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Full Length Research Paper

Protective effect of methanolic extract of *laportea aestuans* on indomethacin-induced kidney damage in male wistar rats

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This study aimed at evaluating the nephroprotective role of different doses of the methanol extract of *Laportea aestruans* leaves on indomethacin-induced nephrotoxicity in adult male Wistar rats. Forty rats were divided into five groups of eight rats and pretreated orally for three days with methanol extract of *Laportea aestruans* before administration of indomethacin. Rats in group one; normal control received 0.5 ml normal saline (0.9 % v /v). Rats in group two received 20 mg/kg body weight indomethacin alone. Rats in group three, four and five received 200, 400 and 800 mg/kg body weight of methanol extract of *Laportea aestruans*. At the expiration of the experiment, rats were sacrificed, blood and the kidney were collected for renal functions tests. The results showed that administration of indomethacin alone caused a significant ($p < 0.05$) increase in the concentrations of creatinine, urea, uric acid, cholesterol and total protein and the activity of glucose-6-phosphatase and renal 5'-nucleotidase in the piroxicam alone treated group. However, pretreatment methanol extract of *Laportea aestruans* showed a dose-dependent decrease in the concentrations and the activities of all the kidney markers assayed for. The results show that methanol extract of *Laportea aestruans* possesses nephroprotective potential against indomethacin-induced renal damage.

Keywords: Nephroprotective, *Laportea aestruans*, indomethacin

INTRODUCTION

Indomethacin, a methyl indole derivative, is a non-steroidal anti-inflammatory drug (NSAID) that exert anti-inflammatory, analgesic and anti-pyretic effects through the suppression of prostaglandin (PG) synthesis, by inhibiting the enzyme cyclooxygenase (COX) the enzymes that catalyzes the synthesis of cyclic endoperoxides from arachidonic acid to form prostaglandins (Tanaka et al., 2001; Buer, 2014). Two isoforms of this enzyme COX-1 and COX-2 are known to exist. COX-1 is constitutively expressed in several tissues and is thought to participate in "housekeeping" functions. In the kidney, it localized to mesangial cells, arteriolar smooth muscle and endothelial cells, parietal epithelial cells of Bowman's capsule, and cortical and medullary collecting ducts and controls homeostatic functions such as regulating renal blood flow (Crofford, 1997). COX-2 expressed inducibly in most tissues in response to injury or inflammation, but also present at detectable levels in normal adult mammalian kidneys. COX-2 is regulated in response to intravascular volume (de Leval et al., 2004). COX-2 functions primarily affect salt and water excretion (Weir, 2002). Blockade of either or both of these enzymes can therefore have different effects on renal function (Nante et al., 1999; Schnermann and Briggs, 1999). Prostaglandins are known to be mediators of inflammation, regulate a variety of renal functions such as vascular tone, salt and water balance, and renin release (Johnson, 1997).

Various biochemical abnormalities produced in the kidney in response to the administration of indomethacin include oxidative stress and mitochondrial dysfunction mediated through the production of free radicals (Basivireddy et al., 2004; Varghese et al., 2009).

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Indomethacin induces also impairment in structure and function of brush border membranes in the kidney mediated by free radicals and the activation of phospholipases (Basivireddy et al., 2004). Reactive oxygen species (ROS) are known to break protein disulphide bonds, resulting in accumulation of unfolded or misfolded protein in cells (Inagi, 2009).

The kidneys are susceptible to oxidant-induced injury due to their high reliance on mitochondria and ATP to facilitate the transport function of the nephron. This is especially true of the proximal tubule cells which have a high abundance of mitochondria to facilitate their transport and secretory functions. The mitochondria are also a key site for mediation of cell death via the release of pro-apoptotic inducers and the production of ATP, which is a master regulator in the decision of a cell to die by apoptosis, necrosis, or autophagy (Linkermann et al., 2014). Recent studies have focused on identifying accurate determinates of mitochondrial function as markers for renal cell death. Studies have also focused on approaches to either preserve mitochondrial function or enhance biogenesis to protect against nephrotoxicity (Bhargava and Schnellmann, 2017; Chen et al., 2017; Collier et al., 2016; Geng et al., 2017; Ni et al., 2017; Zhang et al., 2017).

Laportea aestuans (*Urticaceae*) is a tropical nettle weed commonly called stinging nettle or West Indian wood nettle that is widely distributed in tropical rain forests (Hawaii Pacific Weed Risk Assessment, 2012). The stem is often erect, angular, covered with pilose hair or stinging hairs and green in colour (Cheryl, 2007). Its phytochemical constituents include saponins, tannins, flavonoids, cardiac glycosides, alkaloids and phenolic compounds (Claudia et al., 2006; Mongrand et al., 2000). Paul, 2008 isolated a mixture of methyl esters of aliphatic acids from *Laportea aestuans*. Also, the essential oil from the plant was found to be dominated by methyl salicylate and had significant antioxidant and antimicrobial activities (Oloyede, 2016) Traditionally, *Laportea aestuans* is commonly used to treat laryngitis, arthritis, anemia, hay fever, kidney problems and pain and this, urinary problems, diabetes, bronchitis and filariasi (Focho *et al.,* 2009), stroke, kidney problems and pain (Anely et al., 2007), It is used as abortifacient, laxative, in eye treatments and pain-killers. It is also used in treating pulmonary and stomach troubles, diarrhea and dysentery (Chew, 1969; Burkill, 1985; Friis, 1993; Nadine, 2001), to shorten labour and remove the placenta during childbirth (Lans, 2006, 2007; Cheryl, 2007; Etukudo, 2003). Epidemiological studies indicated that consumption of *L. aestuans* inhibits the damaging activities of free radicals in human body (Morrison and Twumasi, 2010). Phytochemical, toxicity, antimicrobial and antioxidant screening of extracts obtained from *Laportea aestuans* (Gaud) had also been investigated (Oloyede and Ayanbadejo, 2014). The leaves extract of *Laportea aestuans* contains active compounds that reduce pro inflammatory cytokines (Anely et al., 2007). However, there has been no scientific evidence to support its ethnomedicinal use as a therapeutic agent in kidney damage. Thus, the present study sought to investigate the nephroprotective potentials of methanol extract of *Laportea aestuans* (MELA) leaves on indomethacin-induced nephrotoxicity in Wistar rats.

MATERIALS AND METHODS

Chemicals and reagents

Glucose-6-phosphate, adenosine monophosphate (AMP), trichloroacetic acid (TCA), bovine serum albumin (BSA), Folin-Ciocalteau reagent, methanol were purchased from Sigma Aldrich Chemical Co. (St. Louis, MO, USA). Cholesterol kit, urea kit, creatinine kit from Randox Chemicals. Others chemicals and reagents used were of analytical grade.

Plant collection and authentication

Fresh leaves of *Laportea aestuans* (*L. aestuans*) were collected in June 2014 after identification of the plant from fields in Iwo, Osun State, Nigeria and were authenticated at Department of Biological Science, Bowen University, Iwo, Osun State, with voucher identification number BUH 096.

Preparation of extract

The leaves were air-dried at room temperature for 15 days, pulverized with electric blender (model MS-223; Blender/Miller III, Taiwan, China), and the 500 g of the powdered was extracted with 2. 5 liters of methanol by maceration for 48 hours with intermittent shaking. The extract obtained was strained, filtered, evaporated to dryness on a rotatory evaporator (Model 349/2 Corning/England) at 40 ^{0}C. The solid mass obtained (referred to as MELA) and was stored in refrigerator and was used in this study

Experimental Animals

Forty male Wistar strain albino rats (150-170 ± 5 g) were procured from Central Animal House, Faculty of Basic Medical Science, College of Medicine, University of Ibadan, Ibadan, Nigeria. To avoid coprophagy, rats were kept in polyethylene-walled cages in a temperature-controlled room (25 ± 2°C) with 12 h light and 12 h dark cycle prior to the experiments and were fed with standard rat's chow (Ladokun Feeds, Nigeria) with fresh water *ad libitum*. All procedures in this study conformed to the guiding principles for research involving animals as recommended by the Declaration of Helsinki and the Guiding principles in the care and use of animals (2002)

and as approved by the Research Ethical Committee, Bowen University, Nigeria. The "Principle of Laboratory Animal Care" (NIH publication No. 85-23) guidelines and procedures were considered in this study (NIH publication revised, 1985) (1985) The rats were deprived of food for 18 hours but had free access to clean water prior to the commencement of the experiment.

Experimental design

Animals were acclimatized and kept on normal diet for two weeks. The rats were treated orally throughout the experiment. Rats in groups 3-5 were pretreated orally for three days with methanol extract of *Laportea aestuans* (MELA) before the administration of indomethacin (IND). Experimental animals were divided into the following 5 groups of 8 animals each:

- Group I: normal control group received 0.9% normal saline solution.
- Group II: indomethacin control group. Each rat received a dose of 20 mg/kg g body weight of IND.
- Group III: animals were pre-treated with 200 mg/kg body weight of MELA for three days followed by a dose of 20 mg/kg body weight IND
- Group IV: animals were pre-treated with 400 mg/kg body weight of MELA for three days followed by a dose of 20 mg/kg body weight IND
- Group V: animals were pre-treated with 800 mg/kg body weight of MELA for three days followed by a dose of 20 mg/kg body weight indomethacin
- Biochemical studies

Rats were sacrificed 24 hours after the administration of indomethacin under light ether anesthesia, blood samples were collected for renal function tests; creatinine (Tietz, 1995), urea (Webster, 1997), uric acid (Fossati et al., 1980), cholesterol (Haweroft, 1987) and protein (Lowry et al., 1951) via cardiac puncture and the kidneys were harvested for other biochemical tests and histology. The kidneys were removed and rinsed in ice-cold 1.15% KCl and blotted. One gram portion of the kidney from each rat was used to prepare homogenate of the kidney (10%) in ice cold phosphate buffer solution (100mM, pH7.4) using Teflon homogenizer. The homogenate was centrifuged at 3000 g for 15 minutes at 4^{o}C to remove cell debris. The supernatant was used for the estimation of renal 5'-nucleotidase (George *et al* (1982) and glucose-6-phosphatase (Swanson 1950) activities.

Statistical analysis

Results are expressed as mean ± SEM. The Student's t-test was used for calculating level of significance. Statistical significance was accepted at $p < 0.05$.

Results

Creatinine, urea and uric acid levels are the most important clinical parameters for evaluating abnormalities in renal function. As shown in Figure1, administration of 20 mg/kg of indomethacin alone increased the concentration of creatinine significantly ($p < 0.05$), but pretreatment with different doses of MELA decreased this observation in a dose dependent manner. Blood urea nitrogen was significantly ($p < 0.05$) elevated in the IND alone treated group when compared to the normal control group, but pretreatment with MELA at different doses reduced the level of blood urea nitrogen in a dose dependent manner. Uric acid level was significantly elevated ($p < 0.05$) in the IND alone treated group compared to the control group, but pretreatment with different doses of MELA decreased the level of uric acid in a dose dependent manner when compared with the IND alone group. Cholesterol concentration was significantly ($p < 0.05$) increased in the IND group compared to the control group but pretreatment with MELA at varying doses decreased the concentration of cholesterol. Protein concentration was significantly ($p < 0.05$) reduced in the group given IND alone when compared to the control group, but pretreatment with different doses of MELA increased the level in a dose dependent manner (Figure 2).

Activity of glucose-6-phosphatase was significantly ($p < 0.05$) increased in the kidney of the group treated with 20 mg/kg IND alone compared to the control group. In group pretreated with 200 mg/kg showed a significant decrease in the activity of this enzyme and the level of decreased significance were more pronounced at 800 m/kg. Administration of 20 mg/kg IND alone to rats ($p < 0.05$) significantly increased renal 5'-nucleotidase activity when compared to the control rats. However, pretreatment with 200, 400 and 800mg/kg of MELA decreased the activity of this enzyme in a dose dependent manner (Figure 2).

Discussion

The kidneys are the main organs that function in regulation of body fluid and electrolyte balance by filtration, secretion and re-absorption. Due to this property, it is susceptible to toxins, antibiotics, analgesics as well as different metabolites which are harmful to the body. Drug-induced nephrotoxicity is usually associated with the accumulation of drugs in the renal cortex, dependent upon their affinity to kidneys and on kinetics of drug trapping process. The nephrotoxicity of certain drugs are often associated with marked elevations in blood urea nitrogen, serum creatinine and acute tubular necrosis. (Verpooten et al., 1998).

The mechanism by which the kidney metabolizes and excretes various drugs and toxins importantly contributes to drug nephrotoxicity. The high rate of drug and toxin delivery to the kidney, as a result of high renal blood flow,

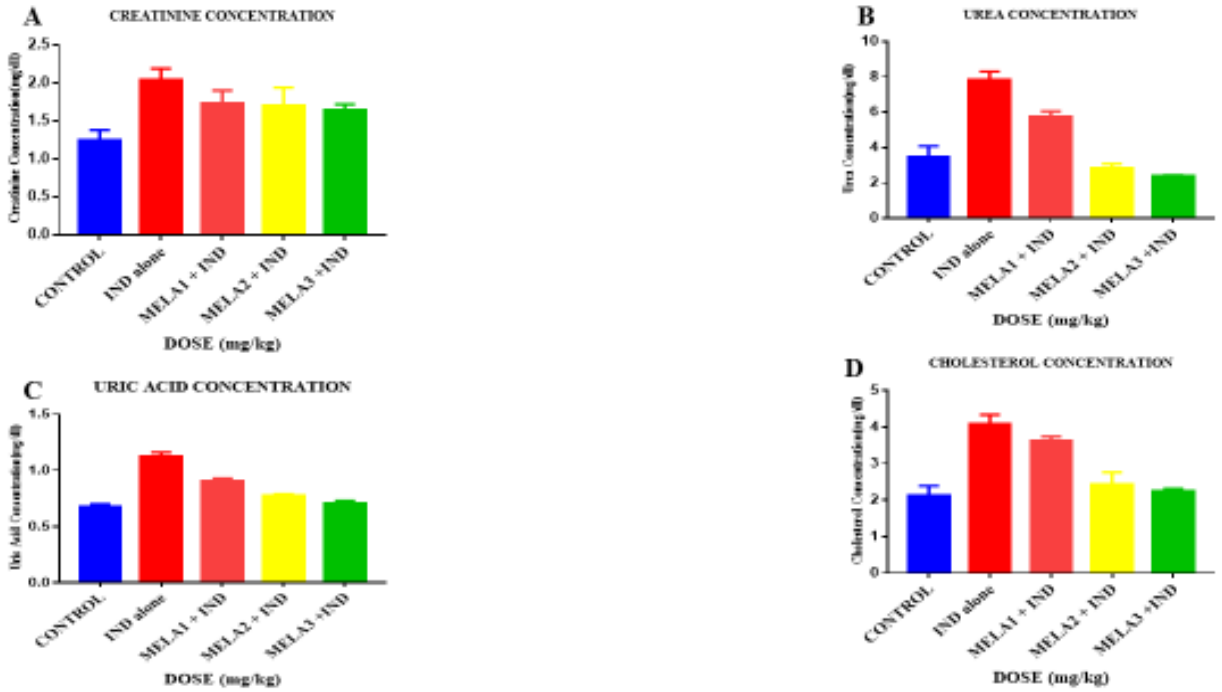

Figure 1: Effect of MELA in IND-induced nephrotoxicity in rats: Creatinine concentration (A), Urea concentration (B), Uric acid concentration (C), Cholesterol concentration (D). [a] significantly different from the control group (p<0.05); [b] significantly different from the IND group (p<0.05). IND: Indomethacin (20 ml/kg b. w.), MELA1: 200 mg/kg methanol extract of *Laportea aestuans* leaf, MELA2: 400 mg/kg methanol extract of *Laportea aestuans* leaf

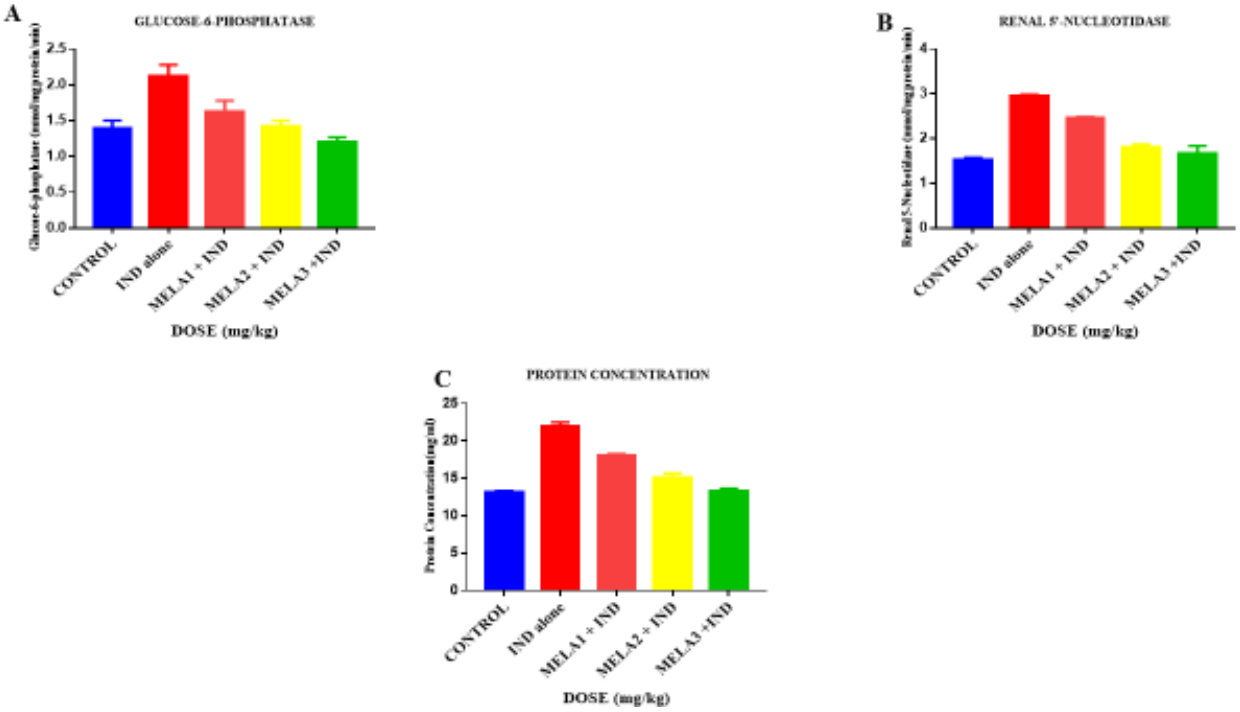

Figure 2: Effect of MELA in IND-induced nephrotoxicity in rats: Glucose-6-phosphatase (A), Renal 5'- nucleotidase activity (B), Protein concentration (C). [a] significantly different from the control group (p<0.05); [b] significantly different from the IND group (p<0.05). IND: Indomethacin (20 ml/kg b. w.), MELA1: 200 mg/kg methanol extract of *Laportea aestuans* leaf, MELA2: 400 mg/kg methanol extract of *Laportea aestuans* leaf.

which approximates 25% of cardiac output, exposes the kidney to significant drug concentrations (Perazella, 2009; Markowitz and Perazella, 2005). In addition, many tubular cells, particularly those in the loop of Henle, reside in a relatively hypoxic environment due to the high metabolic requirements associated with active solute transport by Na^+-K^+-ATPase–driven transport (Cummings and Schnellmann, 2001; Perazella, 2009). Excessive cellular workload of these cells in this relatively hypoxic environment enhances risk for a nephrotoxic-related injury. High concentrations of certain medications and their metabolites develop in the kidney medulla and interstitium from the enormous concentrating ability of the kidney, which can induce kidney injury through direct toxicity as well as ischemic damage from reduced prostaglandin and increased thromboxane production (Perazella , 2009; Kaloyanides et al., 2001).

Indomethacin-induced nephrotoxicity event involves some functional and cellular mechanisms such as glomerular lesions that interfere with glomerular hemodynamics, and altered tubular transport, with the injury ranging from solely functional lesions to the occurrence of necrosis (6).

Creatinine is a break down product of creatine phosphate in muscles, and is usually produced at a fairly constant rate by the body. Chemically, creatinine is a spontaneously formed cyclic derivative of creatine. Creatinine is transported through the blood stream to the kidney. Healthy kidneys filter creatinine and other products from the blood into the urine by the glomerulus. The presence of increased concentrations of creatinine in the kidney indicates a dysfunctional glomerulus. There is little to no tubular re-absorption of creatinine by the kidney tubules. Therefore, creatinine levels in blood and urine may be used to calculate the creatinine clearance (CrCl), which reflects the glomerular filtration rate (GFR). The GFR is important because it is a measurement of renal function. Any condition that impairs the function of the kidney will probably raise the creatinine level in the blood. The result obtained in this study revealed that oral administration of indomethacin to rats caused a significant increase in creatinine concentration (Kaptan and Szasbo, 1983).

Urea is the principal end product of protein catabolism. Enhanced protein catabolism and accelerated amino acid deamination for gluconeogenesis is probably an acceptable postulate to interpret the elevated levels of urea. The presence of some toxic compounds might increase blood urea (Varely, 1976). In addition, the elevation of blood urea is a good indicator for kidney diseases, and may indicate renal damage. In this study, indomethacin increased the concentration of urea in the rats, this result agreed with the result of (Ismail, 2001). However, pretreatment with different doses of MELA decreased this observation.

Uric acid is the end product of the catabolism of tissue nucleic acids i.e., Purine and Pyrimidine bases metabolism (Wolf et. al., 1972). In the present study, the serum uric acid levels exhibited significant increment in the rats treated with indomethacin alone. This may be due to degradation of purines and pyrimidines or to an increase of uric acid level by either over production or inability of excretion (Wolf et. al., 1972). But pretreatment with varying dose of MELA attenuated this observation.

Cholesterol is a constituent of cell membrane, and is a building block of bile, estrogen and testosterone, its presence in an elevated level in the serum may suggest damage to the cellular membrane. In this present study, cholesterol level was elevated in the serum of rats that received indomethacin alone, suggesting that there was damage to the kidney cells. This is in agreement with the report of (Ali, 1987; Hooper et al., 2004). However, different doses of MELA attenuated this increase.

Total protein is filtered by the kidney and transported back into the blood stream. In damaged kidneys, these proteins are not transported back into the blood but are passed into the urine causing proteinuria. The result of this study showed that the indomethacin alone treated group had the highest concentration of protein. This result conform to the result of Shakeerabanu et al., 2011. Pretreatment with different doses of MELA however, attenuate this observation.

Glucose-6 phosphatase (G6Pase), a key enzyme in endogenous glucose production (Cori and Cori, 1952; Shelly et al., 1993). G6Pase catalyzes the hydrolysis of glucose-6-phosphate (G6P), the terminal step of glucose production common to glycogenolysis and gluconeogenesis. In the kidney, the gluconeogenic function is restricted to the proximal convoluted tubules of the cortex (Rajas et al., 2007).The present study revealed an increase in the activity of G6Pase an indication that there was an increase in the production of glucose-6-phosphate for generation of ATP.

Renal nucleotidase, a suitable marker enzyme for plasma membranes which cleaves phosphate group from purine nucleotides AMP and GMP (adenosine monophosphate and guanine monophosphate respectively) to produce adenosine and guanine. It regulates renal hemodynamics, GFR and renin release via specific receptors of the arteriolar walls (Isabel et al., 2012). It was revealed from the result of this study that the activity of the enzyme renal nucleotidase rapidly increased in the indomethacin alone treated group, this is in accordance with the report of Isabel et al., (2012). The increased activity of this enzyme correlates well with elevated activity of G6Pase showing that there was an increased production of Glucose-6 phosphate for continual ATP production to aid the transport function of the nephron especially in proximal tubule cells which have a high abundance of mitochondria. However, while this may seem beneficial it is important to note that, the mitochondria are also a key site for mediation of cell death via the release of pro-apoptotic inducers and the production of ATP, which is a master regulator in the

decision of a cell to die by apoptosis, necrosis, or autophagy (Linkermann et al., 2014). Hence, the observed increase in the activity of this enzyme can be viewed either way. Furthermore, pretreatment with MELA prior to the administration of indomethacin attenuated this increased activity.

Conclusion

This study revealed that methanol extract of *Laportea aestuans* leaves possesses some nephroprotective effect against indomethacin-induced nephrotoxicity and has the ability to complement and maintain the hemodynamics. Studies elucidating the biochemical and molecular mechanisms of action involved in the nephroprotective activity of *L. aestuans* are in progress in our laboratory.

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