The Journal of Phytopharmacology 2019; 8(3): 91-95
Online at: www.phytopharmajournal.com

Research Article

ISSN 2320-480X
JPHYTO 2019; 8(3): 91-95
May- June
Received: 06-04-2019
Accepted: 08-05-2019

DOI: 10.31254/phyto.2019.8302

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Endothelium-dependent and independent effect of *Guibourtia tessmannii* (Caesalpiniaceae) on vascular contractility of rat

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ABSTRACT

The stem barks of *Guibourtia tessmannii* (Caesalpiniaceae) are used in traditional Gabonese medicine as antihypertensive remedies. In the present study, we investigated vasorelaxant properties effect of aqueous extract from *G. tessmannii* and fuller understanding these mechanisms of action *in vitro*. The activity of *Guibourtia tessmannii* was evaluated on isolated aorta rings of rat constricted with KCl (80 mM) and norepinephrine (10^{-4} M). Cumulative concentrations (1 mg/mL - 100 mg/ml) of *G. tessmannii* provoked a dose-dependent relaxation of the thoracic aorta precontracted by norepinephrine or KCl (95.69 ± 0.6% and 91.34 ± 4.90%, respectively). The vasorelaxant effect induced by *G. tessmannii* on the aorta precontracted by KCl was significant decreased in presence of Nω-nitro-L-arginine methyl ester (11.30 ± 4.3 %, $p<0.05$), tétraéthylammonium (52.2 ± 9.20 %, $p<0.01$). Indomethacin and atropine modify the vasorelaxant effect of plant extract (57.13± 6.9 %, $p<0.01$ and 58.83± 5.9 %, $p<0.01$, respectively. These results suggest that the vasorelaxant action of *G.tessmannii* was mediated via the muscarinic receptors. The direct effect of *G.tessmannii* to be mediated by alpha-adrenergic receptors and potassium channels.

Keywords: aqueous extract, *G. tessmannii*, aorta, endothelium, vasorelaxation.

INTRODUCTION

In Africa, more than 80% of the population uses traditional medicine and pharmacopeia for primary health needs [1]. In the developing countries, for example, medicinal plants are used in the treatment of various pathologies among which the arterial hypertension [2, 3, 4].

Hypertension is one of most important risk factors for cardiovascular and cerebrovascular diseases. Blood pressure control reduces the risk for developing arterial coronary disease, heart failure, cerebral vascular disease and renal damage [5]. The cardiovascular diseases are predicted to cause one- fourth of all global deaths in 2030. The number will increase by about 60% to a total of 1.56 billion as the proportion of elderly people will increase. Some reasons are the changes in population lifestyle, which include a diet in sugar and high fat process foods and sedentary behavior [6].

Hypertension is asymptomatic until progresses to a life-threatening condition. Prevent or treatment of hypertension can be done by different means pharmacological, non-pharmacological therapies with the modification of life-style, body weight reduction, alcohol intake reduction, moderation of dietary sodium, increasing physical activity and by means of medicinal plants. The medicinal plant has been used for centuries to treat more diseases such as cardiovascular diseases [7].

Guibourtia tessmannii is a plant used to handle the high arterial blood pressure, it was demonstrated that are hypotensor effect could inhibit the calcic impulse, [4] on one hand and that this plant had an antioxidant activity on the other hand [8].

In our study, in order to provide pharmacological basis for traditional use of *Guibourtia tessmannii* as hypertensive treatment and discover novel vasorelaxant from natural resources, we investigated the vasorelaxant effects of aqueous extract from the stem bark of *Guibourtia tessmannii* on her ability to relaxed isolated ring aorta.

MATERIAL AND METHODS

Plant material and extraction

Stems barks of *Guibourtia tessmannii* were collected in the south of Gabon in august 2010. The plant was authenticated in the Herbier National du Gabon (HNG), Institute of Pharmacopeia and the Traditional Medicine, Libreville, Gabon were a voucher specimen (SRFG 879 LBV) was deposited.

The stems barks of the plant were sundried and crushed to powder using mortar and Culatti micro-crusher. The powder obtained (400 g) was macerated in 1000 mL of distilled water during 24 hours at room temperature and filtered using a watman Millipore filter. The filtrate lyophilized at - 40°C. The powder lyophilized (25 g) was stored at + 5°C until further used.

Tissue preparation

Wistar rat of 180-250 g of both sexes were used. Each rat was acclimated and after anesthesia with urethane (15%:1.5 g/kg of body weight), the aorta was dissected, cleaned of connective tissue and cut into approximately 5-6 mm strips. Aorta rings were placed in petri-dish containing the Mac-Ewen solution with the following composition (mM): NaCl, 130; KCl, 5.6 CaCl2, 2.6; NaH2PO4, 0.91; NaCO3H, 11.9, MgCl2, 0.24; glucose, 11., maintained at 37 C and continuously aerated with 95% O_2, 5% CO_2. The aorta was extracted from the surrounding tissues and strip of aorta was cut into 6-7mm length, for isometric tension recording, as previously described [9].

Endothelium integrity responsiveness was verified through relaxation of norepinephrine-induced contraction with ACh (10^{-4} M). The functional removal was verified by the absence of relaxation evoked by ACh on Ad-induced contraction. The Aortic strip was then washed with Mac-Ewen solution to allow its relaxation to the lowest tension [10, 7].

Experimental protocols

Effects of the extract aqueous of *G. tessmannii* on KCl and NE-induced pre-contractions

This study was aimed to assess the effect of the aqueous extract of *G. tessmannii* on the isolated aorta contracted by KCl (80 mM) or NE (10^{-5} M). When the contraction reached a plateau, the extracts added cumulatively (1 mg/ml, 10 mg/mL, 50mg/mL and 100 mg/mL). The relaxation effect was calculated as the percentage of the contraction response induced to NE or KCl.

Effects of antagonists on the aqueous extract-induced relaxation of de KCl and NE-induced pre-contraction

To study possible mechanism related to relaxant effects induced by the extract, the following protocols were performed: the roll of endothelium was studied adding the extract with NE or KCl contracted rings. To assess whether nitric oxide, K^+ channel, calcium channels, or muscarinic receptor are related to the relaxant mechanism induced by the extract. The extract was added to rings contracted with NE or KCl treated 15min previously with one of the following substances, respectively: L-NAME (10^{-5} M), a NO synthase inhibitor; TEA (10^{-4} M), a non-selective K^+ channels blocker; indomethacin (10^{-4} M), a cyclooxygenase (COX) inhibitor and atropine (10^{-5} M) a muscarinic receptor blocking agent.

Data analysis

All values are expressed as means ± standard error of the mean (S.E.M). Multiple comparisons were performed to GraphPad Prism software (GraphPad Software Corporation, 5.0 version) with ANOVA test followed by Dunett's test to determine the difference between the group means. The 5% significance level ($p<0.05$) was adopted for the differences.

RESULTS

Effects of the t aqueous extract of *G.tessmannii* on KCl and NE-induced pre-contractions

The cumulative concentration of aqueous extract of *G.tessmannii* (1-100mg/mL) produced a dose-dependent relaxing effect on k^+ depolarized smooth muscle. The maximum effect obtained for the highest concentration (100 mg/mL) was 95.69 ± 0.6 % ($p<0.05$) and 91.34 ± 4.90 % with KCl and norépinéphrine, respectively (figure 1).

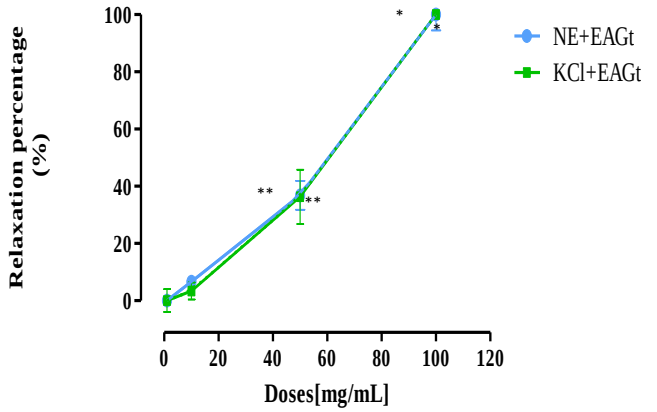
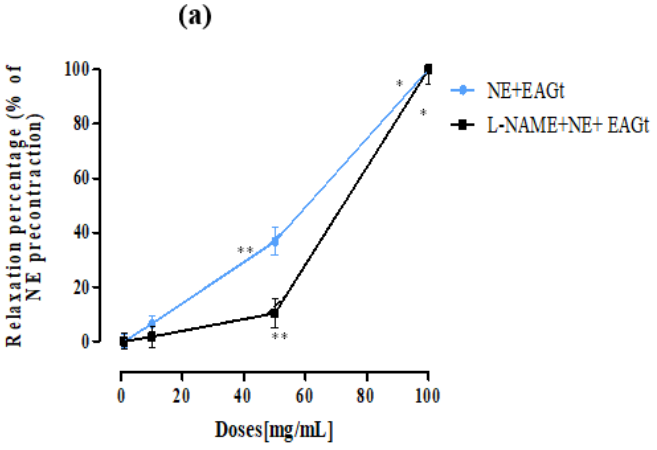


Figure 1: Effect of aqueous extract of *G. tessmannii* (EAGt) on aorta isolated pre-contracted with KCl or norepinephrine (NE). Each point represent mean ± S.E.M of the relaxant effect expressed as % relaxation. (n=6). Significant difference for * $p<0.05$; ** $p<0.01$.

Effects of antagonists on the relaxant effect of aqueous extract of *G. tessmannii*

Pre-incubation of k^+ depolarized tissues with L-NAME resulted in the non-parallel shift to the right of dose response curves (figure 2a and 2b) with significant reduction in the global relaxation response to the extract (figure 2b). The relaxation percentage obtained for the highest concentration of extract were, respectively, 84.83 ± 4.6%vs 91.34 ± 4.90 % (non-significant) and 11.30 ± 4.3 % ($p<0.05$) versus 95.69 ± 0.6 % for NE and KCl, respectively (figure 2).



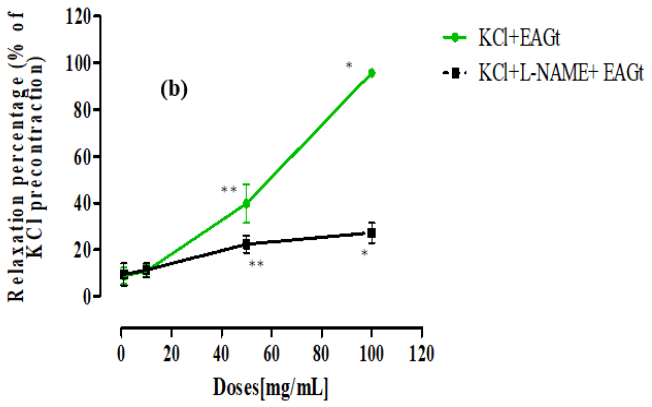


Figure 2: Effect of the L-NAME on the vasorelaxant effect of *EAGt* on aorta rings contracted with norepinephrine (NE) (fig2a) or KCl (fig2b). Each point represent mean ± S.E.M of the relaxant effect expressed as % relaxation. (n=6). Significant difference for * p<0.05; **p<0.01; versus control (KCl+ EAGt).

The vasorelaxant effect of *EAGt* was inhibited partially by the presence of TEA on the aortic strip pretreated with KCl, 52.2 ± 9.20 % versus 95.69 ± 0.6 % (p<0.01) on the aortic non treated respectively for the maximal effect. The effect relaxant was significantly reduced by TEA (figure 3).

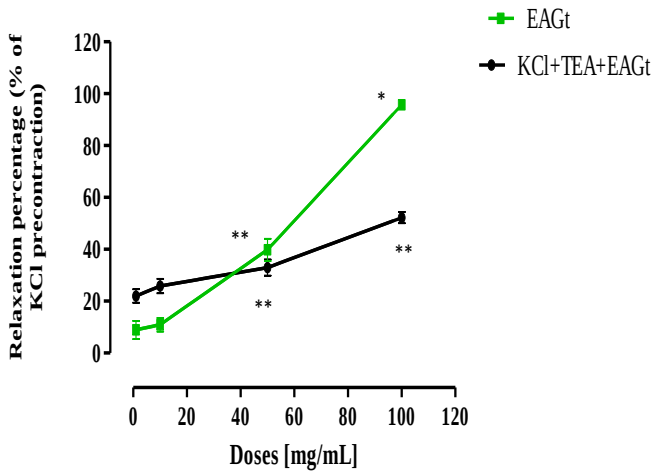


Figure 3: Effect of tetraethylammonium (TEA) on the relaxation induced by EAGT on isolated aorta pre-contracted with KCL. Each point represent mean ± S.E.M of the relaxant effect expressed as % relaxation. (n=6). Significant difference for * p<0.05; **p<0.01; versus control (KCl+ EAGt).

Atropine (Atr) induced a dose–dependent relaxation on the aortic rings isolated. The relaxant effect of extract was reduced, E_{max} = 57.13± 6.9 % (p<0.01) versus control E_{max} = 91.34 ± 4.90 %, for rings without antagonist. The relaxant action of the extract on aorta rings was affect the minimum dose response (p<0.05) which were decreased by the presence of atropine.

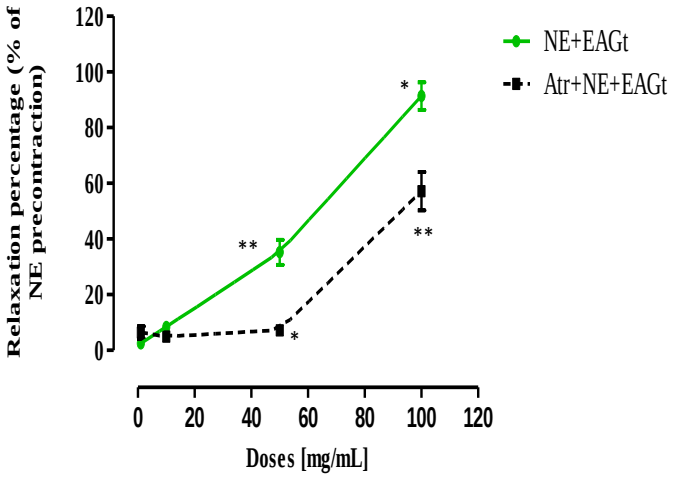


Figure 4: Effect of atropine on the relaxation of EAGt on isolated aorta constricted with NE. Each point represent mean ± S.E.M of the relaxant effect expressed as % relaxation. (n=6). Significant difference for * p<0.05; **p<0.01; versus control (KCl+ EAGt).

The figure 5 showed the effect of Indomethacin (Indo) on the aortic strip isolated. The relaxant effect of EAGt had increase for minimal dose (1 mg/mL) 40.55± 4.8% versus 2.55± 0.14% without indometacin. The relaxation maximal was not significant (p<0.01) versus control.

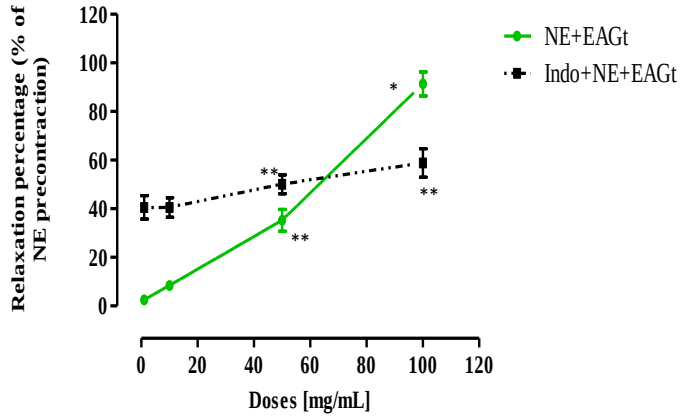


Figure 5: Effect of indomethacin on the relaxant effect of EAGt on the isolated aorta constricted with NE. Each point represent mean ± S.E.M of the relaxant effect expressed as % relaxation. (n=6). Significant difference for * p<0.05; **p<0.01; versus control (KCl+ EAGt).

DISCUSSION

The study on the effects of *G. tessmannii* on the isolated rat aorta and these mechanisms of action showed that the aqueous extract of *G. tessmannii* had vasorelaxant dose-dependent and endothelium-dependent activity.

G. tessmannii macerated is used in the Gabonese pharmacopoeia for antihypertensive properties. The results obtained showed that the aqueous extract induces dose-related vasorelaxant effects, this shows that *Guibourtia tessmannii* contains substances capable of causing vasorelaxation which could be involved in the antihypertensive effects of the plant.

The vascular contraction and relaxation are controlled by changes in cellular Ca^{2+} concentration in the vascular smooth muscle. The Ca^{2+} used for contraction includes intracellular or extracellular sources or both sarcoplasmic reticulum is the major source of intracellular Ca^{2+} [11, 12]. The vascular endothelium plays an important role in regulating vascular tone through the secretion of both relaxing and contractile factor. The endothelium responds to the different chemical and physical stimulation by producing vasoactive substances, which include nitric oxide (NO), prostacyclin and an endothelium-derived hyperpolarizing factor (EDHF) [13].

The vasorelaxant effect of aqueous extract of *Guibourtia tessmannii* persisted in presence of L-NAME (a nitric oxide synthase inhibitor) [14]. This result did not show a significant difference between the effects on the non-treated aorta strips and pre-contracted by NE. These results suggesting that the activity of the plant aqueous extract is endothelium-independent. The extract may therefore act directly on the vascular smooth muscle. By contrast, the relaxant effect of aqueous extract was blunted by L-NAME presence on aorta strips contracted by KCl, suggesting that extract interferes with endothelium-dependent production of relaxing factors. This effect is related to the action of different agonists such as adenosine, acetylcholine, bradykinine and serotonin on endothelial receptors or by an improvement in the availability of the substrate or co-factor for NOS remains to be determined. Similar results were found by Belemnaba and *et al*. [15] with dichloromethane fraction from *Anogeissus leiocarpus*, aqueous extracts of *Terminalia superba* [16] and ethanolic extract of *Marrubium vulgare* [17].

In addition, the relaxation evokes by *Guibourtia tessmannii* in the aortic strips was not significantly affected by indomethacin (a cyclooxygenase inhibitor) [18], showing that the aqueous plant extract did not use the endothelium-derived prostacycline or nitric oxide pathway.

The relaxant effect of aqueous extract was significant in NE-constricted aorta rings than KCl- constricted aorta rings. The use of KCl in the extracellular medium in the constriction of the aorta ring inhibits the EDHF contribution, which depends mainly on K^+ channels activation [19, 13].

This is how the vasodilatation dependent on the contribution of nos and cyclooxygenase becomes dominant during the KCl challenge [20, 7]. Thus, the reduction of the relaxing effect of the extract during constriction with KCl, would suggest that it is partially mediated by the improvement of the EDHF release.

Also, according to experiment with atropine, reduced extract relaxant effect, it can be presumed that extract would action on muscarinic receptors, then a cholinomimetic effect relaxant profile.

Our results show that pretreatment of aortic strips with a non-specific K^+ channels, tétraéthylammonium [16, 21] significantly reduce relaxant effect of the aqueous extract. These results suggesting that aqueous extract of *Guibourtia tessmannii* dilated the vascular smooth muscle via activation of TEA-sensitive K^+ channels.

The aqueous extract of *Guibourtia tessmannii* relaxed without significant difference contraction caused by norepinephrine or KCl. These substances act by activating intracellular and extracellular calcium; norepinephrine acting on intracellular calcium and KCl acting on extracellular calcium. In fact, depolarization related to K+ response leads to calcium input through L-type channels, which is a secondary route for NE, the main one being linked to activation of calcium stores by inositol-1, 4, 5-triphosphat [22].

The aqueous extract of *Guibourtia.tessmannii* in our study inhibited the contractile response induced by KCl and NE. Norepinephrine is an alpha-adrenergic that causes the contraction of the cell muscle smooth by the entry of Ca^{2+} via calcium receptors and by the release of Ca^{2+} from the sarcoplasmic reticulum [11, 23]. The activation of alpha receptor leads to the production of diacylcerol and inosotol tri-phosphate (IP3) inducing the release of Ca^{2+} [24]. Contrary, the contraction elicited by KCl is directly related to the influx of extracellular calcium caused by the depolarization of the cell membrane and the opening of Ca^{2+} voltage- depend channels [25, 26]. This could suggest that the vasorelaxant endothelium-independent effect of aqueous extract is related to its effect on smooth muscle Ca^{2+} homeostasis.

CONCLUSIONS

In conclusion, result of this study indicated that aqueous extract of *Guibourtia tessmannii* had dose-dependent and endothelium-dependent and independent vasorelaxation properties which may be related by activation of K^+channels. Our results suggest that the relaxant effect of *Guibourtia tessmannii* on the contractile response of the isolated aorta may be related to the cholinomimetic and non-cholinomimetic action.

Further experiments are needed to isolated and identify the active principle of this plant.

ACKNOWLEDGEMENTS

This work was supported by Institute of Pharmacopeia and Medicine Traditional (IPHAMETRA) of Libreville/ Gabon. We thank Ada Nguema Sandra, technician, of the, Department of pharmacology and toxicology (IPHAMETRA), for her free availability.

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HOW TO CITE THIS ARTICLE

Orianna KMN, Raissa ASR, Alain S, Alfred SBT. Endothelium-dependent and independent effect of *Guibourtia tessmannii* (*Caesalpiniaceae*) on vascular contractility of rat. J Phytopharmacol 2019; 8(3):91-95.