

Dysregulation of Electrolyte Balance and Lipid Profile in Rats Following Oral Administration of Methanolic Fruit Pulp Extract of *Azanza garckeana* (Tula Kola Nut)

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Abstract

This study investigated the effects of methanolic fruit pulp extract of *Azanza garckeana*(MFEAG) on renal function through acute and subacute toxicity studies. Twenty-nine (29) apparently healthy adult male Wistar rats weighing 100-120 grams were used. For the acute study, 9 rats were randomly grouped into three: Group 1 were administered 300 mg/kg MFEAG once. Group 2 was sequentially administrated the next dose of 2,000 mg/kg, also once, when there were no signs of toxicity or mortality in Group 1 48 hours post-treatment, while Group 3 were administered the vehicle (10% v/v Tween 80). The rats were then observed for two weeks, and then sacrificed. For the subacute study, 20 rats were randomly grouped into four and daily administered once with the extract (at 300, 600, and 1,200 mg/kg doses) and the vehicle, respectively, for four weeks. No significant effects were observed on serum kidney function markers of the treated rats, except for the level of serum chloride that was increased and that of serum triglyceride that was decreased after the acute study, and then the serum sodium that was increased after the subacute study. These findings indicate that MFEAG may cause some level of impairment in renal function.

1 INTRODUCTION

For many years, plants have served as food for humans, as well as medicine in treatment and management of various diseases [1]. About 80% of global population and 95% of those in developing countries depend on natural products of plant origin for basic and primary health care [2, 3]. This is due to their cheap availability to these populations [4]. Also, the use of herbal products has been estimated to increase by 10–20% annually [5]. These medicinal plants have served as alternatives for conventional drugs, as they contain many active phytochemicals that are always in demand and made as focal points in research [6]. They also play a vital role in both the development and production of new drugs [7], with up to 65% of modern drugs directly or indirectly derived from plants [6].

Azanza garckeana belongs to the family ‘Malvaceae’. It is one the most important fruit-bearing plants in Nigeria [8], where it is grown mostly in Tula village of Kaltungo Local Government Area of Gombe State and where the local people who are predominantly Hausa by tribe call it ‘Goron Tula’, meaning ‘Tula kola nut’. The generic name *Azanza* is derived from the word *Azania*, meaning ‘black and surviving in Zanzibar. The specific name *garckeana* is in honor of professor August Garcke (1819–1904), a German botanist and plant collector who specialized in pharmacognosy [9]. Goron Tula is an important medicinal and food plant commonly used in Northern Nigeria as herbal medicine for treatment of various illnesses [10]. Different parts of this small tree/shrub are used locally as herbal remedies for diseases like cough, chest pain, infertility, menstruation abnormalities, sexually transmitted infections and hepatic impairment [9, 11].

The fruit of *Azanza garckeana* is one of the most important parts of the plant both traditional and therapeutic potentials of which have been well documented [10–13]. The fruit has rough and hairy bark. It is grayish brown in colour, also fibrous with longitudinal fissures and brown and yellow slashes. It is

2.5-4 cm in diameter, clearly divided into 5 segments, the fleshy gummy pulp containing five seeds in each segments. The seed is hemispherical in shape with brownish and woolly floss [9].

Generally, wild edible fruits are characterized by remarkable nutrient values as well as being excellent sources of minerals, fibers, vitamins C, A and E, polyphenols, as well as ascorbic and fatty acids that add flavor and colour to diets [14–16]. Among many others, the local uses of *Azanza garckeana* fruit include its being edible – either eaten raw while slightly green or ripe, soaked in a small quantity of water to make jelly of it, or boiled and used as a relish or porridge [12, 13]. It has reportedly been found to have advantage in nutritional contents over many other food substances or other parts of the *Azanza garckeana* [17]. It also records a use as booster for sexual performance (aphrodisiac), which is its most widely known local use [11]. The fruit has also been established to have antioxidant, antihyperglycemic/anti-diabetic, anti-inflammatory/analgesic, antifungal, antimalarial, iron absorption and uterotonic potentials by various researches [16–18].

Despite the undeniable potentials of traditional plants, it has been stressed that the use of these traditional medicines as well as the development of conventional drugs from them should be rationalized by evaluating their safety profile before their approval for consumption or use [19, 20]. A number of studies have attempted to evaluate the safety profile of various parts of *Azanza garckeana*, with a study revealing the ethyl acetate, acetone, methanol and water extracts of the plant's stem bark being toxic to brime shrimps, while its petroleum ether extract proved to be safe [21]. A recent study of ours indicated a general safety of the methanol fruit pulp of the plant at up to a dose of 2,000 mg/kg after acute and subacute oral toxicity evaluations, except for a level of toxicity in the cardiopulmonary and hepatic systems observed after the subacute (4 weeks) treatment with its high dose in rats [17]. Another study reported the methanol extracts of air- and sun-dried fruit pulp of the plant causing some alterations in the functional integrity of rats' kidneys after a two weeks daily treatment at doses of 150, 300 and 600 mg/kg, for which it was recommended that caution should be exercised during its use [22].

This study evaluated the acute and subacute effects of higher doses (300, 600, 1,200 and 2,000 mg/kg) of the methanolic fruit pulp extract of *Azanza garckeana* on serum lipid profile, creatinine, urea and electrolytes (sodium, potassium, chloride, bicarbonate and calcium) in adult male Wistar rats through one time and 28 days daily administrations.

2 MATERIALS AND METHODS

2.1 Drugs and Other Chemicals

Drugs and other chemicals used include Tween 80 (CP Execution Standard QB/440500712208) for dissolving the extract because it was jelly in nature; ketamine (Midazolam injection, USP) and diazepam (Centurion Healthcare Private Limited) for anaesthetizing the rats after the experiments.

2.2 Experimental Animals

Twenty nine (29) apparently healthy growing Male Wistar rats (100-120 grams) were obtained and acclimatized for two weeks before commencement of the experiments. All animals were maintained on standard rodent feed and water *ad libitum* while being housed in the animal house of Department of Human Physiology, Ahmadu Bello University, Zaria-Nigeria in whose laboratory the experimental procedures were carried out. All experimental protocols were carried out in compliance with standard guidelines as approved by the AHmadu Bello University Committee on Animal Use and Care (ethical approval number: ABUCAUC/2021/009).

2.3 Source of Plant Material and Extract Preparation

Azanza garckeana fruit was obtained from Tula village in Kaltungo Local Government Area of Gombe State, Nigeria. The plant material was identified and authenticated by a botanist in Botany Department of Ahmadu Bello University, Zaria-Nigeria, where a voucher specimen (ABU07276) was deposited. The extraction was carried out according to the methods described earlier [23–25]. The fruit pulp was manually separated from its seeds, air-dried, shredded to small chunks and taken for extraction. 1 kg of pulverized *Azanza garckeana* fruit pulp was macerated with 3,000 mL of methanol and allowed to stand for 72 hours, after which it was filtered. The filtrate was collected and concentrated on a water bath at 50°C. The concentrated jelly extract was collected and properly stored in a container before further experiments and analysis.

2.4 Experimental Design

The study was carried out according to a previously described method [26], and it was done in two phases: Acute and Sub-acute, as follows.

2.4.1 Acute toxicity study: The extract was administered to the rats after overnight fasting by using oral gavage in a volume of 10 mL/kg body weight. Nine rats were randomly divided into 3 groups of 3 rats each. The starting dose of 300 mg/kg body weight of the extract (dissolved in 10% v/v Tween 80) was administered to Group 1. Group 2 animals were sequentially administered with the next dose of 2,000 mg/kg when there was no signs of toxicity or mortality shown in Group 1 48 hours post-treatment. In parallel, Group 3 animals were treated with the vehicle (10% v/v Tween 80) to establish a comparative normal control group according to the OECD guideline [27].

2.4.2 Sub-acute toxicity study: Rats were randomly divided into four groups of 5 each. Groups 1-3 rats were administered daily with the extract at different doses (300, 600 and 1,200 mg/kg body weight, respectively), and Group 4 animals (control) were administered with equal volumes of the vehicle (10% v/v Tween 80) via oral gavage once daily for 28 consecutive days.

2.4.3 Sacrifice of Animals and Collection of Blood Samples

At the end of each experiment (15th day for acute and 29th day for sub-acute studies), the rats were anesthetized by intraperitoneal injection of 75 mg/kg of ketamine and 5 mg/kg of diazepam. Blood samples (4-5 mL) were then collected into plain bottles via cardiac puncture. The blood samples were

allowed to stand for 20-30 minutes for them to coagulate, and then centrifuged at 3,000 rpm for 10-15 minutes. The serum was then decanted into clean specimen tubes, and were used for the biochemical assessment of serum kidney function markers and lipid profile, which were done spectrophotometrically using Audicum autoanalyzer and Surechem spectrophotometer.

2.4.4 Data Analysis

Data were analyzed by one-way analysis of variance (ANOVA) followed by Turkey’s post-hoc multiple comparison test to compare the effects across the groups. Mean differences with *P* values less than 0.05 (*P* < 0.05) were considered statistically significant. SPSS (statistical package for social science) version 24.0 was used for the analysis. Results were expressed as a mean ± standard error of mean (SEM).

3 RESULTS

3.1 Acute Study Results

The results in Table 1 below indicates that there was no statistically significant changes (*P* ≥ 0.05) on the serum sodium, potassium, bicarbonate, calcium, urea and creatinine levels of the animals after the acute study. However, a significant increase (*P* < 0.05) in the serum chloride level was observed after treatment with the high dose of 2,000 mg/kg when compared to the control.

Table 1

Acute effects of *Azanza garckeana* fruit pulp extract on rats’ serum kidney function markers.

Treatment	Creatinine (mEq/L)	Urea (mg/dL)	Na ⁺ (mMol/L)	K ⁺ (mMol/L)	Cl ⁻ (mg/dL)	CO ₃ ²⁻ (mg/dL)	Ca ²⁺ (mg/dL)
10% v/v Tween 80	1.07 ± 0.12	16.8 ± 5.2	62.0 ± 10.6	10.7 ± 0.9	30.7 ± 2.3	80.7 ± 2.3	1.50 ± 0.3
300 mg/kg extract	3.27 ± 2.37	48.3 ± 19.2	68.8 ± 11.9	14.6 ± 1.7	31.3 ± 1.9	85.3 ± 5.2	1.07 ± 0.2
2,000 mg/kg extract	1.13 ± 0.09	9.77 ± 2.7	61.5 ± 14.2	16.6 ± 2.3	40.3 ± 0.3 ^{ab}	91.7 ± 1.2	1.5 ± 0.4

Superscripts ‘a’ & ‘b’ indicate statistically significant differences (*p* < 0.05) from 10% v/v Tween 80- and 300 mg/kg extract-treated groups respectively.

Also, the results in Table 2 below shows no significant effects on serum total cholesterol (T/Chol), high density lipoprotein cholesterol (HDL) and low density lipoprotein cholesterol (LDL) when compared to the control (*P* ≥ 0.05) after the acute study. However, the treatment of rats with 2,000 mg/kg of the extract caused a significant decrease in triglycerides (TG) level when compared to the control (*P* < 0.05).

Table 2
Acute effects of *Azanza garckeana* fruit pulp extract on rats' serum lipid profile.

Treatment	T/Chol (mg/dL)	TG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)
10% v/v Tween 80	61.67 ± 15.81	126.73 ± 27.09	7.07 ± 1.41	29.27 ± 12.07
300 mg/kg extract	94.27 ± 22.14	77 ± 9.47	10.77 ± 0.62	68.03 ± 23.84
2,000 mg/kg extract	70.9 ± 15.4	51.37 ± 8.04 ^a	7.13 ± 1.59	53.50 ± 14.83

T/CHol: Total cholesterol; TG: Triglycerides; HDL: High Density Lipoproteins; LDL: Low Density Lipoproteins; superscript 'a' indicates statistically significant difference when compared to 10% v/v Tween 80-treated group.

3.2 Subacute Study Results

The results obtained after the subacute study (Table 3) indicates no significant changes ($P \geq 0.05$) on the serum levels of potassium, chloride, bicarbonate, urea and creatinine when compared to control. However, a significant increase ($P < 0.05$) in the serum level of sodium ion was observed after treatment with 1,200 mg/kg of the extract when compared to the control.

Table 3
Subacute effects of *Azanza garckeana* fruit pulp extract on rats' serum kidney function markers.

Treatment	Creatinine (mEq/L)	Urea (mg/dL)	Na ⁺ (mMol/L)	K ⁺ (mMol/L)	Cl ⁻ (mg/dL)	CO ₃ ²⁻ (mg/dL)
10% v/v Tween 80	1.02 ± 0.09	54.2 ± 12.8	82.6 ± 8.8	9.9 ± 1.7	37.0 ± 2.3	100.6 ± 5.7
300 mg/kg extract	0.81 ± 0.14	22.9 ± 2.5	86.9 ± 8.1	10.2 ± 1.4	35.7 ± 3.1	105.6 ± 5.8
600 mg/kg extract	0.96 ± 0.11	28.7 ± 9.7	87.6 ± 6.9	38.3 ± 28.0	26.6 ± 4.4	97.4 ± 2.7
1,200 mg/kg extract	1.90 ± 1.03	81.8 ± 16.8 ^{bc}	144.7 ± 20.6 ^{ac}	18.0 ± 3.0	27.1 ± 1.9	95.4 ± 3.9

Superscripts 'a', 'b' & 'c' indicate statistically significant difference ($p < 0.05$) from 10% v/v Tween 80-, 300 and 600 mg/kg extract -treated groups respectively.

Furthermore, the results in Table 4 indicates that extract caused no significant effects on serum levels of all the lipids when compared to control ($P \geq 0.05$) after the subacute study.

Table 4
Subacute effects of *Azanza garckeana* fruit pulp extract on rats' serum lipid profile.

Treatment	T/Chol (mg/dL)	TG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)
10% v/v Tween 80	36.44 ± 7.69	49.24 ± 11.24	7.22 ± 1.29	19.66 ± 6.38
300 mg/kg extract	45.44 ± 5.46	66.75 ± 9.39	9.77 ± 1.16	22.24 ± 4.54
600 mg/kg extract	31.14 ± 3.99	77.36 ± 14.05	9.36 ± 0.73	6.32 ± 2.88
1,200 mg/kg extract	57.60 ± 6.36 ^c	97.09 ± 12.80	8.97 ± 1.06	30.43 ± 6.85 ^c

T/CHol: Total cholesterol, TG: Triglycerides, HDL: High Density Lipoproteins, LDL: Low Density Lipoproteins, Superscript 'c' indicates statistically significant difference ($p < 0.05$) from 600 mg/kg extract-treated group.

4 DISCUSSION

The safety of herbal medicines is usually questioned due to reports of certain adverse effects, illnesses and even fatalities, particularly related to nephrotoxicity [5, 28]. The kidney receives the largest blood flow per gram due to its cardinal role in filtering large amounts of toxins and drug metabolites from the blood. These toxins and metabolites can concentrate within the kidney's renal tubules and cause various degrees of damage [29]. Hence, the safety evaluation of herbal medicines on the kidney is said to be very important [5, 28]. Thus, this study evaluated the safety of methanolic fruit pulp extract of *Azanza garckeana* (MFEAG) on kidney function of adult male Wistar rats through acute and subacute oral toxicity evaluations. Both an initial assessment using an acute study and establishing a safety margin using subacute or chronic multiple administrations of different doses of extracts of medicinal plants in laboratory animals, in whom it is almost only possible, are essential in safety screening of these medicines [30, 31]. Therefore, the effects of one time administration of MFEAG as wells as the subacute multiple administrations of the extract on the kidney function were assessed in this study using rat models.

Despite the deficiency of glomerular filtration rate (GFR) in assessment of true tubular health, assessment of kidney function is considered to be more appropriate majorly by evaluating the GFR. However, due to the invasiveness and time consumption in the determination of GFR [32], certain markers of kidney function from the serum or urine are usually implored to indirectly assess kidney function and to diagnose kidney damage/dysfunction despite their imperfections of not fully fulfilling certain criteria for representing GFR [33–35]. Therefore, serum levels of creatinine, urea and electrolytes are the major markers routinely used for assessment of renal function [32, 35].

Creatinine is a product of muscle metabolism. Because it is not normally reabsorbed by the renal tubules after filtration, its serum concentration usually increases when there is a failure of the kidney to excrete it as a result of a damage, especially during chronic kidney injury [33, 35]. Serum creatinine is the most

commonly used marker of kidney function because of the advantages it has of being cheap and quick to assess [35, 36], although its level is often influenced by individual's muscle mass [34]. It is an integrator of both intrarenal and extrarenal kidney functions/injury that indicates a balance between its generation and excretion [37]. In this study, the serum creatinine level was not affected by treatment with MFEAG. This is in contrast to the finding of the study of Yusuf et al. (2020) where the air-dried methanol fruit pulp extract of the plant decreased the serum creatinine level after two weeks daily treatment, thereby indicating renal damage. However, a decrease in serum creatinine level does not really indicate impairment of renal function, because creatinine level is usually increased (rather than decreased) when there is impairment in renal function that affects GFR [33, 34]. Therefore, the decrease in serum creatinine observed in the study of Yusuf et al. might have been through other mechanisms unrelated to renal function. The observation of MFEAG not affecting the serum creatinine of rats treated both at acute and subacute levels and at up to a dose of 1,200 mg/kg in this study (which is relatively higher than the doses of 150, 300 and 600 mg/kg used in the study of Yusuf et al.) further points to the fact that the fruit of *Azanza garckeana* may not have any ability to affect the excretion of creatinine through inflicting renal damage.

Like the creatinine, urea is also a product of muscle metabolism that is considered as a non-protein nitrogenous (NPN) waste. Its concentration is determined by its renal excretion as well as the level of intake and metabolism of protein. Unlike creatinine, it is not excreted only by the kidneys, hence, not as good as creatinine in being a marker of renal function. Nonetheless, its serum level is usually increased in especially acute renal injuries [33, 34]. The serum level of urea was also not affected by both the acute and subacute treatment of rats with MFEAG in this study. This finding is also in contrast to the study of Yusuf et al. (2020) in which the serum urea was significantly decreased by all doses of both the air-dried and sun-dried fruit pulp extracts of *Azanza garckeana*. Nevertheless, a decrease in serum urea level, rather than increase, is also seldom seen in kidney injuries but in rare inherited disorders of the urea cycle and advanced liver disease, hence the decrease in serum urea level was considered to be of less clinical significance and, therefore, does not actually reflect renal injury [33, 34]. This further stresses the safety of the fruit on renal function.

Kidney damage can lead to a build-up of electrolytes such as sodium, potassium, calcium, magnesium and bicarbonate in the body, because the kidneys are not able to properly excrete them, leading to, for instance, hypernatremia. So, in a way, these electrolytes are like the "canary in the coal mine" - they can signal a problem with the kidneys before other symptoms appear. Therefore, abnormalities in these electrolytes are associated with reduced kidney function, as shown in a number of studies [35, 38]. In this study, MFEAG was found to have no effects on serum potassium, bicarbonate and calcium ions. This is in contrast to the findings of Yusuf et al. (2020), where bicarbonate was found to be increased following a two weeks daily administration of the methanolic fruit pulp extract of the plant. However, the serum level of chloride ion was found to be significantly increased by 2,000 mg/kg dose of MFEAG after the acute treatment period, which contradicts the finding of the study of Yusuf et al., where no effects on the electrolyte by the same type of the extract was observed. Again, MFEAG was found to significantly increase the serum sodium ion level at the dose of 1,200 mg/kg after the subacute study, which is in line

with the finding of Yusuf et al., in which sodium level was also increased by the fruit extract. Since an increase in serum sodium (hypernatremia) and chloride (hyperchloreaemia) levels are indicative of kidney injuries [38, 39], the effect of MFEAG may be as a result of a possible damage to the kidney that caused a failure to excrete excess sodium and chloride ions.

Dyslipidaemia, or abnormal levels of lipids in the blood, can have an impact on renal function. Two main types of lipoproteins are high-density lipoproteins (HDL) and low-density lipoproteins (LDL). HDL is often called the "good" cholesterol, because it helps to remove excess cholesterol from the body, while LDL is often called the "bad" cholesterol, because it can deposit cholesterol in the walls of blood vessels. The balance between HDL and LDL is an important factor in cardiovascular health and thus other organs as the kidney that are dependent on that. Changes in this balance can also affect the levels of triglycerides and cholesterol in the body. High levels of triglycerides and LDL as well as lower levels of HDL have been found to be able to cause glomerulosclerosis, thereby causing a decline in GFR [40, 41]. In this study no effects were observed on total cholesterol, high and low density lipoproteins after treatment of rats with MFEAG at both acute and subacute levels. However, a significant decrease in triglyceride level was observed among the 2,000 mg/kg MFEAG-treated rats after the acute study. This is in contrast to the finding of a recent study [42], in which no significant effects were observed in serum triglycerides after 12-weeks treatment with the same type of extract at a dose of 500 mg/kg in rabbit bucks. There is evidence that high levels of triglycerides can be associated with impaired renal function as highlighted above, but it is unclear whether lower levels of triglycerides could also have a negative impact on the kidneys. In fact, there is some evidence that lowering triglyceride levels with certain medications, like statins, can actually improve renal function [43]. This implies that the decrease in triglyceride level by MFEAG seen in this study may not indicate renal damage, rather, it could be considered as beneficial to the body by getting rid of harmful lipids.

Many plant species have been reported to exert their pharmacological activities through their phytoconstituents [44]. For example, excessive dosage of some phytochemicals have been shown to cause certain levels of electrolyte imbalance and nephrotoxicity, e.g. anthraquinones in laxatives [45–47], some cardiac glycosides such as digoxin [48], alkaloids, saponins [49], steroids as well as flavonoids [50]. Anthraquinones and tannins have been shown to be able to inhibit certain chloride channels that are involved in chloride ion secretion [51–53]. Furthermore, certain steroids like glucocorticoids that cause sodium retention via various pathways [54], aldosterone that stimulate sodium and chloride ions absorption via epithelial Na^+ channel (ENaC) and $\text{Na}^+ - \text{K}^+ - 2\text{Cl}^-$ cotransporter 2 (NKCC2) [55], as well as a flavonoid, quercetin, that activate NKCC1 [55] inhibit the excretion or increase the absorption of sodium and chloride ions in the renal tubules, thereby increasing the body's levels of these electrolytes. The increase in serum sodium and chloride ions observed as a result of treatment with MFEAG in this study might have been as a result of the effects of these phytochemicals that have been found to be present in the extract in our previous study [17], or extracts of other parts of the plant [8, 11, 15, 56, 57].

Again, certain phytochemicals present in the fruit of *Azanza garckeana* have also been implicated to play roles in lipid metabolism. For instance, flavonoids [58, 59] and saponins [60, 61] increase the activities of

lipoprotein lipase (LPL), which is an enzyme that helps in breaking down triglycerides into free fatty acids and glycerol, thereby playing an important role as a regulator of serum triglyceride level [62]. Although it is found in many other organs, LPL is also expressed in the kidneys, where it plays a role in triglyceride metabolism [62, 63]. It is possible that the extract is increasing the activity of LPL through these phytochemicals that are found to be present in it, thereby increasing the metabolism of triglyceride and decreasing its level. A study reported that LPL appears to have a minor role in the kidney [64]. Therefore, the effect of MFEAG on triglyceride level may not necessarily be related to its effect on renal function.

Suffice it to say that, while there is no direct evidence yet, there is some indication that *Azanza garckeana* fruit pulp might affect certain chloride channels or enzymes involved in sodium and chloride transport as well as triglyceride metabolism through its active phytochemicals, thereby increasing the retention of these electrolytes and the metabolism of triglycerides, hence, affecting their levels in the body.

5 CONCLUSION

From the findings of this study, it can be deduced that MFEAG may confer some level of toxicity to the kidney owing to its ability to increase serum sodium and chloride levels in rats. However, further studies may be required to investigate the effects of the fruit or its active ingredients on certain molecular targets that are involved in renal handling of electrolyte and lipid metabolism.

Declarations

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Compliance with Ethical Standards

Competing interests: The authors declare that they have no competing interests.

Funding: No funding was received for conducting this study.

Ethics approval and consent to participate: All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. The study protocol was reviewed and approved by the Ahmadu Bello University Committee on Animal Use and Care, with approval number ABUCAUC/2021/009.

Availability of data and materials

The data supporting the findings of this study are available upon reasonable request from the corresponding author.

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