stored in plastic containers. The filtrate obtained were concentrated in vacuo using rotary evaporator (BUCHI R-210, Switzerland) set at low temperatures. The methanol remaining in the extract was finally removed by placing small volumes in porcelain dishes in the oven set at low temperature of 30°C. The extracts obtained were refrigerated at 4°C and fresh batches of the extracts were reconstituted with distilled water daily for administration to the rats.

**Antihypertension Study:** Forty normotensive male Wistar rats (150-180g) were used for the study. The rats were housed at the Experimental Animal House of the Department of Veterinary Pharmacology & Toxicology, University of Ibadan. They were allowed access to feed (commercially available rat pellets) and clean water ad libitum. The experiment was conducted in accordance with Experimental Animal Care and Use Regulations Ethics Committee (ACUREC) of the University of Ibadan, Nigeria which is acceptable internationally.

The rats were randomly divided into eight groups of five rats each. Group one served as the normotensive control while hypertension was induced in groups 2 – 8 by intraperitoneal injection of deoxycorticosterone acetate (DOCA)

salt twice weekly and daily inclusion of 1% sodium chloride (NaCl) in water. The hypertensive rats were fed with commercially available rat pellets and NaCl (1%) in drinking water daily. Group 2 were hypertensive and untreated through the course of the study and groups 3 and 4 were orally administered with Lisinopril 5mg/70kg or Hydrochlorothiazide 12.5mg/70kg body weight respectively. Groups 5, 6, 7 and 8 were administered with 100 or 200mg/kg of the whole fruit extract of *L. breviflora* Roberty or corm extract of *X. sagittifolium* (L) Schott respectively. This study was conducted for the period of 35 days for treatment of hypertension after induction of hypertension.

**Sample Collection:** Cardiovascular parameters (mean arterial pressure, systolic and diastolic blood pressures) were measured on day 34 by non-invasive tail plethysmography method without anaesthesia using an electro-sphygmomanometer (CODA, Kent Scientific, USA). On day 35, blood samples (3ml) were collected into lithium heparinized bottles from the retro-orbital sinus of each rat for determination of markers of oxidative stress. Serum was obtained by centrifugation of blood for 10 minutes and at 3000rpm and the serum was stored at -20 °C until use.

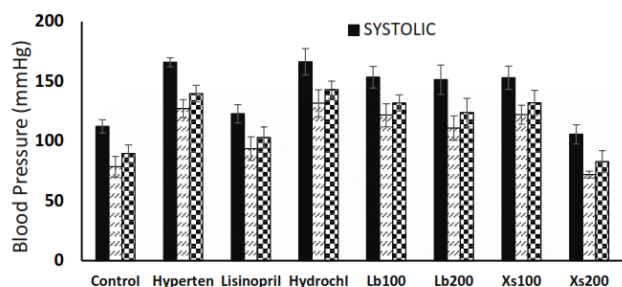
The rats were thereafter humanely sacrificed and the heart, liver and kidney of each rat was carefully removed, perfused immediately with normal saline and blotted with filter paper. It was homogenized in cold potassium phosphate buffer (0.1 M, pH 7.4) using a Teflon homogenizer. The homogenate was centrifuged at 10000 rpm for 10 minutes with a cold centrifuge at 4 °C to obtain post mitochondrial fraction (PMF). The supernatant was used as the sample for determination of markers of oxidative stress assay which estimated levels of total protein, protein thiol, reduced glutathione (GSH), glutathione peroxidase (GPx), glutathione S-transferase (GST), malondialdehyde (MDA), catalase, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and nitric oxide (NO). The biochemical assays were determined by standard protocols according to the methods described by Olaleye et al. (2007).

**Statistical analysis:** Data on biochemical analysis were reported as mean ± standard deviation and were analyzed using ANOVA and subsequently Tukey Kramer multiple comparison test in GraphPad Prism, version 5.0 0 (Graph Pad Software, San Diego, CA, USA) and values of p < 0.05 were considered significant.

## RESULTS

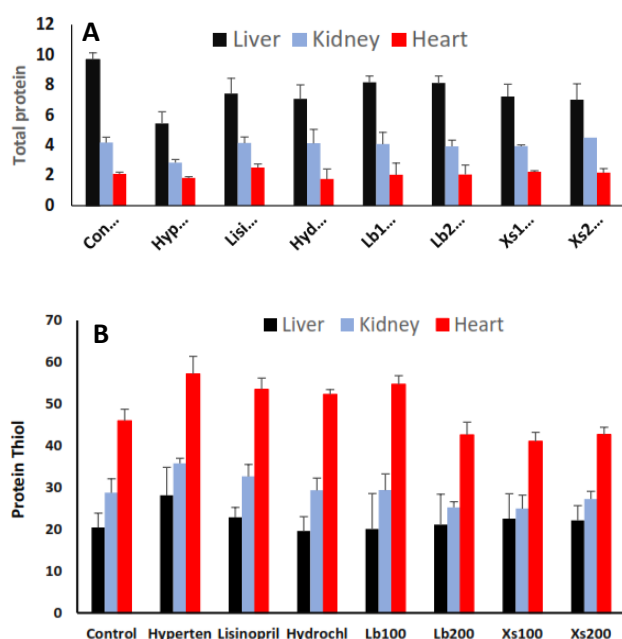
Systolic, diastolic and mean arterial pressures were significantly reduced by the higher doses (200mg/kg) of the extracts compared to the untreated hypertensive rats, especially by *X. sagittifolium* which was statistically unchanged compared to the control rats. Total protein levels in the liver and kidney of hypertensive untreated rats (5.45±0.56mg/dl and 2.85±0.20mg/dl) were significantly lower than that of the normotensive control (9.68±0.75mg/dl and 4.18±0.21mg/dl). Although total proteins in test animals were lower than the control rats, the

levels were significantly higher than those of the hypertensive untreated rats.



**Figure 1:**

Blood pressure of hypertensive rats treated with methanol extract of *Lagenaria breviflora* whole fruit or *Xanthosoma sagittifolium* corm



**Figure 2:**

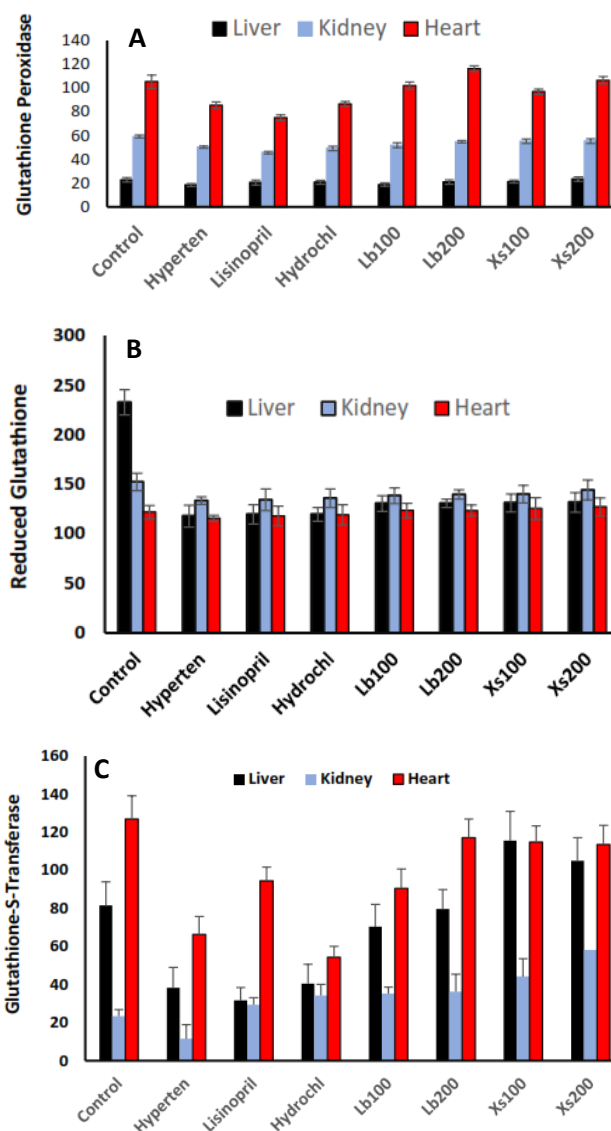
Serum protein (A) and protein thiol (B) levels of hypertensive rats treated with methanol extract of *Lagenaria breviflora* whole fruit or *Xanthosoma sagittifolium* corm

Total protein levels in the heart, although reduced, were not significantly changed when the test rats were compared to the control rats. Protein thiol levels were significantly ( $p < 0.05$ ) increased in all the organs, particularly in the kidney and heart of hypertensive untreated rats ( $28.13 \pm 1.22$   $\mu\text{mol/L}$ ,  $35.79 \pm 1.71$  and  $57.20 \pm 2.61$   $\mu\text{mol/L}$ ) compared to the control rats ( $20.44 \pm 2.31$ ,  $28.77 \pm 2.77$  and  $45.98 \pm 1.67$   $\mu\text{mol/L}$ ). Rats treated with *L. breviflora* (200mg/kg) or *X. sagittifolium* (100 or 200mg/kg) extracts had protein thiol levels that were statistically unchanged compared to the control rats.

The result showed that glutathione peroxidase (GPx) expression was statistically unchanged in liver, but the kidney and heart of hypertensive untreated rats showed significant ( $p < 0.05$ ) decline compared to the controls rats. This decline was reversed in hypertensive rats with the *L. breviflora* or *X. sagittifolium* extracts. *L. breviflora* (200mg/kg) showed significant increase ( $p < 0.05$ ) in GPx expression in the heart. Hypertensive untreated rats showed the least levels of glutathione expression (GSH) in all the organs with marked decline in GSH levels from the liver.

*Oxidative stress amelioration by L breviflora and X sagittifolium in hypertensive rats*

Slight increases in the hypertensive rats treated with the extracts were however observed compared with the untreated rats. Glutathione-S-Transferase (GST) levels were significantly ( $p < 0.05$ ) decreased in the hypertensive untreated rats, but markedly high levels were observed in rats administered with the extracts, especially in the liver of rats administered with *X. sagittifolium* at 100mg/kg ( $151.55 \pm 15.44$ ).

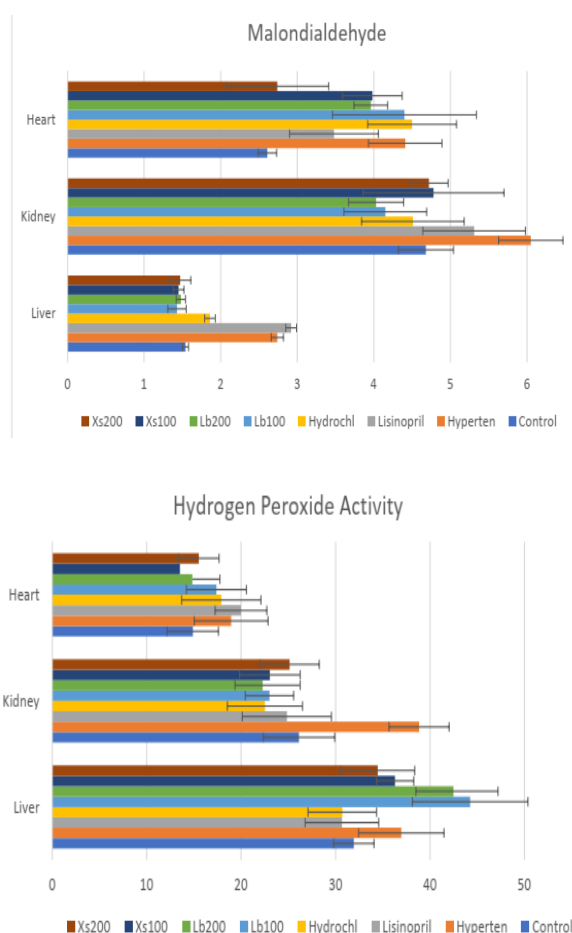


**Figure 3:**

Glutathione peroxidase (A), reduced glutathione (B) and glutathione-S-transferase (C) levels in hypertensive rats treated with methanol extract of *Lagenaria breviflora* whole fruit or *Xanthosoma sagittifolium* corm

Malondialdehyde (MDA) levels in the organs of hypertensive untreated rats were significantly ( $p < 0.05$ ) higher compared to rats treated with the extracts, particularly in the liver. MDA levels in extract treated rats were comparable to that observed in control rats. Rats treated with *X. sagittifolium* extract showed the most consistently comparable levels to the control rats. Catalase levels in the liver and kidneys of all hypertensive rats were reduced, but a non-significant increase was observed in

heart of rats administered with the *L. breviflora* (100 and 200mg/kg) or *X. sagittifolium* (100mg/kg) extracts. Hydrogen peroxide ( $H_2O_2$ ) levels in the liver of hypertensive rats treated with *L. breviflora* were significantly ( $p < 0.05$ ) increased compared to normotensive rats. Although, the levels in liver of hypertensive rats treated with *X. sagittifolium* were increased, it was non-significantly lower than that observed in untreated hypertensive rats. Levels of  $H_2O_2$  in the kidneys of all treated rats were non-significantly lower than that of control rats but significantly ( $p < 0.05$ ) lower than in untreated hypertensive rats. The heart of treated hypertensive rats showed varying levels of non-significant ( $p > 0.05$ ) reductions in the level of  $H_2O_2$  compared to untreated hypertensive rats.



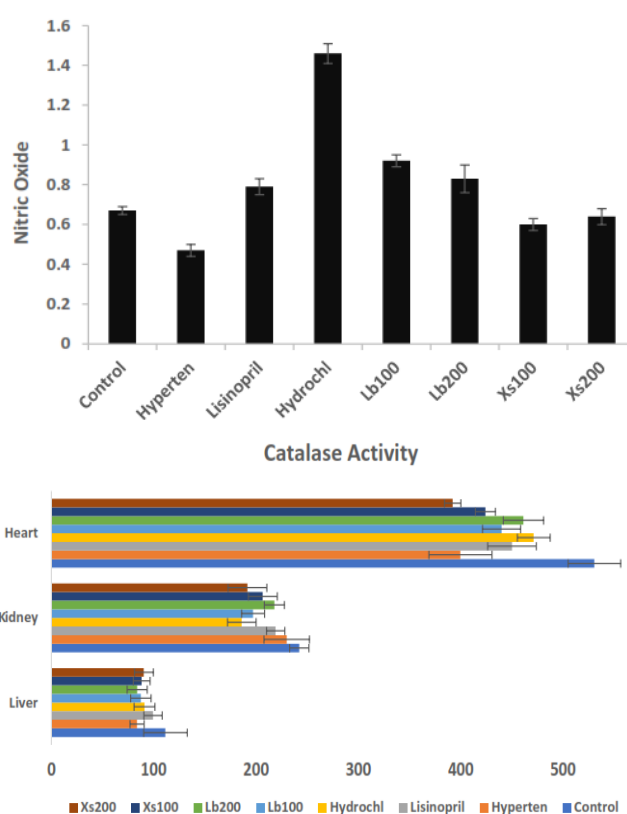
**Figure 4:** Malondialdehyde and hydrogen peroxide levels in hypertensive rats treated with methanol extract of *Lagenaria breviflora* whole fruit or *Xanthosoma sagittifolium* corm

Liver of hypertensive rats treated with *L. breviflora* had significantly higher levels of nitric oxide compared to that of control rats while those treated with *X. sagittifolium* was statistically unchanged. Nitric oxide level in the liver was highest in hydrochlorothiazide group and least in the hypertensive control.

## DISCUSSION

This study evaluated the antihypertensive effect of methanol extracts of *Lagenaria breviflora* whole fruit and *Xanthosoma sagittifolium* corm in experimentally induced

hypertensive rats. The result showed the higher doses of the extracts lowered the blood pressure, particularly *X. sagittifolium*. The main target of management of hypertension is blood pressure control (Rosei, 2016) which these plants gave promising results warranting further study on the exact mechanism involved. A major complication of hypertension, clinically presented as oedema in some hypertensive patients is hypoalbuminaemia (Blankfield *et al.*, 2000). The significantly low protein levels seen in the liver, kidneys and heart of hypertensive untreated rats was reversed in the plant extracts treated hypertensive rats and this was comparable to the hypertensive rats treated with lisinopril, an ACE inhibitor (Khan, 2015) and hydrochlorothiazide, a thiazide diuretic (Vongpatanasin, 2015).



**Figure 5:** Nitric oxide levels and catalase activity in hypertensive rats treated with methanol extract of *Lagenaria breviflora* whole fruit or *Xanthosoma sagittifolium* corm

In this study, hypertension was experimentally induced by bi-weekly intraperitoneal injection of DOCA salt and oral loading with 1% sodium chloride. High sodium diet is associated with increased intra renal angiotensin II (Chen *et al.*, 2020) which may result in renal vasoconstriction and increased renal  $O_2$ - production due to activation of NADPH oxidase. Over production of superoxide anions and other free radicals due to activation of NADPH oxidase may overwhelm the antioxidant capability and cause imbalances between oxidant and antioxidant status which result in oxidative stress (Muhammad *et al.*, 2012). The production of reactive oxygen species (oxidative stress) is the important etiological factor in the development of various diseases

including hypertension (Barrows *et al.*, 2019; Verma *et al.*, 2019).

A major antioxidant defense against free radicals is protein thiol (Birben *et al.*, 2012). Most protein thiols occur bound to albumin in the blood, forming bonds with the sulfhydryl group at its cysteine-34 portion (Rossi *et al.* 2008). Albumin maintains colloid osmotic pressure in the blood and a shift in the balance such as seen in hypoalbuminaemia, clinically presents as excess circulating thiols (Toyoda *et al.*, 2020). Increase protein thiol levels in untreated hypertensive rats further showed hypoalbuminaemia, with more unbound thiols in circulation. This was reversed in hypertensive rats treated with the extracts of *L. breviflora* or *X. sagittifolium* and were comparable to that observed in normotensive rats. Thiols have other roles separately from their role in defense against free radicals and this include their role in signal transduction, detoxification, apoptosis and various other functions at the molecular level (Brown and Griendling, 2015). Protein thiol has also been associated with diabetes mellitus, alcoholic cirrhosis, disorders related to kidney particularly chronic renal failure, cardiovascular disorders such as stroke and several neurological disorders (Prakash *et al.*, 2009).

Glutathione, another form of thiol which occurs in the free oxidized or reduced state, is a known antioxidant in plants and animals which prevents cellular damage caused by reactive oxygen species to important cellular component (Pompella *et al.*, 2003). Glutathione serves as an electron donor to reduce disulfide bonds formed within cytoplasmic proteins to cysteines. This process converts glutathione to its oxidized form; glutathione disulfide (GSSG). GSH act directly as an antioxidant or a cofactor for several protective enzymes important for cellular defense against oxidative stress (Lushchak, 2012; Koohpeyma *et al.*, 2020). In this study, GSH levels were statistically unchanged in all hypertensive rats. However, extract treated rats had significantly increased glutathione peroxidase and glutathione-s-transferase levels. Glutathione peroxidases are a family of peroxidases that protect the body from oxidative damage by reducing lipids and hydrogen peroxides (Espinoza *et al.*, 2008), while glutathione-s-transferases, a family of phase II detoxification enzymes, catalyze GSH conjugation to a wide assortment of endogenous and exogenous electrophilic compounds (Townsend and Tew, 2003).

Another antioxidant is nitric oxide which was restored to slightly above normal levels by the extract in this study. Nitric oxide protects against increased blood pressure and cardiac hypertrophy which are very key in the hypertensive state (Liu *et al.*, 2005). The nature of nitric oxide as a messenger molecule is unusual with many physiologic roles, in many systems including the cardiovascular, neurologic and immune systems. Nitric oxide is known to mediate pathogen suppression, blood vessel relaxation and neurotransmission (Toda and Okamura. 2003). Nitric oxide in excessive amounts may cause host cell injury triggering neurotoxicity during strokes, and initiating hypotension related to sepsis (Madan and Rao. 1996). Furthermore, this study showed catalase activity increased significantly particularly in the heart of extract treated rats compared to the decline in untreated hypertensive rats. Catalase is an enzyme which occur in approximately all living organisms with oxygen exposure. This enzyme catalyzes hydrogen

peroxide breakdown to water and oxygen, an important protective mechanism against ROS-induced oxidative damage (Chelikani *et al.*, 2004).

This study also showed significant reduction in malondialdehyde and hydrogen peroxide, major markers of oxidative damage. MDA is the main product from lipid peroxidation and can be estimated to determine the extent of tissue damage (Davey *et al.*, 2005). A previous clinical investigation by Verma *et al.* (2019) showed that MDA concentrations were high in hypertensive patients than in normal individuals. This current study corroborated this finding and suggested the extracts inhibited lipid peroxidation further enhancing the antioxidant system. Generation of hydrogen peroxide was also significantly inhibited especially in the kidneys.

In conclusion, the antihypertensive effect of *L. breviflora* whole fruit or *X. sagittifolium* corm extract was demonstrated with effective reduction in blood pressure and was comparable to lisinopril. The extracts also reversed hypoproteinaemia with the consequently increased circulating protein thiols, while enhancing the antioxidant systems of the glutathione enzymes, nitric oxide and catalase. The extracts also ameliorated oxidative damage associated with hypertension determined in this study by decreased MDA levels and hydrogen peroxide activity. Further study is needed to elucidate the bioactive principle(s) responsible for this pharmacological activity.

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