Original Research Article

Antihyperglycemic, antidyslipidemic and antioxidant properties of hydromethanol extract of *Eremomastax speciosa* leaf in alloxan monohydrate-induced hyperglycemic rats

SO Onoja1*, UO Effiong2, BK Chukwuocha2, CC Okorie-Kanu3, MI Ezeja1, YN Omeh2, AO Anaga4

1Department of Veterinary Physiology, Pharmacology, Biochemistry and Animal Health and Production, Michael Okpara University of Agriculture, Umudike, Umuahia, 2Department of Biochemistry, Michael Okpara University of Agriculture, Umudike, Umuahia, 3Department of Veterinary Pathology, Michael Okpara University of Agriculture, Umudike, Umuahia, Nigeria, 4Department of Veterinary Physiology and Pharmacology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria

*For correspondence: Email: samonreal@yahoo.com; Tel: +234-8030613032

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Abstract

**Purpose:** To investigate the antihyperglycemic, anti-dyslipidemic and antioxidant properties of hydromethanol extract of *Eremomastax speciosa* leaf in alloxan monohydrate-induced hyperglycemic rats.

**Methods:** Hydromethanol extract of *Eremomastax speciosa* leaf was tested at dose range of 25 to 100 mg/kg against vehicle (distilled water) and glibenclamide (2 mg/kg, reference standard) in alloxan-induced hyperglycemic rats. Gas chromatography mass spectrometry (GC-MS) was employed in the identification of phytoconstituents. The fasting blood glucose (FBG), serum lipid profile, malondialdehyde, catalase and superoxide dismutase levels as well as the histopathology of the pancreas of the animals were evaluated.

**Results:** The extract (25 mg/kg) significantly reduced FBG, lipid profile and malondialdehyde levels relative to vehicle treated group but increased the superoxide dismutase and catalase levels and reversed alloxan-induced pancreatic islet cell degeneration. GC-MS analysis showed the presence of 4,5-dimethylthiazole (4.38%), benzenesulfonic acid, methyl ester (15.62%), benzenesulfonyl chloride (40.62%), benzopyran-4-one, 5,7-dihydroxy-2-phenyl (21.25%), 2(5H)-furanone, 4-methoxy-5-phenyl (12.50%), and p-chlorobenzenesulfonyl chloride (5.62%)

**Conclusion:** The hypoglycemic effect of the extract validates the folkloric uses of *E. speciosa* leaf in the ethnobotanical care of diabetes mellitus.

**Keywords:** antihyperglycemic, antioxidant, anti-dyslipidemic, *Eremomastax speciosa*

INTRODUCTION

Diabetes mellitus is described as chronic endocrine and/or metabolic disorder usually characterized by insulin deficiency, insulin resistance or a combination of both [1]. The insulin resistance is associated with obesity, aging and reduced physical activities [2]. Insulin
resistance give rise to hyperglycemia, dyslipidemia, diabetes and its complications [3]. Hyperglycemia inhibits superoxide dismutase and catalase by protein glycation, therefore aggravating free radical generation with resultant increase in lipid peroxidation and oxidative stress [4]. Oxidative stress is incriminated in the progression of diabetes mellitus and its complications; cardiovascular disease, neurological disorder, cancer, nephrotoxicity, liver damage, inflammation etc [5,6]. The most common form of diabetes is type 2 and it constitutes about 90% of the diabetic patients’ population [7]. The treatment strategy for type 2 diabetes involves change of lifestyle (diet and physical exercise), insulin and oral hypoglycemic drug therapy [8]. The limitation of insulin and oral hypoglycemic drugs are the development of some side effects like hypersensitivity reaction, hypoglycemia, lactic acidosis, obesity, sodium retention, osteoporosis etc [9-11]. Despite the side effects of insulin and oral hypoglycemic drugs most diabetic patients from the under-developed countries depend on herbal preparations for the management of their health conditions. This has been attributed to poverty, cultural inclination, high cost of treatment and inadequate access to health facilities [12].

Eremomastax speciosa (Acathaceae) commonly called “blood plant” in Cameroon is an erect medicinal plant used in the management of anaemia and stomach disorders [13]. The leaf decoction is used in traditional management of diabetes mellitus in Ikot-Ekwere Akwa-Ibom state (oral communication), but this is yet to be reported in scientific literature. The pharmacological activities; antianaemic [14,15], antiulcer [16], antisecretory [17], antimicrobial [14], antifungal [13] and antidiarrhoea [18] have been reported. The purpose of this study was to investigate the antihyperglycemic, antioxidant and anti-dyslipidemic activities of hydromethanol extract of E. speciosa leaf on alloxan monohydrate-induced hyperglycemic rats.

**EXPERIMENTAL**

**Extract preparation**

*E. speciosa* (aerial parts) were harvested from Itu in Ikot-Ekwere Itam, Akwa-Ibom State, Nigeria and identified by Mr. Okon Etefia. The plant material was chopped, dried at normal environmental temperature (27 ± 2 °C) for two weeks and ground into coarse powder with electric hammer mill. The coarse powder (410 g) was soaked in 80% methanol (Sigma Aldrich, USA) for 48 hours. The mixture was shaken gently at 3 hours intervals during this period, after which it was sieved with Whatmann number 1 filter paper. Later, the extract was dried in an oven (Surgifriend Medical, England) at 40 °C to exhaustively evaporate the methanol leaving a dark-green viscous *E. speciosa* extract (ESE) weighing 136.4 g (35.7% w/w).

**Gas chromatography-mass spectrometry (GC-MS) of ESE**

The GC-MS analysis of ESE was carried out using GCMS-QP2010 PLUS (SHIMADZU, JAPAN) equipped with a DB-5MS column (J & W Scientific, Folsom, CA) [19]. The compounds were identified by comparing with the available data at National Institute of Standards and Technology mass spectral database.

**Animals**

Following approval by the Ethical Committee in University of Nigeria, Nsukka, albino Wistar rats (40) weighing between 110-140 g were obtained from reliable source. The rats were acclimatized for 14 days under ambient temperature of 27 ± 2 °C and natural light/dark cycle. They were fasted of food (16 hours) prior to the experiments. The animal house was well ventilated and rats were given standard animal chow and tap water *ad libitum*.

**Acute oral toxicity test**

This was conducted using Up and Down method [20] and dose limit of 2000 mg/kg [21].

**Experimental design**

The induction of diabetes and experimental protocol were conducted as modified by Okwudili et al [22]. Briefly, 30 diabetic rats were randomly separated into 5 groups (I-V) (n = 6). The group I received vehicle (distilled water, 5 ml/kg) while groups II-V received ESE at 25, 50 and 100 mg/kg, respectively. ESE and drug were administered *per os* and FBG levels were determined at 1, 3 and 6 h after treatment. Thereafter, the rats were dosed once daily for 14 consecutive days. On day 14, the rats were fasted for 16 hours and blood was collected via the median canthus for serum sample preparation and determination of lipid profile. Thereafter, the liver was excised following cervical dislocation. Ten percent liver homogenate in phosphate buffer saline (pH 7.0) was used for estimation of lipid peroxidation, catalase and superoxide activity. The pancreas was also excised and preserved in 10% formal saline for histopathologic examination.
relative organ weight of kidney, liver and heart were also determined.

Estimation of serum lipid profile

The serum cholesterol, triacylglycerol and high-density lipoprotein cholesterol (HDL-C) levels were estimated with commercial assay kits (Randox Laboratory Diagnostics, United Kingdom) according to the manufacturer’s procedures. Serum low density lipoprotein cholesterol (LDL-C) was calculated using Friedewald’s equation [23]. \[ \text{LDL-C} = \text{cholesterol} - \{\text{HDL-C} + \left(\frac{\text{triacylglycerol}}{5}\right)\} \]

\[ \text{VLDL-C} = \left(\frac{\text{triacylglycerol}}{5}\right) \] [4].

In vivo antioxidant status

The determination of lipid peroxidation (LPO) in the liver homogenate was done by thiobarbituric acid reactive substances (TBARS) [24], assay of superoxide dismutase [25] and catalase [26].

Histopathology

The tissue sections were prepared according to the methods used by Ezeja et al [1]. Photomicrographs were captured at 400 magnifications with digital microscope (Olympus Scientific Equipment, USA).

Data analysis

The data were presented as mean ± standard error of mean (SEM) and analysed using ANOVA, and variant means were separated by least significant difference (LSD) test with SPSS version 21.0. The \( p \) values <0.05 were statistically significant.

RESULTS

Acute toxicity study

The ESE produced no sign of toxicity and mortality at 2000 mg/kg (LD50 of ESE was >2000 mg/kg).

Table 1: Chemical composition of \( E. \ speciosa \) extract as identified by the GC-MS

| S/N | Retention time (min) | % | Molecular weight (g) | Molecular formula | Chemical name |
|-----|----------------------|---|----------------------|-------------------|--------------|
| 1   | 30.60                | 40.63 | 176 | \( \text{C}_8\text{H}_7\text{ClO}_2\text{S} \) | Benzenesulfonfyl chloride |
| 2   | 33.00                | 21.25 | 254 | \( \text{C}_{13}\text{H}_{10}\text{O}_4 \) | Benzopyrane-4-one, 5,7-dihydroxy-2-phenyl- |
| 3   | 21.80                | 15.63 | 172 | \( \text{C}_8\text{H}_6\text{O}_3\text{S} \) | Benzenesulfonic acid, methyl ester |
| 4   | 28.80                | 12.50 | 190 | \( \text{C}_7\text{H}_6\text{O}_3\text{S} \) | 2(5H)-Furanone, 4-methoxy-5-phenyl- |
| 5   | 34.50                | 4.38  | 113 | \( \text{C}_9\text{H}_7\text{NS} \) | 4,5-Dimethylthiazole |
| 6   | 32.00                | 5.63  | 210 | \( \text{C}_6\text{H}_4\text{Cl}_2\text{O}_2\text{S} \) | p-Chlorobenzensulfonfyl chloride |

Legend: % = percentage composition

Chemical composition of ESE as identified by the GC-MS

A total of six (6) compounds corresponding to the 6 peaks in the GC-MS chromatogram were identified (Figure 1). These were 2(5H)-furanone, 4-methoxy-5-phenyl- (12.50%), benzenesulfonic acid, methyl ester (15.62%), benzenesulfonfyl chloride (40.62%), benzopyrane-4-one, 5,7-dihydroxy-2-phenyl- (21.25%), 4,5-dimethylthiazole (4.38%) and p-chlorobenzensulfonfyl chloride (5.62%) (Table 1). Molecular structures of the compounds are presented in Figure 2.

Figure 1. GC-MS chromatogram of \( E. \ speciosa \) extract. Legend: 1 = benzenesulfonfyl chloride, 2 = benzopyrane-4-one, 5,7-dihydroxy-2-phenyl-, 3 = benzenesulfonic acid, methyl ester, 4 = 2(5H)-furanone, 4-methoxy-5-phenyl-, 5 = 4,5-dimethylthiazole, 6 = p-chlorobenzensulfonfyl chloride

Acute effects of ESE on FBG

Glibenclamide and ESE 25 mg/kg increased (\( p < 0.05 \)) percentage reduction in FBG of treated rats relative to vehicle treated rats. ESE also produced non-dose-dependent anti-hyperglycemic effect (Figure 3).

Sub-acute effects of ESE on FBG

The FBG of the various treatment groups did not vary significantly (\( p > 0.05 \)) on day 0 while on day 7 and 14 the FBG of glibenclamide and ESE treated rats were within the normal range (< 120 mg/dl) and significantly lower (\( p < 0.05 \)) than those of the vehicle treated rats (> 126 mg/dl) (Table 2).
Effect of ESE on lipid profile

The ESE significantly reduced (p < 0.05) serum cholesterol, triacylglycerol, VLDL-C, LDL-C and significantly increased (p < 0.05) HDL-C levels in the treated group relative to vehicle treated group. Glibenclamide produced increased (p < 0.05) cholesterol and HDL-C levels but did not cause (p > 0.05) change in triacylglycerol, VLDL-C and LDL-C levels relative to distilled water treated rats (Table 3).

| Treatment                  | Fasting blood glucose ± SEM (mg/dl) |
|----------------------------|-------------------------------------|
|                            | Day 0                               | Day 7                           | Day 14                          |
| Distilled water 5 ml/kg    | 467.75 ± 113.44                      | 206.67 ± 47.88                   | 148.00 ± 33.45                   |
| Glibenclamide 2 mg/kg      | 326.75 ± 16.72                       | 97.50 ± 10.17*                   | 73.25 ± 3.71*                    |
| ESE 25 mg/kg               | 354.50 ± 50.02                       | 94.00 ± 5.99*                    | 75.40 ± 2.94*                    |
| ESE 50 mg/kg               | 409.75 ± 101.08                      | 74.00 ± 8.98*                    | 82.60 ± 6.10*                    |
| ESE 100 mg/kg              | 360.00 ± 6.35                        | 112.20 ± 17.96*                  | 71.20 ± 6.21*                    |

*p < 0.05 relative to vehicle treated rats. ESE = Eremomastax speciosa extract, SEM = standard error of mean

In vivo antioxidant activities of ESE

Malondialdehyde levels of ESE and glibenclamide treated rats were low (P < 0.05) relative to vehicle treated rats (Table 4). The ESE (25 mg/kg) and glibenclamide treatment increased (P < 0.05) activity of catalase, while ESE (50 and 100 mg/kg) decreased (P < 0.05) the activities of catalase in the treated rats relative to vehicle treated rats (Table 4). Also, ESE (25 mg/kg) and glibenclamide elevated (P < 0.05) activity of superoxide dismutase in treated rats relative to vehicle treated rats (Table 4).

Table 2: Sub-acute effects of E. speciosa extract on fasting blood glucose

| Treatment                  | Fasting blood glucose ± SEM (mg/dl) |
|----------------------------|-------------------------------------|
|                            | Day 0                               | Day 7                           | Day 14                          |
| Distilled water 5 ml/kg    | 467.75 ± 113.44                      | 206.67 ± 47.88                   | 148.00 ± 33.45                   |
| Glibenclamide 2 mg/kg      | 326.75 ± 16.72                       | 97.50 ± 10.17*                   | 73.25 ± 3.71*                    |
| ESE 25 mg/kg               | 354.50 ± 50.02                       | 94.00 ± 5.99*                    | 75.40 ± 2.94*                    |
| ESE 50 mg/kg               | 409.75 ± 101.08                      | 74.00 ± 8.98*                    | 82.60 ± 6.10*                    |
| ESE 100 mg/kg              | 360.00 ± 6.35                        | 112.20 ± 17.96*                  | 71.20 ± 6.21*                    |

*p < 0.05 relative to vehicle treated rats. ESE = Eremomastax speciosa extract, SEM = standard error of mean
Table 3: Effects of *E. speciosa* extract on lipid profile

| Treatment            | Cholesterol (mg/dl) | Triacylglycerol (mg/dl) | VLDL-C (mg/dl) | HDL-C (mg/dl) | LDL-C (mg/dl) |
|----------------------|---------------------|-------------------------|----------------|---------------|---------------|
| Distilled water 5 ml/kg  | 45.55±0.72          | 30.82±6.54              | 6.16±1.31      | 6.98±0.32     | 32.40±2.15    |
| Glibenclamide 2 mg/kg  | 60.07±8.38*         | 39.51±17.58             | 7.90±3.51      | 21.95±6.3*    | 30.22±6.19    |
| ESE 25 mg/kg          | 48.19±3.26          | 20.54±7.05              | 4.11±3.51      | 16.96±3.20*   | 27.12±3.15    |
| ESE 50 mg/kg          | 39.61±1.25*         | 15.01±1.32*             | 3.00±0.26*     | 18.45±2.07*   | 18.15±0.66*   |
| ESE 100 mg/kg         | 38.29±3.26*         | 6.32±1.00*              | 1.26±1.0*      | 14.96±1.09*   | 22.06±2.34*   |

*P< 0.05 relative to vehicle treated rats. ESE = *Eremomastax speciosa* extract; VLDL-C = very low-density lipoprotein cholesterol; HDL-C = high density lipoprotein cholesterol; LDL-C = low density lipoprotein cholesterol

Table 4: Effect of *E. speciosa* extract on some in vivo antioxidant markers

| Treatment            | Malondialdehyde (nanomole/g protein) | Catalase (micromole/g protein) | Superoxide dismutase (U/g protein) |
|----------------------|--------------------------------------|-------------------------------|-----------------------------------|
| Distilled water (vehicle) 5 ml/kg  | 442.62 ± 98.59                      | 73.49 ±15.59                  | 11.68 ± 0.22                     |
| Glibenclamide 2 mg/kg  | 275.96 ± 12.25*                     | 619.57 ± 95.58*               | 13.94 ± 1.98*                    |
| ESE 25 mg/kg          | 161.74 ± 25.51*                     | 146.24 ± 16.44*               | 14.57 ± 0.70*                    |
| ESE 50 mg/kg          | 273.16 ± 44.46*                     | 55.85 ± 6.12*                 | 12.39 ± 0.87                     |
| ESE 100 mg/kg         | 259.77 ± 17.58*                     | 39.03 ± 7.46*                 | 8.86 ± 0.83*                     |

*p < 0.05 relative to vehicle treated rats. ESE = *Eremomastax speciosa* extract

Table 5: Effect of ESE on mean body weights

| Treatment            | Body weight in gram ± SEM (weight gain %) |
|----------------------|------------------------------------------|
|                      | DAY 0                                      | DAY 7                                      | DAY 14                                    |
| Distilled water (vehicle) 5 ml/kg  | 100.40 ± 3.06                             | 95.70 ± 9.84 (-4.68)                       | 98.33 ± 12.36 (-2.06)                      |
| Glibenclamide 2 mg/kg  | 84.95 ± 4.35*                             | 90.25 ± 6.22 (6.21)                       | 92.00 ± 6.57 (8.30)                       |
| ESE 25 mg/kg          | 103.33 ± 3.70                             | 106.20 ± 5.04 (2.78)                      | 109.22 ± 5.18 (5.71)                      |
| ESE 50 mg/kg          | 114.50 ± 1.34*                            | 105.00 ± 3.18 (-8.29)                     | 106.26 ± 3.64 (-7.20)                     |
| ESE 100 mg/kg         | 99.25 ± 0.43*                             | 96.16 ± 6.34 (-3.11)                     | 94.74 ± 5.21 (-4.54)                      |

*p<0.05 relative to vehicle treated rats. ESE = *Eremomastax speciosa* extract

Table 6: The effects of ESE on relative organ weight

| Treatment            | Relative organ weight (%) |
|----------------------|---------------------------|
|                      | kidney | liver | heart |
| Distilled water (vehicle) 5 ml/kg  | 1.23 ± 0.03 | 5.45 ± 0.32 | 0.55 ± 0.04 |
| Glibenclamide 2 mg/kg  | 1.09 ± 0.05*                  | 5.06 ± 0.10 | 0.47 ± 0.03* |
| ESE 25 mg/kg          | 0.95 ± 0.07*                   | 3.97 ± 0.20* | 0.47 ± 0.02* |
| ESE 50 mg/kg          | 0.97 ± 0.02*                   | 4.67 ± 0.16* | 0.47 ± 0.01* |
| ESE 100 mg/kg         | 1.16 ± 0.01                    | 5.01 ± 0.05 | 0.59 ± 0.02 |

*p < 0.05 relative to vehicle treated rats. ESE = *Eremomastax speciosa* extract

Effect of ESE on body weights (g)

The vehicle, ESE 50 and 100 mg/kg treated groups had a persistent weight loss throughout the period of experiment relative to day 0 while the groups treated with glibenclamide and ESE 25 mg/kg had time-dependent weight gain relative to day 0 (Table 5).

Effects of ESE on relative organ weight

Glibenclamide, ESE 25 and 50 mg/kg reduced (p < 0.05) the ROW of liver, kidney and heart of treated rats relative to vehicle treated rats (Table 6).

Histopathology

The vehicle treated group showed fatty infiltration and no visible pancreatic islets. Glibenclamide and ESE (25, 50 and 50 mg/kg) treated groups showed pancreatic islet cells without fatty infiltration. Of ESE With 25 and 50 mg/kg of ESE, the islet cells were numerous and had evidence of cell regeneration (Figure 4).
Figure 4: Photomicrograph sections (H&E ×400) of pancreas. Star = pancreatic islets; arrow = Acinar cell; Triangle = fat cells; Figure 4 A, A, islet cells of rats treated with distilled water (vehicle) 5 ml/kg; B, islet cells of rats treated with glibenclamide 2 mg/kg; C, islet cells of rats treated with ESE 25 mg/kg; D, islets cells of rats treated with ESE 50 mg/kg; E, islet cells of rats treated with ESE 100 mg/kg.

DISCUSSION

The hydromethanol extract of *E. speciosa* had LD50 greater than 2000 mg/kg and elicited hypoglycemic, antidyslipidemic and antioxidant properties as well as reversed pancreatic islet cell degeneration in alloxan-induced hyperglycemic rats. GC-MS of ESE identified 6 bioactive compounds. The observed hypoglycemic, antidyslipidemic and antioxidant activities of *E. speciosa* may be related to 5,7-dihydroxy-2-phenyl-benzopyran-4-one (chrysin) [27].

Usually, alloxan treatment cause islet cell degeneration and fatty infiltration of the pancreas (Figure 4) with resultant hyperglycaemia and dyslipidemia which characterize diabetes mellitus [28]. Alloxan structurally (molecular shape) resemble glucose and transported into the beta-cell of pancreatic islet by Glut-2 glucose transporter in plasma membrane as glucose analogue and accumulate in cytosol where it impairs glucose-stimulated insulin secretion via deactivation of glucokinase [29]. The compound also generates free radicals through cyclic reaction of dialuric acid which leads to plasma membrane damage and necrosis [30].

Chrysin is present in the leaves of the genus, *Oroxylum*, *Chamomile* and *Passiflora* [31]. Its antidiabetic, aromatase inhibitory and antioxidant effects have been reported [27]. Antidiabetic activity of chrysin is via the impaired function of 11-hydroxysteroid dehydrogenase type 1, which leads to reduced cortisol elaboration and increased insulin action [32]. The observed antihyperglycemic activity of *E. speciosa* may be mediated through enhanced insulin sensitivity [33]. ESE caused regeneration of pancreatic islet cells damaged by alloxan (Figure 4) which may be due to inhibition of spontaneous generation of free radical from the reduced form of alloxan, dialuric acid [34]. Antioxidants, especially catalase and superoxide dismutase, can inhibit pancreatic toxicity of alloxan [35]. The extract (25 mg/kg) increased (p < 0.05) activities of catalase and superoxide dismutase in treated group. This suggests that antihyperglycemic activity of ESE can be linked to its antioxidant activity [36]. *Eremomastax speciosa* also, reduced (p < 0.05) malondialdehyde equivalent in the treated rats. Malondialdehyde, product of lipid peroxidation, causes DNA fragmentation and plasma membrane damage which will lead to cell death [6,37]. The decrease in the level of malondialdehyde suggests that ESE inhibits lipid peroxidation and thus may prevent development of cardiovascular disease [38]. *E. speciosa* decreased (p<0.05) serum triacylglycerol, LDL-C and elevated HDL-C levels in the treated groups. This suggests that the extract can ameliorate the dyslipidemia which characterizes diabetes mellitus and prevent vascular complications of diabetes [39]. The mechanism of the hypolipidemic effect has not been established but could be via the inhibition of cholesterol absorption, increased expression of LDL-C receptor, increased fecal bile acid excretion and inhibition of HMG-CoA reductase [40].

Alloxan induced diabetes is usually characterized by weight loss due to muscle wasting as a result of negative energy balance and dehydration due to polyuria [41]. The extract (25 mg/kg) and glibenclamide treatment produced time-dependent weight gain in treated rats groups suggesting that the extract may have ameliorated the muscle wasting and polyuria as well as corrected the tissue toxicity which characterizes alloxan treatment as a result of its conversion to butylalloxan [29]. At doses beyond 50 mg/kg, the decrease in the pharmacological activities of *E. speciosa* extract may be as a result of receptor site saturation and inhibition [42].

CONCLUSION

The results of this study have shown that the hydromethanol extract of *Eremomastax speciosa* possesses significant antidiabetic, antidyslipidemic and antioxidant properties. This study therefore validates the use of *E. speciosa* leaf in diabetes mellitus treatment.
DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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