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RESEARCH ARTICLE

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Effects of Methanol Fraction of Anarcardium Occidentale (Cashew) Bark Extract on Salt-Induced Hypertension in Rats

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ABSTRACT

Background: Hypertension is an important public health problem that needs multiple treatment approach. Anacardium occidentale Linn. (Anacardiaceae) on the other hand is a plant largely used locally in for treatment of various ailments. The present study therefore assessed the antihypertensive effects of methanol extract of Anacardium occidentale (AO) root back on cardiovascular parameters in high salt fed Wistar rats

Methods: Twelve (12) male weaning Wistar rats (40-60g) were randomly divided into four groups (n=3). Group A and B were placed on a normal salt diet while Group C and D were placed on a high salt diet for ten (10) weeks. Carotid artery was then isolated, canulated and connected to a SP844 physiological pressure transducer attached to a PowerLab 8SP unit for blood pressure measurement. AO bark extract (0.1mg/ml) was then administered intravenously in different concentrations (0.01, 0.1, 1.0, and 10, 100 µg/ml) through the jugular vein in 0.1ml/dose to Group A and C while Nifedipine was administered in different concentrations (0.04, 0.4, 4.0, and 40, 200 µg/ml) to groups B and D. The data were analyzed using unpaired-student t test and non-linear regression curve were generated using Graphpad-prism version 8 at 95% confidence interval

Results: SBP, DBP, HR and MAP (141.06±4.27, 109.99±4.26, 398±13.25, 120.35±4.25 were significantly higher in HS animals than the NS animals (103.69±11.56, 79.34±4.07, 354±66.96, 77.07±8.17) respectively. Nifedipine was able to reduce DBP and MAP were reduced in a dose dependent faction while AO bark extract reduced all blood pressure parameters in dose dependent faction in HS and NS animals

Conclusion: We therefore concluded that Anacardium occidentale bark extract was able to reduce blood pressure parameters in a dose-dependent manner than nifedipine in this study

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Introduction

Hypertension is the most common risk factor for the development of cardiovascular disease, heart attack, heart failure and stroke and the most common causes of adult morbidity and mortality in Africa [1, 2]. Hypertension is an important public health problem defined as the persistent high blood pressure, clinically indicated as when the systolic pressure remains elevated above 140 mmHg and, or diastolic pressure remains elevated above 80 mmHg. In industrialized countries, hypertension affects over 20% of adult population, and it is implicated in thousand to millions of death from various heart diseases witnessed each year while in underdeveloped countries like Nigeria the prevalence of hypertension is about 28.9%, with a prevalence of 29.5% among men and 25.0% among women [3, 4]. The condition is one of the most common complex disorders and its etiology differs amongst several individuals within a large population [5]. Genetic factors, aging, obesity, excessive alcohol, elevated renin level, vitamin D deficiency, lack of exercise, smoking and high salt intake are all risk factors for hypertension [6, 7]. Extensive evidence of a causal relationship between dietary sodium intake and high blood pressure has emerged from animal experiments, observational epidemiological studies, and randomized controlled clinical trials [8-11].

The known mechanisms of conventional antihypertensive drugs include inhibition of phosphodiesterase, reduction of intracellular Ca2+, control of angiotensin converting enzyme activities, induction of nitric oxide (NO) in smooth muscles [12, 13]. Among them, NO induction from vascular endothelium resulting from anti-oxidative activity of flavonoid and phenolic compounds has an important contribution in their anti-hypertension properties. Oxidative stress continues as an appealing target for cardiovascular diseases prevention and treatment. This is due to abnormal reactive oxygen species (ROS) production, which leads to a decrease in NO bioavailability in the vascular system, and consequently contributes to the pathogenesis of endothelial dysfunction in the development of hypertension [14-16].

Furthermore, evidence suggests that approximately three-quarters of hypertensive patients on anti-hypertensive medication

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are unable to achieve the target blood pressure, due to ineffective and side effects of the drugs [17-19]. Consequently, a significant number of individuals with cardiovascular diseases, including hypertension, make use of alternative therapies [20]. The use of herbs and medicinal plants for the treatment of disease has become a worldwide phenomenon [21]. Nonetheless, the efficacy of many of such herbs are still not scientifically validated.

Anacardium occidentale popularly known as cashew has long been used in folk medicine to treat hypertension [22]. This plant is from Brazil and belongs to the Anacardiaceae family. It is also grown in India, Sri Lanka, Kenya, and Tanzania. The Greek prefix ana-, which means 'up or upward,' the Greek cardia, which means 'heart,' and the New Latin suffix -ium make up the generic name Anacardium [23]. The plant has different local names depending on language groups. In Nigeria, it is called Kashu by the Hausa people; Kashuu by the Igbo speaking tribes and Kaju by the Yoruba people. The English name of the plant is derived from the Portuguese pronunciation: [kaˈʒu], which is derived from the Tupian word acaju, which literally means "self-producing nut" [24]. This plant has been used for the treatment of various ailments [25,26]. Therefore, the current study was designed to highlight and further study the possible anti-hypertensive properties of Anacardium occidentale using male rats as the animal model.

Materials and Methods

Collection of Plant and Extract Preparation

The stem bark of Anacardium occidentale was collected at Abeokuta, Ogun State between May and June, 2019. The plant was authenticated by Mr D.P.O. Esimelekuai of the Department of Botany, University of Ibadan, Ibadan, Nigeria and deposited with herbarium no.: UIH-22599.

The stem bark was dried under shade and pulverized. The obtained pulverised sample (500 g) was packed into a soxhlex extractor and extracted with methanol. Methanol was later removed from the resultant mixture with the aid of a rotatory evaporator under reduced pressure and temperature of 52oC. The solid sample of the extract obtained was kept in the refrigerator. The crude methanol extract was designated as AoME and used for this study. To prepare the extract solution used for this invitro study, 0.005g of the extract was weighed and dissolved in 5ml of 100% ethanol. The solution was then diluted with water in serial concentration which are in 10^{-2} µg, 10^{-1}µg, 1µ, 10µg, 100µg from lower to higher concentration.

Animals and Induction of Hypertension

The experiment was carried out on 12 male weaning Wistar rats weighing 40-60g randomly assigned into four groups (n=3). The animals in groups A and B were normotensive rats given a normal salt feed (0.3% NaCL), while hypertension was induced in the animals in groups C and D by placing them on a high salt diet (8% NaCL) for a period of ten weeks as already established in previous studies [27-29].

Design of Study

The experiment was carried out on 12 male weaning Wistar rats weighing 40-60g randomly assigned into four groups (n=3). The animals in groups A and B were normotensive rats given a normal salt feed (0.3% NaCL), while hypertension was induced in the animals in groups C and D by placing them on a high salt diet (8% NaCL) (27) for a period of ten (10) weeks as already established in previous studies [28, 29]. After the induction of hypertension, the animals in group A and C were treated with serial concentration (0.04µg, 0.4µg, 4µg, 40µg and 200µg) of nifedipine while animals in group B and D were treated serially with 10^{-2} µg, 10^{-1}µg, 1µ, 10µg, 100µg of AoME

Sacrificing of Animals and Determination of Blood Pressure

After ten (10) weeks of feeding, the animals were anesthetized via intra-peritoneal route using urethane solution (5 ml/kg bw). The jugular vein and carotid artery were isolated, catheterized and heparinized using heparin saline solution with the cannula connected to a SP844 physiological pressure transducer attached to a PowerLab 8SP unit (AD Instruments, Australia) for reading of blood pressure parameters. After proper cannulation, heparinized saline solution was injected into the jugular vein, and the blood pressure and heart rate were recorded to determine the baseline values. After that, different doses of the extract were injected and blood pressure was measured. This procedure was repeated using all the animals in the different study groups (A, B, C and D). To compensate for fluid loss, the normal saline was continuously infused at intervals throughout the period of the measurement of blood pressure parameters.

Preparation of Nifedipine

Nifedipine used for this study was purchased from local pharmacy and the solution was prepared following same procedure for Anacardium occidentale above. 0.005 g of Nifedipine was dissolved in 5 ml of distilled water and Serial concentrations of 0.04µg, 0.4µg, 4µg, 40µg and 200µg (from lowest to highest concentration) were prepared. This was then administered through the jugular vein after 15 minutes of stabilizing the animals by administering a dose of 0.1ml for each concentration sequentially. The next preceding dose is administered after the effect of the previous administration wears off and the blood pressure returns to normal.

Statistical Analysis

Data were presented as Mean ±Standard Error of Mean (SEM) for the in vivo study. Comparisons between groups were made using unpaired two-tailed t-test analysis with the help of the statistical software Graphpad version 8, with $p<0.05$ considered statistically significant. Non-linear regression curve were also generated using same statistical software

Results

Effect of High Salt Diet on Blood Pressure Parameters

The result is as shown on Table 1. According to the result, SBP [(141.06±4.27) (P=0.0128)], DBP [(109.99±4.26)(P<0.0082)], MAP [(120.35±4.25)(p= 0.0027)], and H.R [(398±13.25)(P=0.0225)] were significantly higher in animals fed with high salt diet for a period of ten weeks when compared with values obtained in animals fed with normal salt diet with a confidence interval of 95%. Although RPP and PP were also higher, these differences were not statistically significant when compared with the group fed with normal salt diet at confidence interval of 95%.

Effect of Nifedipine on Systolic Blood Pressure in Normal and High Salt Rats

In normal salt animal, the systolic blood pressure started off by

decreasing after the first concentration of nifedipine but there was no significant decrease thereafter. However, in high salt animal, there was significant decrease (with 95%confidence interval) in the systolic blood pressure from the initial pressure to the final pressure after the last concentration of nifedipine was administered (Figure: 1a). According to the result also, there was a significant decrease (with 95%confidence interval) in the diastolic blood pressure from the initial pressure to the final pressure after the last concentration of the nifedipine was administered in the normal salt fed as well as the high salt fed animals. However, there was no concentration dependent differences (with 95%confidence interval) in the heart rate in the normal salt fed as well as the high salt fed animals following the administration of nifedipine (Figure: 1c). The mean arterial blood pressure was also significantly reduced from the initial pressure to the final pressure after the last concentration of the nifedipine was administered to the normal salt fed and the high salt fed animals (Figure: 1d). Nifedipine also reduced the rate pulse pressure in the high salt diet only (Figure: 1e) while the pulse rate was not affected (Figure: 1f) at 95%confidence interval.

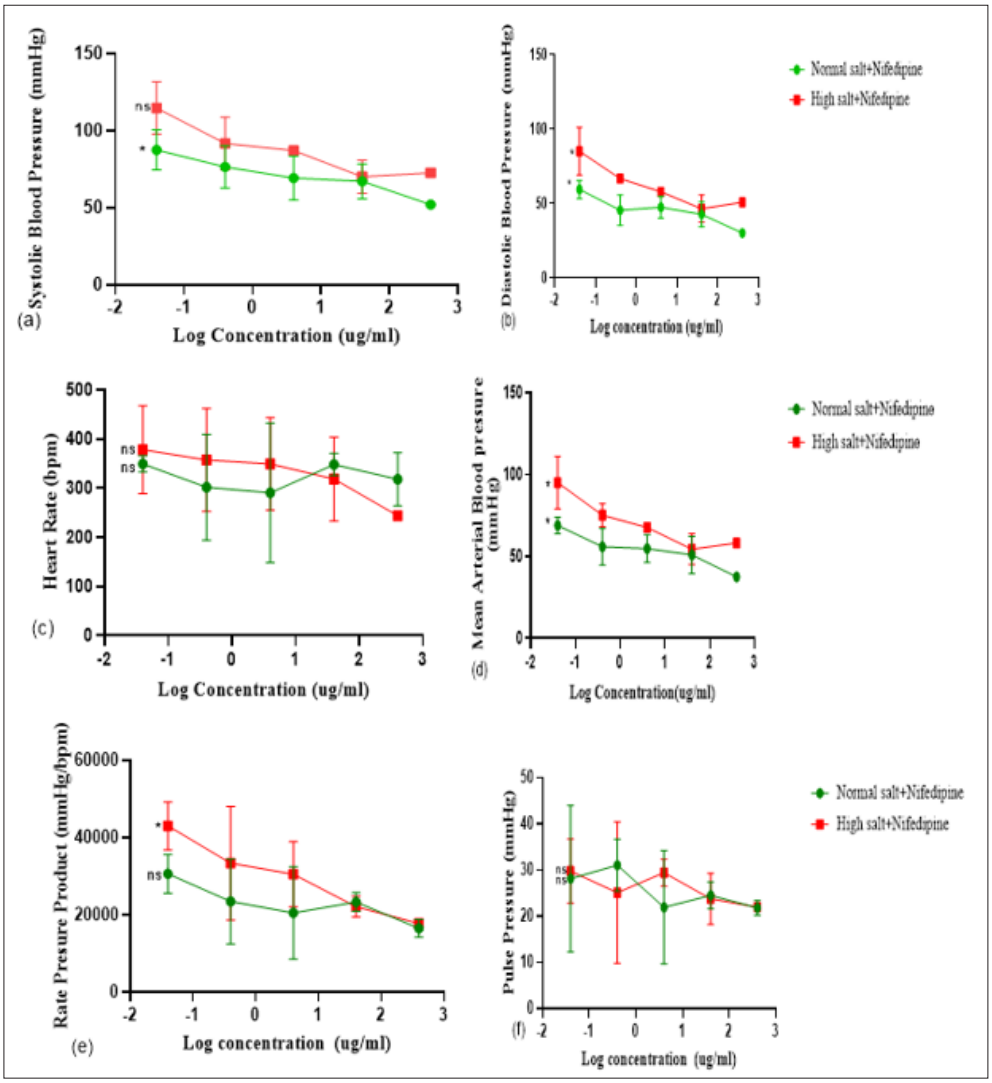

Figure 1: Noncumulative log concentration –response curve showing effect of serial Concentrations of Nifedipine on Blood Pressure Parameters in normal and High Salt Rats.

*Significantly different from initial to final concentration P<0.05

ns No significantly different from initial to final diastolic blood pressure p<0.05

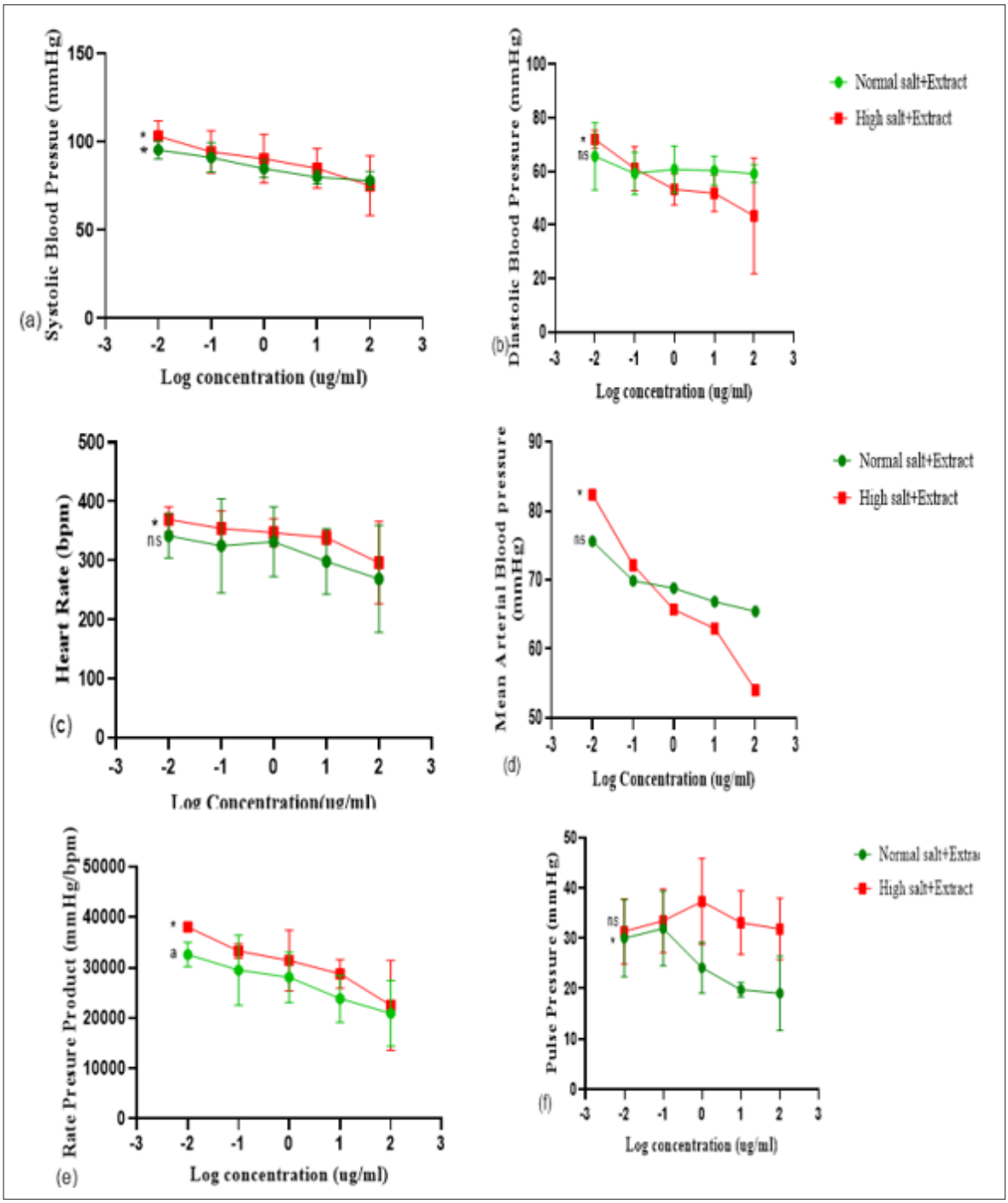

Figure 2: Noncumulative log concentration-response curve showing effect of Serial Concentrations of methanol *extract of Anacardium occidentale* on Blood Pressure Parameters in normal and High Salt Rats.

*Significantly different from initial to final concentration P<0.05

ns No significantly different from initial to final diastolic blood pressure p<0.05

Discussion

The reversal effects of methanol extract of Anacardium occidentale back was investigated in salt-induced hypertension rats using nifedipine as a standard drug in this study. A large body of evidence suggests that salt excess plays a significant role in the pathogenesis of many types of human and experimental hypertension (10); high salt intake has also been shown to cause functional and structural changes in the vasculature that are unrelated to blood pressure (BP) elevation, whereas salt restriction has been shown to act in the opposite direction [30]. In the study, high salt diet caused a rise in SBP. DBP, MAP and HR after a high salt dieting over a period of 10 weeks. A high-salt diet is known to suppress the renin-angiotensin system. Although the exact mechanisms are unknown, endothelial dysfunction is likely to play a role in the influence of high sodium intake on blood pressure [31]. Furthermore, salt loading causes renal sodium retention, which eventually causes ECF volume expansion, resulting in higher cardiac output with tissue perfusion that exceeds metabolic needs. The peripheral tissue vasculature responds by activating autoregulatory vasoconstriction, increasing peripheral resistance and, as a result, blood pressure [32]. High salt also induce hypervolumia also increase blood flow and shear stress in the central arterial vessels and induces expression of endothelial nitric oxide synthase, consequently causing accounting for the increase peripheral resistance and the resulting increasing in blood pressure [33]. Therefore, feeding the animals for 10 weeks in this study could have induced hypertension via changes in the activities of the renin-angiotensin system and expansion of ECF volume, resulting in an increase in SBP, DBP, MAP, and increased peripheral resistance, resulting in an increase in HR.

Prior to this study, it was known that the mechanism of action of nifedipine, a dihydropyridine calcium channel blocker, involves peripheral arterial vasodilation and, as a result, a decrease in peripheral vascular resistance but potassium sparing diuretics are typically recommended when used [34,35]. In vitro treatment with nifedipine in the present study was able to reverse the salt-induced hypertension by exerting a concentration dependent reducing effects on SBP, DBP, MAP possibly via the reduction of arterial vasodilation and decrease in peripheral vascular resistance but was not able to reduce the HR due to it's the earlier explained reason by Murphy et al. Similarly, Snider et al. reported a reduction in blood pressure in human model following long term treatment with nifedipine stating same modalities also stated in this study [36].

The current study also found that in vitro treatment with methanol extract of the stem back of Anacardium occidentale has a dose-related hypotensive effect on the animal's arterial blood pressure, lowering SBP, DBP, MAP, and HR. This demonstrated that extract could have a direct inhibiting effect on cardiac activity, as well as a vasodilating effect on vascular muscles at the same time, possibly replenishing potassium levels and further lowering heart rate, as also observed in the study [37]. This latter property would make the extract more effective in the treatment of hypertension than nifedipine. These effects may be related to the extract's influence on the central nervous system, inhibition of phosphodiesterase, reduction of intracellular Ca2+, and induction of nitric oxide in smooth muscles [12, 13, 38, 39].

It is also important to note that the rate pressure product which signifies the myocardial work load was shown to be high in hypertensive animals due to the effort exerted on the cardiac muscles in pumping blood in hypertension [40]. On administration of Anacardium occidentale, the rate pressure product significantly reduced due to the hypotensive properties of the extract.

According to the qualitative phytochemical compositions done in a previous study by omolaso et al. on the methanol extract of Anacardium occidentale stem bark was found to contained high amount of steroids, flavonoids, and phenols. It also includes alkaloids, saponins, and tannins, as well as oleic and hexadecanoic acids, which make up 45.51 percent and 20.57 percent of the fatty acid acid compositions, respectively [41]. The antihypertensive effects of the methanol extract of Anacardium occidentale stem bark in the present study can thus be attributed to the extract's Octadecenoic acid (oleic) acids as well as the saponins and tannins component. Similarly, previous research found that treatment with saponins, tannins, alkaloid and oleic acid (45) reduced various blood pressure parameters [42-45].

Conclusion

Conclusively, this study established the potency of methanol extract of Anacardium occidentale root back in the treatment of salt-induced hypertension in rats due to its ability in reducing blood pressure parameters in a dose-dependent manner than nifedipine in this study. The reduction in blood pressure parameters observed in this study may therefore support its use in the treatment of hypertension on an empirical basis. We therefore suggest isolation of the active compounds in the extract for a follow-up analysis.

Declarations

Ethical Approval: This study was done under close supervision and as approval of the Research ethics committee of University of Medical Sciences was collected and preserved by the authors.

Competing interests

There is no known conflict of interest by authors at the time of this report

Consent for Publication: Not required

Availability of Data and Material: All data used for this study are available on request from the authors

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Authors Contributions

The research was conceptualized, designed and supervised by BOO and, BZA carried conducted the experiment and contributed reagents. VE helped in literature search, analyzed the data and drafted the article. All authors approved the article

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