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Original Research Article

Acute Oral Toxicity of a Novel Pharmaceutical Excipient (Grewia gum) in Wistar Rats

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Abstract

Purpose: Grewia gum is reported to possess significant potential as a natural pharmaceuti cal excipient. However, there is currently a lack of information regarding its toxicity in animal models.
Methods: The acute oral toxicity of Grewia gum (*G. mollis*) was evaluated in female Wistar rats using a fixed-dose procedure as outlined in Organisation for Economic Co-operation and Development Guideline No. 420. The animals were monitored for mortality, behavioural, biochemical, and haematological changes and their vital organs were preserved for histopathological analysis.
Results:The results indicated no mortality, systemic toxicity, or behavioural changes in the rats administered Grewia gum, with the oral median lethal dose (LD_{50}) exceeding 2000 mg/kg. Grewia gum did not affect food and water consumption in the animals. However, the concentrations of serum bicarbonate, total bilirubin and total protein increased significantly ($p < 0.05$) in the Grewia gum-treated animals. Furthermore, the administration of Grewia gum resulted in a significant reduction in mean corpuscular haemoglobin concentration (MCHC) values, which suggests subtle effect on haematopoietic tissue. Microscopic examinations of organ sections from both the control and Grewia gum-treated groups revealed no abnormalities.
Conclusion: Thus, oral administration of Grewia gum resulted in mild effects on serum liver and haematological indices, indicating that the gum is safe at the present level of exposure in rats.

Keywords: Grewia gum, Excipient, Acute toxicity, Oral, Rat

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INTRODUCTION

Grewia gum, a novel pharmaceutical excipient, has wide potential applications in many drug formulations. The gum is a natural polysaccharide extracted from the inner stem bark of the *Grewia mollis* plant.[1] The *G. mollis* plant is a member of the Malvaceae family and grows in many African countries and some parts of Asia and the Middle East. In Nigeria, the plant is found predominantly in the northern part of the country.
The stem bark of the *G. mollis* plant is widely used as a thickening agent in various locally produced

foods in Nigeria. Grewia gum, which is extracted from the inner stem bark of *G. mollis*, has been shown to be an effective alternative to synthetic and semi-synthetic excipients.[2]The gum polysaccharide contains glucose and rhamnose as the main monosaccharides, while galacturonic acid is the main sugar acid.[3-5]

Grewia gum serves effectively as a binder, mucoadhesive, and disintegrant in solid dosage forms, as well as a stabiliser or suspending agent in liquid formulations.[2]In a previous study, paracetamol was used as a model drug to assess the binding capacity of Grewia gum in comparison with the widely used binder, polyvinylpyrrolidone (PVP).[6] The binding ability of grewia gum was comparable to that of PVP. In another study, the suspending properties of Grewia gum were evaluated against hydroxymethylpropyl cellulose (HPMC) and sodium carboxymethyl cellulose (Na-CMC) in the formulation of ibuprofen (Ogaji and Hoag, 2011).[7] The results demonstrated that Grewia gum was a better suspending agent compared to HPMC and Na-CMC. The sustained-release characteristics of Grewia gum in cimetidine were assessed in comparison with ethyl cellulose (Ethocel), carboxymethyl cellulose (Blanose), gum arabic, and hydroxypropylmethyl cellulose (Methocel). The findings indicated that Grewia gum demonstrated superior sustained-release properties compared to these conventional excipients. These findings, along with additional reports[8-14] regarding Grewia gum, suggest that it is a promising pharmaceutical excipient.

Despite the advantages of Grewia gum, there is currently no available information regarding its potential toxicity. Hence, this study investigated the potential toxicity of Grewia gum in Wistar rats.

MATERIALS AND METHODS

Equipment

The equipment includes a spectrophotometer (Jenway, 7315), precision balance (C78530), centrifuge (Hettich, 78532), thermostat oven (DHG-9030A), water bath (DK-420), and freeze dryer (DW-10ND).

Chemicals and reagents

The chemicals were procured from BDH Chemicals Limited (Poole, Dorset, England) and Sigma-Aldrich Chemical Corporation (Missouri, USA). All chemicals were of analytical grade, while the test reagents were obtained from Agape Diagnostics (Switzerland) and Randox Laboratories Limited (Antrim, United Kingdom).

Animals

Eight-week-old female Wistar rats were purchased from the National Veterinary Research Institute in Vom, Plateau State, Nigeria. The Wistar rats were allowed a week to acclimatise prior to the commencement of the investigation. The rats were housed in plastic cages fitted with stainless steel tops and filled with hardwood chip bedding. The environment was well-ventilated, with a 12-hour light and dark cycle maintained at a temperature range of 24-25°C. The laboratory animals had unrestricted access to standard laboratory feed (Vital Feeds, Jos, Nigeria) and water. The Institutional Animal Care and Use Committee of Modibbo Adama University in Yola approved the experiment, referencing MAU/FLS/2021/062. Animal care practices adhered to the guidelines established by the National Institutes of Health for the care and use of laboratory animals.[15]

Collection and preparation of plant material

The fresh inner stem bark of *G. mollis* was collected from *Bati village* (Lat. 12^{o} 27'E -12^{o} 29'E and Long. 08^{o} 52'N -08^{o} 54'N), in the Yadim Development Area of Fufore Local Government Area in Adamawa State, Nigeria. A taxonomist from the Department of Plant Sciences at Modibbo Adama University (MAU), Yola, Nigeria, identified and authenticated the plant. The specimen was then deposited in the MAU Yola herbarium under the voucher number MAUH/GM/2021/04-162. The freshly shredded inner stem bark was dried in the laboratory unit of the Biochemistry Department at Modibbo Adama University, Yola, in a shaded area at a temperature of approximately 40°C. The dried plant material was stored in clean, airtight containers until required.

Extraction of Grewia gum

The extraction of Grewia gum was conducted as reported previously.[1] Dried and shredded bark from the inner stem of *Grewia mollis* (100 g) was macerated in water containing 0.1% sodium metabisulphite. This mixture was subsequently diluted for a total of three (3) litres using the same solvent. Thereafter, the swollen gum was filtered through a muslin bag to separate it from the residue. The filtrate was precipitated using two volumes of absolute ethanol over a period of four hours. Further purification was achieved by redispersing the gum in water, followed by a final precipitation with two volumes of absolute ethanol for another four hours. The extracted Grewia gum (GG) was then oven-dried at 50°C for 24 hours. An aqueous stock solution of Grewia gum (GG) (100 mg/mL) was prepared immediately prior to administration.

Experimental design

The assessment of acute oral toxicity was performed in accordance with the fixed dose procedure specified in Guideline No. 420 of the Organisation for Economic Co-operation and Development.[16] During the sighting study, a rat was administered a single oral dose of GG at 2000 mg/kg of body weight and monitored for mortality during a 24-hour period. A second rat was orally administered the same dose of GG to confirm the absence of mortality. In the main study, ten (10) healthy adult female nulliparous Wistar rats were randomly allocated into two groups consisting of five rats each. Before dosing, the food was withheld overnight while allowing unrestricted access to water. The animals in the experimental group received a single dose of GG orally at 2000 mg/kg body weight. The animals in the control group received distilled water (2 ml/kg). Food was withheld for an additional three hours. The animals in both groups were monitored for any signs of general systemic toxicity and behavioural alterations during the initial 4-hour period, followed by daily observations lasting 14 days.

Collection of blood sample

At the end of the experiment, the rats were fasted overnight. Subsequently, euthanasia was performed through intraperitoneal injection of ketamine hydrochloride and xylazine at 90 mg/kg body weight and 5 mg/kg body weight,[17]respectively. Blood samples were then obtained via heart puncture.

Determination of animal body weight

Animal body weights were measured at three specific time points: immediately prior to dosing, on the 7th day, and on the 14th day.

Determination of food consumption

Food consumption was assessed by measuring the amount of food (g) given and the quantity remaining after a 24-hour period.

Determination of water consumption

Water consumption was assessed by measuring the volume of water (ml) supplied and the amount remaining after a 24-hour period.

Determination of serum biochemical indices

Blood samples were collected in sterile containers. To extract serum, the blood was allowed to coagulate before being centrifuged at 3000 g for 10 minutes. The serum markers for liver and kidney function were measured using test kits obtained from Randox Laboratories Limited. Additionally, the parameters of the serum lipid profile were evaluated using test kits obtained from Agape Diagnostics. The concentration of low-density lipoprotein cholesterol in the serum was determined using the Friedewald equation.[18] Serum electrolyte (sodium, potassium, chloride, and bicarbonate) concentrations were measured using an automatic electrolyte analyser (KH-996, Kinghawk).

Determination of haematological indices

A fraction of each blood specimen obtained from animals in both experimental groups was placed in clean containers containing ethylenediaminetetraacetic acid (EDTA). Haematological parameters were assayed using the auto-haematologyanalyser (RT-7200).

Organ weight

The vital organs, specifically the livers, kidneys, hearts, lungs, and spleens, were surgically removed and weighed on an analytical balance. The relative organ weight of each animal was calculated using the formula (equation 1):

$$\text{Relative organ weight (\%)} = \frac{\text{Final body weight (g)}}{\text{Absolute weight of organ (g)}} \times 100 \quad \text{.. Equation 1}$$

Histopathological analysis

The collected organs (heart, kidney, liver, lungs, and spleen) were subsequently preserved in a solution of 10% neutral buffered formalin. Following this, the organs underwent a dehydration process that included multiple alcohol solutions. The organs were then treated with xylene, placed in paraffin blocks, and sliced into very thin pieces that are 5 μm thick. They were then stained with haematoxylin and eosin dyes to demonstrate general tissue structure.[19]The sections were examined using a light microscope, and photomicrographs were captured using a Moticam Images Plus 2.0 digital camera (Motic China Group Ltd., 1999-2004).

Statistical analysis

The results are presented as the mean ± standard error of the mean based on five replicates. Statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS) (version 26), employing the independent samples t-test. The mean values from treatment and control groups were compared at the 5% significant level.

RESULTS AND DISCUSSION

The acute toxicity test is a vital toxicological assessment conducted on animal models to evaluate the safety of a substance. It involves investigating the substance's impact on mortality, organs, and biochemical and haematologic indicators. In the present study, no mortality or systemic toxicity was observed in the animals administered Grewia gum (GG) (Table1). Consequently, it was determined that the oral median lethal dose (LD50) of GG in rats exceeded 2000 mg/kg of body weight.Variations in body weight serve as a clear indicator of the harmful effects resulting from exposure to toxicants.[20,21]Nevertheless, the study showed that the body weight of the group administered with GG was similar to the control group (Figure 1).

Table 1: General appearance and behavioural observations of rats administered an acute oral dose of Grewia gum

Parameters	Clinical observations									
	Control					Treatment (GG 2000 mg/kg)				
	30min	4hrs	24hrs	7days	14days	30min	4hrs	24hrs	7days	14days
Changes in Skin and fur	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Changes in Eye colour	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Itching	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Diarrhoea	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Salivation	Absent	Normal	Absent	Absent	Absent	Absent	Normal	Absent	Absent	Absent
Changes in behaviour pattern	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Tremors and convulsion	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Death	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent

GG, Grewia gum.

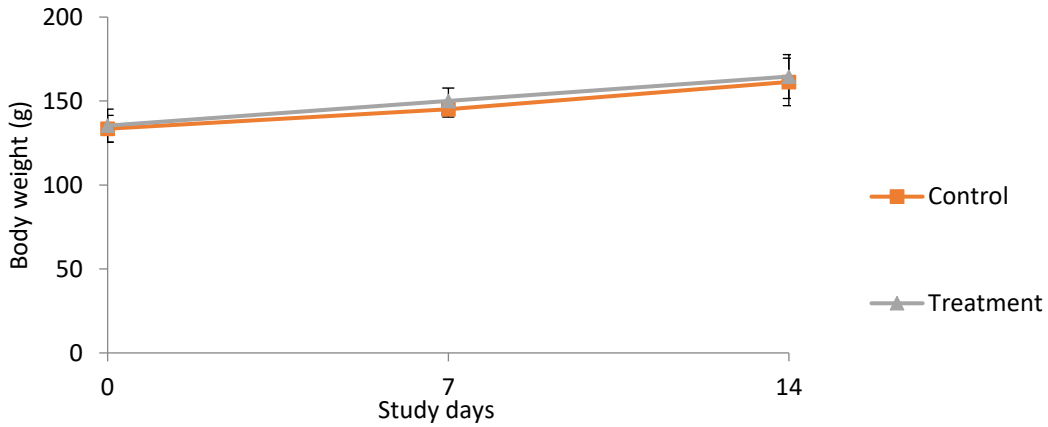

Figure 1: Effect of acute oral administration of GG on body weights of rats. There is no significant ($p > 0.05$) difference in body weight of GG-treated rats and the control.

This finding suggests that the administration of GG did not have a significant effect on the overall

health condition of the rats. Additionally, the rats administered GG did not show any significant change in their food consumption in week 1 and week 2 (Figure 2). In general, changes in food consumption are commonly linked to appetite, nutrient intake and the toxicological effects of chemical substances.[22,23] The lack of substantial changes in the consumption of food may indicate the absence of an adverse effect and adequate nutrient intake in rats.

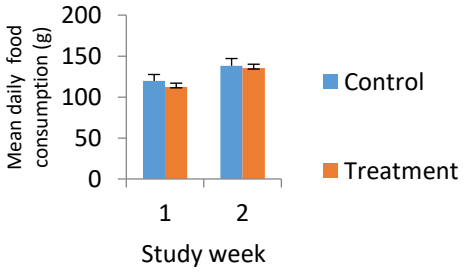

Figure 2: Effect of acute oral administration of GG on the food consumption of rats. There is no significant ($p > 0.05$) difference in food consumption between GG-treated rats and the control.

Table 2 shows the effect of acute oral administration with GG on the serum biochemical parameters of rats. Serum biochemical analysis is commonly used to evaluate organ function and detect any abnormalities.[24] Hepatocytes are the primary source of aspartate aminotransferase and alanine aminotransferase in the blood.[25] Increased release of these enzymes into the bloodstream is seen in cases of hepatic injury.[26] The fact that the serum activity of these enzymes is similar in the GG and the control groups suggests that the gum did not have any toxic effects on the hepatocytes. In contrast, oral administration of GG led to increased concentrations of serum total bilirubin. An increased level of serum total bilirubin typically suggests liver dysfunction or haemolysis. Bilirubin is a byproduct of heme degradation, originating from the lysis of red blood cells.[27,28] During the process of haemolysis, the rupture of red blood cells leads to the release of bilirubin into the blood.High levels of bilirubin and alkaline phosphatase activity are indicative of biliary obstruction or liver disease.[29-34] However, the serum alkaline phosphatase activity in the GG-group was comparable to that of the control group.

Table 2: Effect of acute oral administration of Grewia gum on some serum biochemical indices

Parameter	Control	Treatment
AST (U/L)	89.00 ± 5.78	110.20 ± 12.52
ALT (U/L)	76.20 ± 6.30	82.20 ± 5.18
ALP (U/L)	111.80 ± 11.84	92.40 ± 8.18
Total bilirubin (µmol/L)	0.80 ± 0.05	1.04 ± 0.06*
Total protein (g/dL)	7.34 ± 0.32	8.82 ± 0.28*
Albumin (g/dL)	3.96 ± 0.15	3.98 ± 0.09
Triacylglycerol (mg/dL)	66.40 ± 8.42	69.40 ± 7.22
Total cholesterol (mg/dL)	78.40 ± 4.71	81.20 ± 6.58
HDL cholesterol (mg/dL)	27.20 ± 6.48	23.60 ± 5.57
LDL cholesterol (mg/dL)	59.80 ± 4.42	65.00 ± 7.67
Urea (mg/dL)	79.20 ± 3.89	82.20 ± 3.98
Creatinine (mg/dL)	1.04 ± 0.22	1.40 ± 0.08
Sodium (mmol/L)	121.20 ± 5.59	117.00 ± 4.98
Potassium (mmol/L)	18.20 ± 1.39	16.60 ± 1.17
Chloride (mmol/L)	90.80 ± 6.67	105.00 ± 3.08
Bicarbonate (mmol/L)	21.80 ± 0.37	28.20 ± 1.53*

Mean ± standard error of mean for five replicates; *****significantly different compared to the control ($p < 0.05$). AST = aspartate aminotransferase; ALP = alkaline aminotransferase; ALP = alkaline phosphatase; HDL = high density lipoproteins; LDL= low density lipoproteins.

Furthermore, the likelihood of increased red blood cell haemolysis remains low, as no changes were noted in red blood cells or haemoglobin concentrations. This evidence indicates that the oral administration of GG had no impact on either red blood cell or liver function in rats.

Furthermore, the administration of GG resulted in an increased serum total protein concentration;

however, serum albumin levels in the group receiving GG were comparable to those in the control group. Albumin, along with other proteins, is exclusively synthesised in the liver. Consequently, the levels of these proteins serve as indicators of hepatic function and overall liver health.[26] Hence, the observed increase in total protein concentration, without any corresponding changes in serum albumin concentration, was considered to have no toxicological relevance.

Similarly,the animals administered GG showed no significant changes in their lipid profile parameters.The assessment of lipid profiles holds significant importance in the evaluation of cardiovascular disease.[35-40] The findings indicate that the acute oral administration of GG did not pose a significant atherosclerotic risk.

The blood bicarbonate is important for maintaining blood pH and electrolyte balance.[41]The kidney is largely responsible for the regulation of blood bicarbonate concentrations.[42] Therefore, alkalosis, a disease condition where bicarbonate concentration is high, is related to kidney function.In this study, the group that received GG showed a significant increase in bicarbonate concentrations, which may suggest impaired kidney function. However, the levels of other related indicators, including urea, creatinine, and additional electrolytes (sodium, potassium, and chloride), remained unchanged. The altered serum bicarbonate level in the group administered GG was considered toxicologically insignificant.

The effect of acute oral treatment on blood haematological parameters is given in Table 3. The mean corpuscular haemoglobin (MCHC) measures the average concentration of iron-rich haemoglobin in red blood cells. MCHC is employed to evaluate the presence of anaemia.[43,44]However, the small reduction in MCHC with no comparable decrease in haemoglobin, packed cell volume, or red blood cell levels found in the group receiving GG may indicate a subtle effect on the haematopoietic tissue.

Figure 3 shows the effect of acute oral administration of GG on organ weight in rats. The weights of all the organs examined in the group administered GG were similar to those in the control group. This may indicate that oral administration of GG did not cause any toxic effect on the organs.[45,46]

Table 3: Effect of acute oral administration of Grewia gum on some haematological parameters in rats

Group	WBC (x 10^9/L)	RBC (x 10^{12}/L)	HCT (%)	HGB (g/dl)	MCV (fl)	MCH (pg)	MCHC (g/dl)	PLT (10^9/l)	LYM. (%)	MON. (%)	NEU. (%)	EOS. (%)	BAS. (%)
Control	12.78 ± 1.56	7.15 ± 0.22	44.62 ± 2.27	12.56 ± 0.57	62.26 ± 1.34	17.54 ± 0.26	28.18 ± 0.23	227.60 ± 19.07	65.26 ± 2.66	6.84 ± 1.77	18.00 ± 0.85	2.06 ± 0.25	7.84 ± 1.98
Treatment	13.19 ± 0.51	7.52 ± 0.32	47.12 ± 2.17	12.60 ± 0.49	62.62 ± 0.85	16.76 ± 0.25	26.78 ± 0.48*	204.20 ± 9.88	65.96 ± 3.36	9.92 ± 3.51	16.24 ± 3.26	1.58 ± 0.46	6.30 ± 1.12

Mean ± standard error of mean for five replicates; *significantly different compared to the control; WBC, white blood cells; RBC, red blood cells; HCT, haematocrit; HGB, Haemoglobin; MCV, mean corpuscular volume (MCV); MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; NEU, neutrophils; LYM, lymphocytes; MON, monocytes; EOS, eosinophils (EOS); BAS, basophils; PLT, platelets.

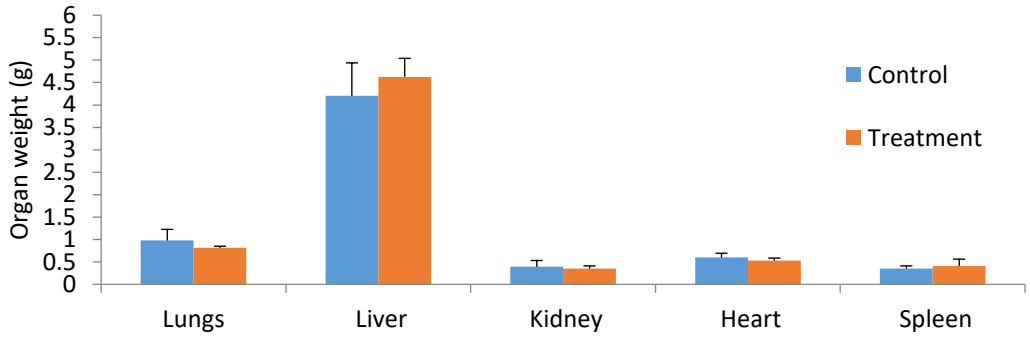

Figure 3: Effect of acute oral administration of GG on organ weight in rats. There is no significant ($p > 0.05$) difference in organ weight between the GG-treated rats and the control.

The microscopic examination of the organs did not reveal any pathological abnormalities in the liver, kidney, heart, spleen, or lungs (Figure 4). Furthermore, the absence of such abnormalities, combined with a lack of significant biochemical and haematological changes, suggests normal organ function.

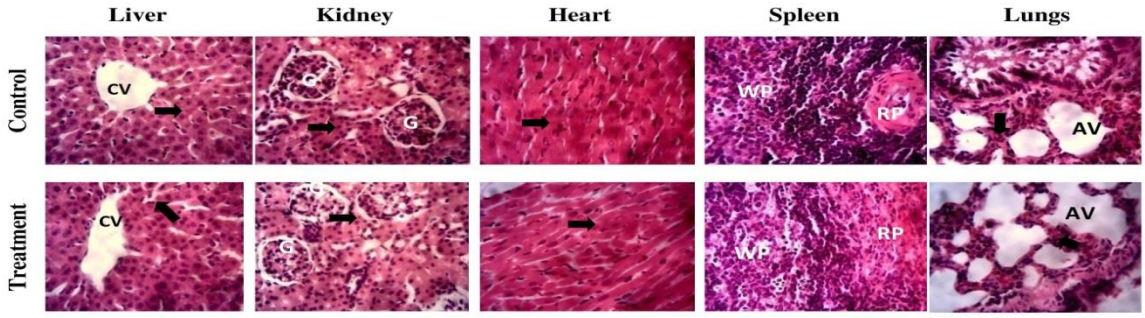

Figure 4: Photomicrographs of tissue sections from the control and treatment (GG 2000 mg/kg) groups. Liver, central vein (CV), hepatocytes (arrows); kidney, glomerulus (G), renal tubules (arrows); heart, cardiac muscle fibres (arrows); white pulp (WP), and red pulp (RP); and lung, alveoli (AV), and interstitium (arrows) with no observable histological changes. H & E, × 400

CONCLUSION

The administration of Grewia gum caused a significant increase in serum total bilirubin, total protein, and bicarbonate concentrations and a decrease in mean corpuscular haemoglobin levels. This observation was considered toxicologically irrelevant as other related indices were not altered. Hence, Grewia gum is safe at the present level of exposure. However, there is a need for further research and comprehensive studies to evaluate the long-term effects of Grewia gum administration and its possible impact on overall health.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS DECLARATION

The authors hereby declare that the works presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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