Original Research Article

AMELIORATIVE EFFECT OF GRAPE SEED OILON ALUMINIUM CHLORIDE INDUCEDNEUROTOXICITY ON RATS

ABSTRACT

Aims: Neurodegenerative disorder is characterized by progressive loss of structure and function of neurons. Exposure to aluminium causes neurodegenerative disorder like dementia, Alzheimer's disease (AD). The present study was designed to examine the ameliorative effect of grape seed oilextracton aluminium chloride induced neurotoxicity in Wistar rats.

Methodology: Wistar rats administered with aluminium chloride (175 mg/kg. p.o.) for 28-days to generate neurotoxicity model.Attenuation effect of grape seed oilagainst aluminium chloridetoxicity by oral administration adjunctly from day 18.Behavioral and locomotor activity were determined using passive avoidance test, open field test, actophotometer and rota rod test. Biochemical parameter such as acetylcholinesterase (AChE) activity andsuperoxide dismutase (SOD), catalase (CAT) and glutathione reductase (GR) activity were assessed in brain samplesand histology of brain tissue were assessed on the final day of the experiment.

Results: Aluminium chloridetreatment significantly decreases cognitive function and open field test with significance decrease in the acetylcholinesterase and antioxidant (SOD, CAT, GR)activity level.Also, the histopathological examination shows significance decrease in the neuronal cell density at hippocampal region. Grape seed oiltreatedwith aluminium chloride neurotoxic groups alleviates all the toxicity induced by the aluminium chloride. Histopathological studies in the hippocampus and cortex of the rat brain also supported that the grape seed oil markedly reduced the toxicity of aluminium chloride.

Conclusion: Supplementation of grape seed oilexhibited with beneficial and neuroprotective role on aluminium chloride induced neurotoxicity in Wistar rat modelby improving the cognitive memory and antioxidant enzyme level.

Keywords: ,Aluminium chloride, neurotoxicity, antioxidant, acetylcholinesterase, grape seed oil.

1. INTRODUCTION

Neurodegenerative disorders are characterized by progressive loss of structure and function of neurons and have created great burden to the individual and the society. The global number of AD patients was 44 million in 2015; however, this number is expected to triple, reaching 115 million individuals by 2050 [1]. Each year around 4.6 million new cases were arising globally [2]. In healthcare, the actual cause of various neurodegenerative diseases remains a mystery. Protein degradation, oxidative stress, inflammation, environmental factor, mitochondrial defects, familial history, and abnormal protein accumulation are some of the environmental factors that causes neurodegenerative diseases. Aluminium is a potential causative agent for causing AD reported by numerous studies [3]. Aluminium (Al) affects the human population around the world, which is abundantly present. Besides the presence in nature, Aluminium is presented in antacids, foils, cosmetics, deodorants, vaccines, also added in tap water as a flocculating agent during the water purification process. It is important to study the factors that might increase the absorption of aluminium across the gastrointestinal barrier. Hence, aluminium in the environment makes human at high risk of its exposure via air, water and food [4]. Normal Al concentration in human body is in the range of 0.1-0.4 µg Al/ gm tissue in dry wt. [5]. The multiple pathological conditions including Alzheimer's disease, Autism, multiple sclerosis [6], Parkinson's disease and Dementia [7] are

caused when the presence of aluminium is beyond this limit. It promotes aggregation of β-amyloid protein. Al can penetrate the blood–brain barrier (BBB) [8]; accumulate in different brain regions, including the cortex, cingulate bundles, corpus callosum and hippocampus [9]; and enter different parts of the cell, including the mitochondria, lysosomes and nucleus. To treat psychological and neurodegenerative diseases, therapeutic plants are utilized. Different western medications being utilized in cognitive decline are taken from plants. Plant inferred alkaloids, for example, anticholinesterase have been utilized to treat AD. One of the most widely grown fruit crops in the world is black grapes. Wine, Jams and raisins are also important commodities in the market of the whole world. Grape seed is a by-product of winemaking process [10], and its oil content is traditionally extracted using either an organic solvent or mechanical techniques [11], which is used as a skin care product. Numerous studies are carried out in grapes and its by-products for health-promoting and antioxidant effects. As the black grapes contains high phenolics contents, the interest in health benefits has been increased. Most phenolics in Black grapes are located in the seeds [12]. The main phenolics found in Black grapes seeds are gallic acid, catechin and epicatechin, while ellagic acid and myricetin are the major ones in the skins. Black grapes shown promise as novel antimicrobial agents [13], anti-cancer properties [14],anti-inflammatory activity [15], antimicrobial activity against Escherichia coli O157:H7 [16], nootropic activity, antiulcerative, antiarthritic, anti-viral prevent skin ageing, inhibit UV-radiation induced peroxidation activity [17] and scavenge free radicals [18]. In view of these facts the present study was designed to test the hypothesis whether a nutritional strategy like administration of grape seed oil could prevent aluminium chloride-induced neurotoxicity in rats.

MATERIALS AND METHODS

Grape seed collection and cold pressed extract preparation:

The seeds obtained from fresh whole black grapes (Vitis vinifera) was purchased from Madhavaram, Chennai district of Tamil Nadu in the month of January 2022 and the voucher deposited in the corresponding author's laboratory, Department of Pharmacology, C.L.BaidMetha College of Pharmacy, Affiliated to The Tamil naduDr. M.G.R Medical University, Chennai, Tamil Nadu, India.

Seeds of the grapes were carefully removed using strainer/ hands, washed and dried for approximately 24 hours. Cold pressing is adopted to collect the extract from the dried seeds using mortar and pestle (~100 g of dried seeds) until ground as fine powder with dropwise addition of methanol. Pressing the extracts until the oil shows the visible separation from the grape seed powder. Approximately after 16-18 hours, the cold pressed oil was separated and stored in closed glass bottles (~50 ml).

2.1 EXPERIMENTAL ANIMALS

Male Wistar rats weighing 180-200g of around 2-3 months old were used for the study. All experimental protocols were approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and Institutional Animal Ethical Committee (IAEC).

IAEC Approval no: 01/321/PO/Re/S/01/CPSEA/dated 17/11/2021 valid up to 18/11/2021.

EXPERIMENT DESIGN:

On the first day of experiment the animals had been divided randomly into 5 groups of 4 animals each. Neurotoxicity is induced using Aluminium chloride (175mg/kg, p.o.) for the II, III, IV, V groups have been carried out for 30 days (29/01/2022 – 27/02/2022) and GSO (3.7g/kg) treatment was carried for 10 days (17/02/2022 – 27/02/2022). Control animals were given (0.9% NaCl 5ml/kg, p.o.) by means of using oral gavage. The final dose was given 60 min prior to behavioural checking and on 30^{th} day, scarification of animal has been done for exvivo studies.

Animals	Treatment
Group-I	Normal saline (0.9% NaCl 5mg/kg) p.o.
Group-II	Aluminium chloride (175mg/kg) p.o.
Group-III	Aluminium chloride (175mg/kg) p.o. + Donepezil HCl (1mg/kg) p.o.
Group-IV	Aluminium chloride (175mg/kg) p.o. + 2ml Grape seed oil (3.7g/kg) p.o.

Group-V	Aluminium chloride (175mg/kg) p.o. + 4ml Grape seed oil (3.7g/kg) p.o.

2.2 ASSESSMENTS
PASSIVE AVOIDANCE TEST:
This test is used to assess short term memory. Pole climbing apparatus chamber is used for passive avoidance response where pole is replaced by a wooden platform fixed on electrified grid floor. When rats stepped off the platform, they receive a continuous foot shock from grid floor. The normal reaction of rat was to jump back to the wooden platform. After about 4–5 trials, the animals acquired the passive avoidance response and they refrained from stepping down. The criterion was reached when the animal remained on the platform for at least 60 seconds [19].

2.3 OPEN FIELD TEST:
Exploratory behaviour was evaluated in an open discipline paradigm. The open arena was made of plywood and accommodates of 40 x 50 x 60 cm dimension. The entire apparatus become painted black and divided into 16 squares with white strains at the floor. Each animal was located on the nook of the equipment and for next 5 mins they have been discovered for ambulation which includes line crossings and head dipping [20].

2.4 MEASUREMENT OF LOCOMOTOR ACTIVITY:
The spontaneous locomotor activity of each rat was recorded individually for 10 min using actophotometer. The locomotor activity (horizontal activity) can be easily measured using an actophotometer which operates on photoelectric cells which are connected in circuit with a counter. When the beam of light falling on the photocell is cut off by the animal, a count is recorded [21].

2.5 ROTA-ROD TEST:
The effect of aluminium as well as grape seed oil treatment on muscle performance was evaluated using Rota-rod (Techno) test. All the rats were given two initial training trials of 300 s, approximately 10 min apart, to maintain posture on the Rota-rod (3 cm in diameter and rotating at a constant 20 rev/min). After the initial training trials, a baseline trial of 120 s was conducted. The time each animal remained on the Rota-rod was recorded. The animals that did not fall off the Rota-rod were given a maximum score of 120 seconds [21].

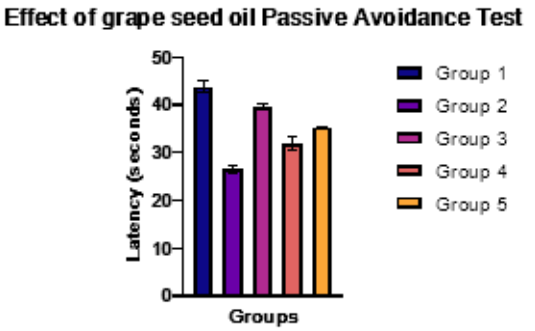
2.6 ASSESSMENT OF ANTIOXIDANT AND NEUROTRANSMITTER:
The animals were anaesthetized using chloroform and sacrificed. The whole brain was carefully removed from the skull. For preparation of the homogenate, the fresh whole brain was weighed and transferred to a glass homogenizer and a 10% (w/v) tissue homogenate was prepared in 0.1M phosphate buffer (pH 7.4, stored at −2 to–8°C). The homogenate was centrifuged at 3000 rpm for 10 min, and the resultant cloudy supernatant liquid was used for antioxidant and neurotransmitter assessments.

2.7 HISTOPATHOLOGICAL STUDIES
The animals were anaesthetized using chloroform and sacrificed. The whole brain was carefully removed from the skull. The brain sample from each group was selected and stored in 10% buffered formalin
solutions andfurther embedded in paraffin with wax. The blocks were processed for sectioning; the sections were then stained with haematoxylin and eosin as nuclear and cytoplasmic stains, respectively to assess the ameliorative effect of grape seed oil. Pathological changes, if any, were viewed under light microscope and recorded.

2.8 STATISTICAL ANALYSIS
The statistical analysis was carried out with the aid of one-way ANOVA followed through Dunnett's-t test. P values <0.05 (95%confidence restrict) was considered to be significant, by the use of software graph pad 9.

3. RESULTS

Figure 1:



PASSIVE AVOIDANCE TEST:

The Step-Down Latency (SDL) of Group II animals shows significant decrease ($p<0.0001$) when compared with Group I animals. On treatment with Grape seed oil (2ml), the Step-Down Latency (SDL) of Group IV animals shows significant decrease ($p<0.001$) when compared with Group III animals. On treatment with Grape seed oil (4ml), the Step-Down Latency (SDL) of Group V animals shows significant increase ($p<0.05$) when compared with Group III animals. Results are plotted in Figure 1.

Figure 2a:

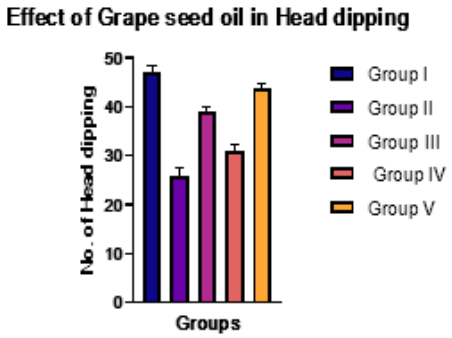
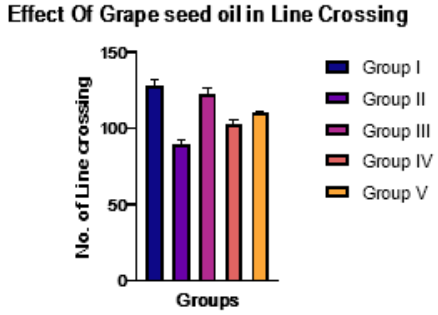


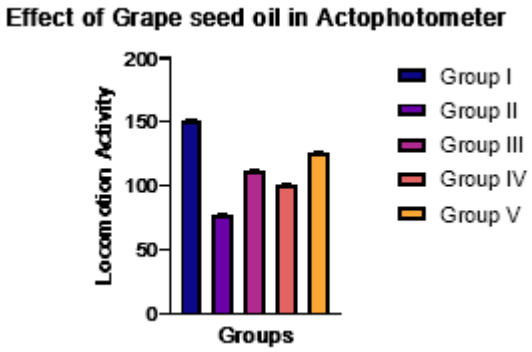
FIGURE 2b:



OPEN FIELD TEST:

The Group II animals shows significant decrease in head dipping and line crossing when compared with Group I animals ($p<0.0001$). The Group IV animals shows significant decrease in head dipping and line crossing beh
avior statistically when compared with Group III animals ($p<0.001$ and $p<0.01$). The Group V animals shows significant increase in head dipping and significant decrease in line crossing when compared with Group III animals ($p<0.05$ and $p<0.05$). Results are plotted in Figure 2a and 2b.

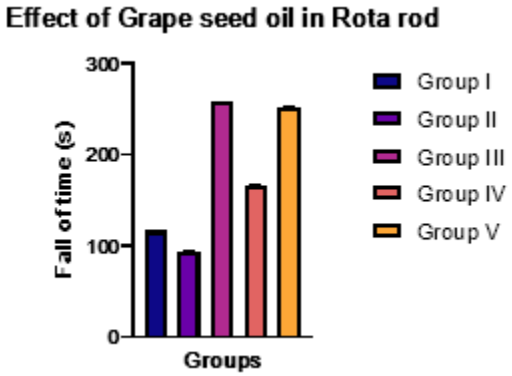
FIGURE 3:



LOCOMOTOR ACTIVITY:

The locomotion activity of Group II animals shows significant decrease ($p<0.0001$) when compared with Group I animals. On treatment with Grape seed oil (2ml), the locomotion activity of Group IV animals shows significant decrease ($p<0.001$) when compared with Group III animals. On treatment with Grape seed oil (4ml), the locomotion activity of Group V animals shows significant increase ($p<0.001$) when compared with Group III animals. Results are plotted in Figure 3.

FIGURE 4:

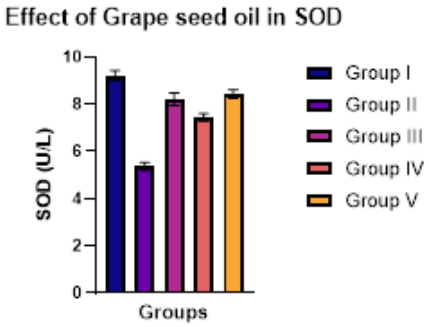


ROTA ROD TEST:

The fall of time of Group II animals shows significant decrease ($p<0.0001$) when compared with Group I animals. On treatment with Grape seed oil (2ml), the fall of time of Group IV animals shows significant decrease ($p<0.001$) when compared with Group III animals. On treatment with Grape seed oil (4ml), the fall of time of Group V animals shows significant increase ($p<0.001$) when compared with Group III animals. Results are plotted in Figure 4.

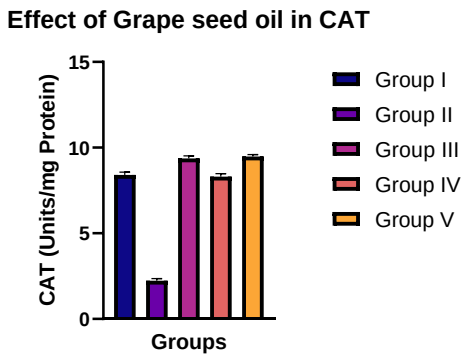
ASSESSMENT OF ANTIOXIDANT

FIGURE 5:



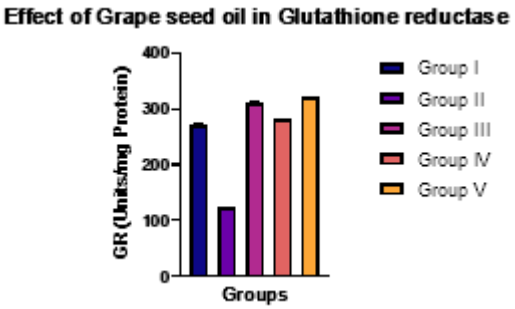
The SOD level of Group II animals shows significant ($p<0.0001$) decrease when compared with Group I animals. On treatment with Grape seed oil (2ml), the SOD level of group IV animals shows significant decrease ($p<0.001$) when compared with Group III animals. On treatment with Grape seed oil (4ml), the SOD level of Group V animals shows
significant ($p<0.05$) increase when compared with Group III animals. The results are plotted in Figure 5.

FIGURE 6:



The CAT level of Group II animals shows significant ($p<0.0001$) decrease when compared with Group I animals. On treatment with Grape seed oil (2ml), the CAT level of group IV animals shows significant decrease ($p<0.001$) when compared with Group III animals. On treatment with Grape seed oil (4ml), the CAT level of Group V animals shows significant ($p<0.05$) increase when compared with Group III animals. The results are plotted in Figure 6.

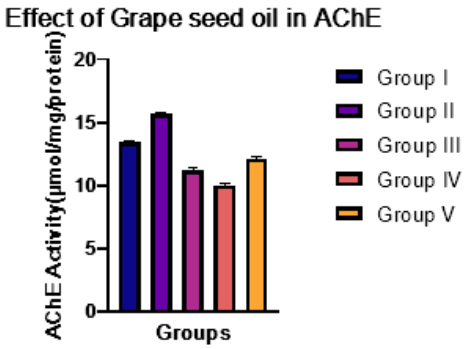
FIGURE 7:



The GRD level of Group II animals shows significant ($p<0.0001$) decrease when compared with Group I animals. On treatment with Grape seed oil (2ml), the GRD level of group IV animals shows significant decrease ($p<0.0001$) when compared with Group III animals. On treatment with Grape seed oil (4ml), the GRD level of Group V animals shows significant ($p<0.001$) increase when compared with Group III animals. The results are plotted in Figure 7.

ASSESSMENT OF NEUROTRANSMITTER

FIGURE 8:



The AChE activity of Group II animals shows significant ($p<0.0001$) increase when compared with Group I animals. On treatment with Grape seed oil (2ml), the AChE of group IV animals shows significant decrease ($p<0.001$) when compared with Group III animals. On treatment
with Grape seed oil (4ml), the AChE of Group V animals shows significant ($p<0.001$) decrease when compared with Group III animals. The results are plotted in Figure 8.

ASSESSMENT OF HISTOPATHOLOGY

On observation, it was found that there was decrease in density of neuronal cells and disruption in the normalarrangement of neuronal cells in hippocampal region of Group

II animals when compared to GroupI animals. Treatment groups (Group IV and V) revealed improved neuronal configuration thanGroupIII.GroupIV,Vshowssignificantincrea seinthedensityofneuronalcellsandhippocampal regions of brain when compared with neuronal loss in negative control group (GroupII). Histopathologicalpictures areshown inFIG 1to16.

FIG 9: BRAIN HISTOPATHOLOGY OF CONTROL GROUP

CORTEX HIPPOCAMPUS

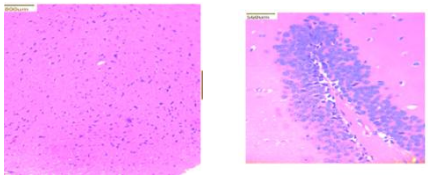


FIG 10: BRAIN HISTOPATHOLOGY OF NEGATIVE CONTROL GROUP

CORTEX HIPPOCAMPUS

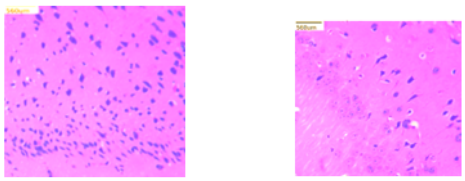


FIG 11: BRAIN HISTOPATHOLOGY OF STANDARD CONTROL GROUP

CORTEX HIPPOCAMPUS

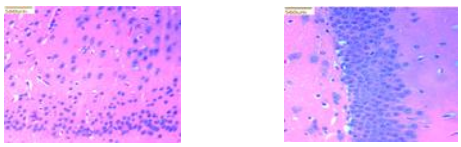


FIG 12: BRAIN HISTOPATHOLOGY OF GRAPE SEED OIL (2ml)

CORTEX HIPPOCAMPUS

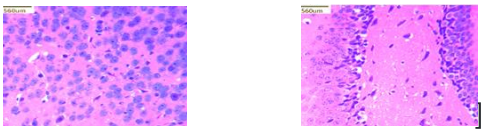
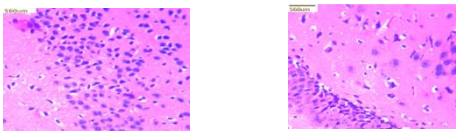


FIG 13: BRAIN HISTOPATHOLOGY OF GRAPE SEED OIL (4ml)

CORTEX HIPPOCAMPUS



DISCUSSION:

- Aluminium chloride causes a class of neurodegenerative disorder. The incidence of neurodegenerative disorder increases with age. Damage of short-termmemory is the characteristic feature. When thecondition proceeds, extra cognitive capabilitiesare impaired, likeability tocalculate, anduseobjectsandequipment.Acetylcholine esteraseinhibitorsarethebestmarketersauth orized by means of the Food and Drug Administration (FDA) for the treatment of AD.Alldifferent retailersprescribed fortheremedy of ADareused on anoff-labelfoundation.
- Asperprecedingstudies*grape seed oil* hassuperbantioxidantbelongings,conseque ntly it's far believed to have actions on CNS problems and neurodegenerationillnesses.
- Thecurrentstudyhasrevealedthe ameliorative effect of *grape seed oil* on Aluminium chloride induced neurotoxicity in rats. Aluminium chloride induced impairment of reminiscence became assessed by using the behavioural parameters like Passive avoidance task, open field test and locomotor activity using actophotometer, rota rod. Itbecamediscoveredthatremedywith*grape seed oil* shieldscognitivedeficitsin Aluminium chloride induced neurotoxicity.
- Spatial studying inside the open area habituation was used to access mastering and memory.The decline in response to regular surroundings after repeated exposures to the acquaintedsurroundingsiscitedtospatiallear ning.Recurrentpublicityproducesalowerinth eexploratorytasks,that'simplicativeofmemor

yreferringtoaselectedfeatureofthatsurroundings. Exploratory activities may be reduced on subsequent touch with open subject.In the end of the study the animals which are treated with *grape seed oil* indicated elevated spatialroutineand sleep deprivation decreased spatialroutinelearning.

- Passive avoidance behavior is based on poor reinforcement became used to observe thelevelof memory. An electro-shock is given during training period for about 15 sec inside the Step-Down Latency (SDL) which is then recorded. SDL was determined by the time taken via the rat to step down from the wooden platform to grid ground with its whole paw on the gridfloor. SDL is enhanced as an indication of long-term memory in which Aluminium chloride treated animalsshowedreducedSDL.Treatmentwith *grape seed oil* showeddevelopmentinlongtimereminiscences index ofelevationin SDL.
- Muscular and locomotion activities using Actophotometer, and Rota rod were found to be significantly decreased after Al treatment. Therefore, high levels of Al not only interfere with the memory but also affect the motor functions and leads to decreased motor activities and grip strength. On treatment with *grape seed oil*, animals enhanced the locomotor activity.
- Antioxidants SOD, CAT, GRD and neurotransmitter AChE activity was evaluated where there is a significant decrease in antioxidant level and increased AChE after Al treatment. On treatment with *Grape seed oil,* there was increase in antioxidant level and decrease in AChE activity.
- The histopathology studies of the brain shows that on treatment with *Grape seed oil,* there is a significantincreaseinthedensityofneuronalcellsandhippocampal regions of brain while compared with neuronal loss in Aluminium chloride treated group.

CONCLUSION:

The *Grape seed oil*possessedsignificant recovery in memoryand locomotor activity processesbut higher dose4ml showed better activitythan lowerdose2ml.

The current study reveals the ameliorative effect of *Grape seed oil* on Aluminium chloride induced neurotoxicity in rat. From the effects it can be concluded that*Grape seed oil* has extraordinaryimpact in behavioural & locomotor activity enhancement and oxidative stress.Further studies are required for the identification of molecular level activity and individualphytochemicalconstituent which could beresponsible forneuroprotective action.

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