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Sub-effective doses of a bendroflumethiazide-imipramine combination offer greater synergistic antidepressant effect compared to a bendroflumethiazide-fluoxetine combination: an isobolographic analysis

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Abstract

Background: Bendroflumethiazide is often prescribed with fluoxetine or imipramine for patients with both depression and hypertension. However, there is little data on the potential interactions between these drugs.

Objective: The objective of this study was to investigate the potential antidepressant effects of bendroflumethiazide, as well as sub-effective dose combinations of bendroflumethiazide with fluoxetine or imipramine.

Methods: Forced swimming and tail suspension tests were used to investigate the behavioural effects of bendroflumethiazide [5-20 mg/kg; *per os* (*p.o*], imipramine (3-30 mg/kg; *p.o*) and fluoxetine (3-30 mg/kg; *p.o*). Mean immobility, swimming, climbing, curling, and swinging scores were measured. Median effective dose (ED_{50}) values were calculated from the immobility scores. The antidepressant effect of the combination of bendroflumethiazide with imipramine or fluoxetine at sub-effective doses was then investigated. Isobolographic analyses were performed on these combinations to investigate possible synergism, additivity or antagonism.

Results: Bendroflumethiazide produced a significant diminution in mean immobility scores, suggestive of an antidepressant-like effect, while increasing swimming, climbing and swinging scores. Imipramine and fluoxetine also exhibited antidepressant-like effects. A combination of bendroflumethiazide and imipramine at sub-effective doses showed a synergistic antidepressant-like effect with an interaction index of 0.31 as did the bendroflumethiazide-fluoxetine combination (interaction index: 0.41).

Conclusion: This study demonstrated the acute antidepressant-like effect of bendroflumethiazide. Moreover, bendroflumethiazide-imipramine combinations offer greater synergy when compared to bendroflumethiazide-fluoxetine combinations.

Keywords: Bendroflumethiazide, fluoxetine, imipramine, isobolographic, depression, hypertension

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INTRODUCTION

Neuropsychiatric disorders and cardiovascular diseases constitute a significant proportion of the global disease burden. Depression is a global health issue affecting an estimated 280 million people worldwide, which accounts for 3.8% of the global population. Among adults, the prevalence of depression is 5.0%, and among adults older than 60 years, it is 5.7% [1]. Hypertension is a multifactorial cardiovascular disease affecting up to 40% of the world`s population [2]. In 2015, there were over 1 billion adults living with hypertension with the majority of cases originating from Low- and Middle-Income Countries

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[3,4]. Depression is a common comorbidity in hypertensive patients, and its presence can significantly impact clinical outcomes. In 2015, Li et al. estimated that the summarized prevalence of depression among hypertensive patients was 26.8% [5], which is higher than seen in healthy persons in the community [6-9]. Despite the heterogeneity of the pathophysiology of depression and hypertension, research suggests that these conditions share some common pathophysiological pathways [10-12]. Thus, certain drugs used to manage hypertension, such as diuretics, which have demonstrated effects on shared targets in the pathophysiological pathways of depression and hypertension, may have added benefits in the treatment of depression, particularly when used in combination with other antidepressants [13]. Depression is thought to be caused by a dysfunction in the monoamine pathway, where deficiencies in serotonin, dopamine, and noradrenaline in the central nervous system play a major role. In support of this, all clinical antidepressant drugs augment the effects of one or more of these monoamine neurotransmitters [14-15].

Similar to patients with depression, individuals with hypertension display elevated sympathetic tone and increased secretion of adrenocorticotropic hormone and cortisol [16-17]. Moreover, dopamine and related neurotransmitters which are implicated in depression, as well as dopamine receptor agonists such as bromocriptine show antihypertensive actions [18-19]. Dopamine deficiency in the brain has been shown to increase blood pressure and/or trigger depression [20]. The role of carbonic anhydrase in depression is well-known [21]. The findings on the relationship between brain carbonic anhydrase I level and depression have been inconsistent. While some studies have observed higher levels of the enzyme in individuals with depression, others have yielded inconclusive results. However, in cases of bipolar disorder, inhibiting the enzyme with acetazolamide, a carbonic anhydrase inhibitor, has shown promise in significantly improving depressive symptoms [21]. Furthermore, hypertension-induced cerebrovascular and ischemic changes in the brain predispose these individuals to depression [22]. Thiazide diuretics block electroneutral sodium hydrochloride (NaCl) reabsorption at the distal convoluted tubule, connecting tubule, and early collecting duct, evoking a NaCl diuresis [23-24]. Importantly, they also exhibit a carbonic anhydrase inhibitory effect in both clinical and preclinical studies [23].

When patients are diagnosed with both depression and hypertension, a significant concern is the potential for drug-drug interactions between medications used to manage each condition, which can negatively affect treatment effectiveness [25-27]. Though some of these interactions may be beneficial or deleterious, there are not many studies investigating these interactions. This study evaluates the synergistic antidepressant potential between antihypertensives and antidepressants in combination therapy. Previous studies indicate that hydrochlorothiazide, a thiazide diuretic, inhibits carbonic anhydrase, an enzyme that has been linked with depression pathophysiology [13]. Furthermore, thiazide diuretics are frequently prescribed to manage hypertension, but they can interact with antidepressants and cause potential complications [28]. Thus, we put forward a hypothesis that bendroflumethiazide, a thiazide diuretic known to affect the noradrenergic system and potentially modulate mood, could affect the treatment outcomes of depression [27, 29]. This study is an attempt to test this hypothesis. In our current study, we examined the potential antidepressant-like effects of bendroflumethiazide, both alone and in combination with fluoxetine and imipramine, using two well-established and validated models of depression in mice: the forced swimming and tail suspension test.

MATERIALS AND METHODS

Drugs and chemicals

Imipramine hydrochloride (IMI) was obtained from Mallinckrodt Pharmaceuticals (Ireland), fluoxetine (FLX) from Eli Lilly and Company (England) and bendroflumethiazide (BFT) was from Teva Pharmaceutical Industries (Israel). Imipramine and fluoxetine were dissolved in physiological saline (0.9% NaCl) while bendroflumethiazide was suspended using 20% Tween-80 (in normal saline) because it does not easily dissolve in normal saline. Normal saline was used as a control and not more than 10 mL/kg was administered. All solutions were freshly prepared on the day of the experiment. Tween-80 is a commonly used vehicle in pharmacological and physiological studies and 20% of Tween-80 has been shown to have no significant effect on behaviour [30]. Doses of IMI and FLX used were selected from previous work doses in our lab [31] and that of bendroflumethiazide was selected from the literature [32,33].

Animals

Treatment and behavioural naïve Institute of Cancer Research (ICR) male mice, aged 6 to 8 weeks (25 to 30 g), were obtained from Noguchi Memorial Institute for Medical Research, University of Ghana. The animals were brought to the neuropsychopharmacology research laboratory seven days before the experiment to allow them to acclimatize to the laboratory environment. The animals were kept in stainless steel cages (47 cm × 34 cm × 18 cm) at a controlled room of temperature 20 ± 1 °C with a 12-hour light and dark cycle, and access to food and water *ad libitum*.

Tail Suspension Test (TST)

The tail suspension test was conducted following the method described by Steru et al. [34] with slight modifications. In our version of the test, an aluminium suspension bar measuring 1 cm in height, 1 cm in width, and 60 cm in length was utilized to suspend the tail of each mouse. An adhesive tape was used to secure the tail to the bar, with the tape placed 2 cm away from the tail tip. Additionally, we distinguished different modes of action which were not done in the traditional TST [35]. Groups of mice (n = 7) were treated with BFT (5, 10, 20 mg/kg *p.o.*), FLX (3, 10, or 30 mg/kg, *p.o.*), IMI (3, 10, or 30 mg/kg,

p.o.) or normal saline (as a control vehicle). One hour following oral administration of the test drugs, the mice were individually suspended by their tails from a horizontal bar that was positioned 20 cm above the floor. An adhesive tape was used to secure the tail to the bar, with the tape being placed at a distance of 2 cm away from the tail tip. Immobility (the absence of all movements except for those required for respiration), curling (active twisting movements), and swinging (vertical movement of the paws and/or side-to-side movement of the body) behaviours were recorded for 6 minutes. Predominant behaviour in every 5 seconds of the last 5 minutes was scored and the means were computed. This is due to the finding that mice tend to manifest immobility earlier in the TST [36]. Mice that climbed up on their tails during the test session were gently pulled down and testing continued, but those that continued to climb up on their tails were excluded from the study. The dose-response percentage (dose%) decline in immobility score curves was generated and the median effective dose concentration that is responsible for 50% of the maximal effect of the drugs (ED_{50}) was determined as described in the statistical analysis section. The saline-treated control group was considered as 0 mg/kg of test compounds.

Forced swimming test (FST)

The FST was based on methods described by Porsolt et al. [37]. Mice were randomly assigned to 10 groups of seven animals each. Normal saline (used as a control), BFT (5, 10, 20 mg/kg *p.o.*), FLX (3, 10, or 30 mg/kg, *p.o.*), and IMI (3, 10, or 30 mg/kg, *p.o.*) were administered to their respective groups. One hour following oral administration of the test drugs, the mice were gently placed individually into transparent cylindrical polyethene tanks. Each tank had a height of 25 cm and an internal diameter of 10 cm. The tanks were filled with water at a temperature of 25 to 28°C up to a level of 20 cm, and the mice were left in the water for a period of 5 minutes. Each session was recorded by a video camera suspended approximately 100 cm above the cylinders. An observer scored immobility (when the mouse floated upright in the water and made only small movements to keep its head above water), swimming (active horizontal movements across water), and climbing (active vertical movement by the walls of the cylinder) behaviours during the 5 minutes test. The dose% decline in immobility score curves was generated and ED_{50} of the drugs was determined as described in the statistical analysis section.

Isobolographic Analysis

In a separate experiment, mice were treated with various ED_{50} combinations (n = 7) of BFT/IMI and BFT/FLX in proportions as follows: $ED_{50}/4$ (referred to as Zmix/4), $ED_{50}/2$ (referred to as Zmix/2) and ED_{50} (referred to as Zmix). The ED_{50} values were obtained from the forced swimming test for isobolographic analysis.

Statistical analysis

GraphPad Prism for Windows, version 8.02 (GraphPad Software, USA) was used for statistical analyses. Statistically, significance was considered at $p < 0.05$. In all the tests, a sample size (n = 7) was used. Differences in means were analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keuls' post hoc test. The ED_{50} of BFT, IMI, FLX, and their combinations were determined using a repetitive computer least-squares method with a four-parameter logistic equation for nonlinear regression: $Y= [a + (a – b)] / [1 + 10^{(\log ED_{50} - X) \times (\text{Hill slope})}]$, where X is the logarithm of concentration. Y is the response, starting at 'a' and ending at point 'b'. The fitted midpoints (ED_{50}s) of the curves were compared statistically using the F test.

Isobolographic calculations and graphs were plotted with the program Sigma plot, version 11.0 (Systat Software, Germany). Isobologram (a Cartesian plot of pairs of doses that, in combination, yield a specified level of effect) was then built by connecting the theoretical ED_{50} of fluoxetine or imipramine plotted on the abscissa with that of bendroflumethiazide plotted on the ordinate to obtain the additivity line. For each drug mixture, the experimental ED_{50} and its associated 95% confidence intervals were determined by linear regression analysis of the log dose-response curve and compared by a t-test to a theoretical additive ED_{50} obtained from equation 1 [$Z_{add} = f(ED_{50})$ FLX + $(1 – f) ED_{50}$ BFT] or equation 2 [$Z_{add} = f(ED_{50})$ IMI + $(1 – f) ED_{50}$ BFT], where f is the fraction of each component in the mixture. The variance (Var) of Z_{add} was calculated as equation 3 [$VarZ_{add} = f^2 Var(ED_{50})$ FLX/IMI + $(1\text{-}f)^2$ Var ED_{50} BFT]. From these variances, the standard error means (SEM) were calculated and resolved according to the ratio of the individual drugs in the combination. A synergistic effect is defined as the effect of a drug combination that is higher and statistically different (ED_{50} significantly lower) than the theoretically calculated equivalent effect of a drug combination in the same proportion. If the ED_{50}s are not statistically different, the effect of the combination is additive, meaning each constituent contributes its potency to the total effect. The degree of interaction was also calculated using fractional analysis by dividing the experimental ED_{50} (Z_{mix}) by the theoretical ED_{50} (Z_{add}). A value close to 1 is considered additive. Values < 1 are an indication of synergistic interactions (Zmix/Zadd < 1), and values higher >1 correspond to sub-additive or antagonistic interactions [38].

RESULTS

Effect of BFT treatment in TST

Low doses of BFT (5 and 10 mg/kg) caused a significant ($p < 0.05$) diminution of the mean immobility score in the tail suspension test. One-way analysis of variance with Newman-Keul's *post hoc* test showed significant variation in the means. Similarly, IMI (at 3mg/kg, 10 mg/kg, and 30 mg/kg) and FLX (at 3mg/kg, 10 mg/kg, and 30 mg/kg) each produced a significant ($p < 0.0001$) dose-dependent reduction of immobility score (Figure 1A). The BFT (at 5 mg/kg, 10 mg/kg, and 20 mg/kg) treatments each produced an increase ($p < 0.0001$) in the swinging score (Figure 1B) while BFT at 20 mg/kg produced a reduction ($p < 0.001$) in

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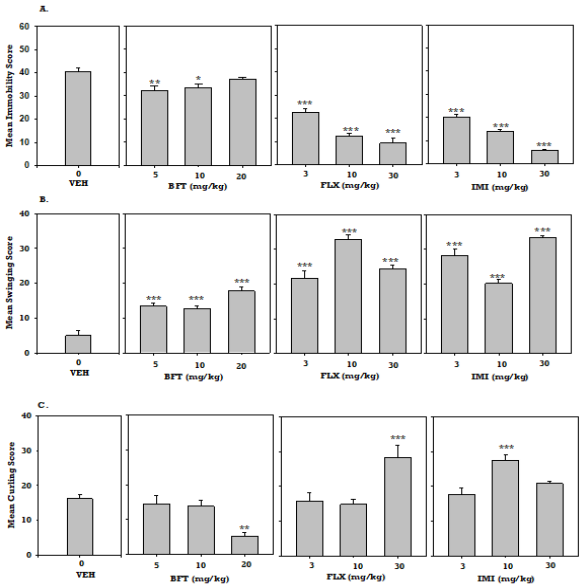

Figure 1: Effect of oral administration of bendroflumethiazide (BFT; 5-20 mg/kg), fluoxetine, (FLX; 3-30 mg/kg) and imipramine, (IMI; 3-30 mg/kg) treatment on: (A) mean immobility, (B) swinging and (C) curling scores in the tail suspension test in ICR mice. The analysis was done by one-way ANOVA followed by a Newman Keul's *post hoc* test. ***P<0.001, **P<0.05; *P<0.01; compared with the vehicle-treated (VEH) group. Data are presented as group means± SEM, (n=7).

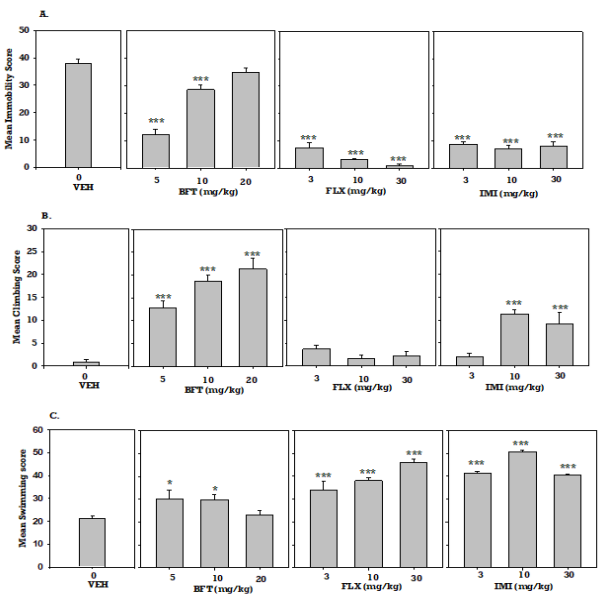

Figure 2: Effect of oral administration of bendroflumethiazide (BFT; 5-20 mg/kg), fluoxetine, (FLX; 3-30 mg/kg) and imipramine, (IMI; 3-30 mg/kg) treatment on: (A) mean immobility, (B) swimming and (C) climbing scores in the forced swimming test in ICR mice. The analysis was done by one-way ANOVA followed by a Newman Keul's *post hoc* test. ***P<0.001, **P<0.05; *P<0.01; compared with the vehicle-treated (VEH) group. Data are presented as group means± SEM (n=7).

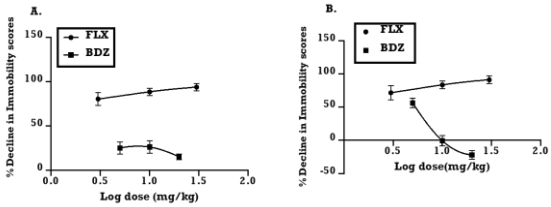

Figure 3: Log dose of bendroflumethiazide and fluoxetine against % decline in immobility score. (A) TST (B) FST

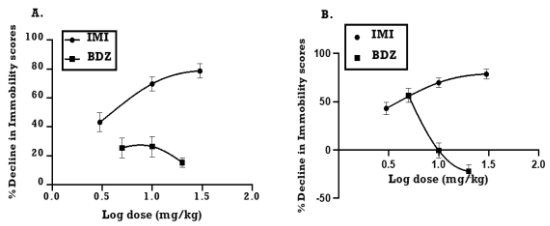

Figure 4: Log dose of bendroflumethiazide and imipramine against % decline in immobility score. (A) TST (B) FST

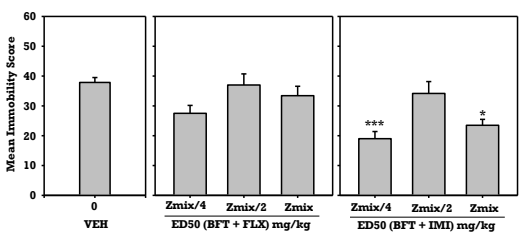

Figure 5: Effect of combination of sub-effective doses of bendroflumethiazide/fluoxetine, and bendroflumethiazide/imipramine treatment on mean immobility scores in the forced swimming test in ICR mice. The analysis was done by one-way ANOVA followed by a Newman Keul's *post hoc* test. ***P<0.001, **P<0.05; *P<0.01; compared with the vehicle treated (VEH) group. Data are presented as group means± SEM (n=7)

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Table 1: ED_{50} values of mean immobility scores for bendroflumethiazide, imipramine and fluoxetine in TST and FST

Drug	ED_{50} (mg/kg) ± SEM	
	TST	FST
Bendroflumethiazide	12.23 ± 1.39	9.21 ± 1.81
Imipramine	3.22 ± 0.71	1.91 ± 1.19
Fluoxetine	1.94 ± 1.00	3.84 ± 0.99

*SEM, standard error mean; TST, tail suspension test; FST, forced swimming tests

Table 2: Interaction indices for bendroflumethiazide-imipramine and bendroflumethiazide-fluoxetine combinations in the forced swimming test

Drug	Bendroflumethiazide-Fluoxetine	Bendroflumethiazide-Imipramine
Z_{add}	7.65 ± 1.27	5.92 ± 0.89
Z_{mix}	3.12 ± 1.08	1.82 ± 0.41
Interaction index (Z_{mix}/Z_{add})	0.41	0.31

* Zmix is the experimental ED_{50} from the drug combinations while Zadd is the theoretical ED_{50}

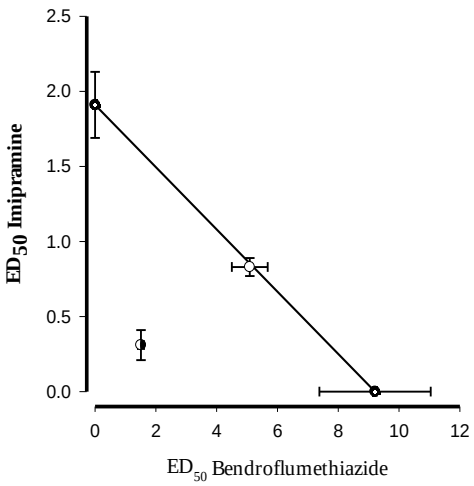

Figure 6: Isobologram for the combination of bendroflumethiazide and imipramine in the forced swimming test. Open clear circle represents the theoretical ED_{50} ± S.E.M and partially filled circle for the experimental ED_{50} ± S.E.M (n=7)

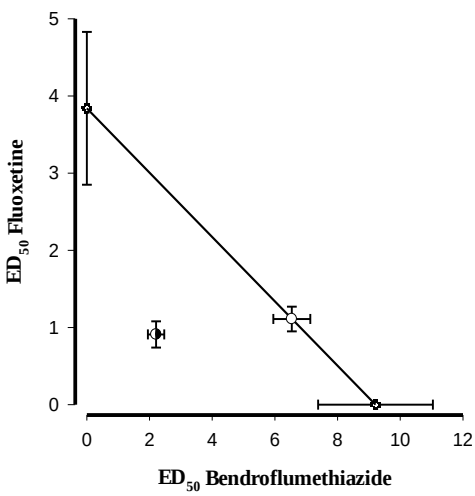

Figure 7: Isobologram for the combination of bendroflumethiazide and fluoxetine in the forced swimming test. Open clear circle represents the theoretical ED_{50} ± S.E.M and partially filled circle for the experimental ED_{50} ± S.E.M (n=7)

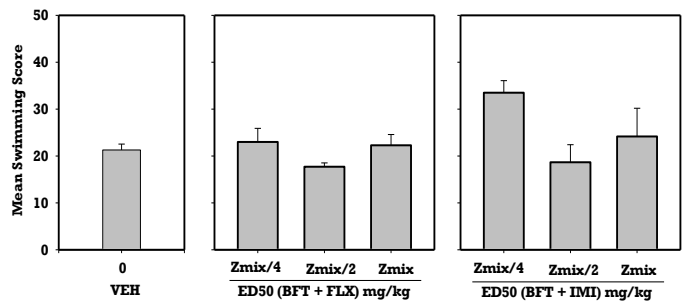

Figure 8: Effect of combination of sub-effective doses of bendroflumethiazide/fluoxetine (BF), and bendroflumethiazide/imipramine (BI) treatment on mean swimming scores in the forced swimming test in ICR mice. The analysis was done by one-way ANOVA followed by a Newman Keul's *post hoc* test. Data are presented as group means± SEM

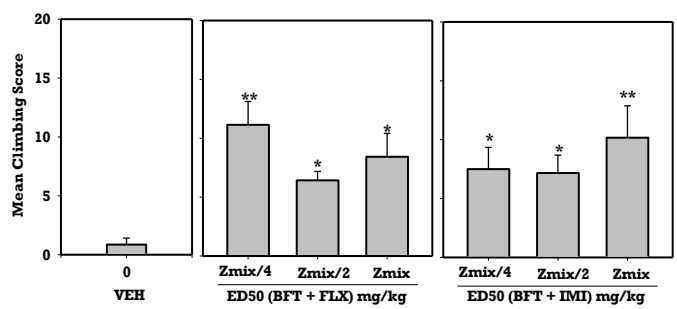

Figure 9: Effect of combination of sub-effective doses of bendroflumethiazide/fluoxetine (BF), and bendroflumethiazide/imipramine (BI) treatment on mean climbing scores in the forced swimming test in ICR mice. The analysis was done by one-way ANOVA followed by a Newman Keul's *post hoc* test. **$p<0.05$; *$p<0.01$; compared with the vehicle treated (VEH) group. Data are presented as group means± SEM (n=7).

the curling score (Figure 1C). In contrast, FLX (at 3 mg/kg, 10 mg/kg, and 30 mg/kg) and IMI (at 3 mg/kg, 10 mg/kg, and 30 mg/kg) each produced increases ($p < 0.0001$) in both swinging score and curling score (Figure 1B & 1C). The potencies of these drugs are shown in Table 1.

Effect of BFT in the FST

The BFT treatment (at both 5 and 10 mg/kg) significantly ($p < 0.0001$) decreased immobility score (Figure 2A). Additionally, BFT increased swimming scores at 5, 10, and 20 mg/kg (Figure 2B), as well as climbing scores at 5, 10, and 20 mg/kg (Figure 2C). Similar effects were observed with IMI treatments at 3, 10, and 30 mg/kg ($p < 0.0001$) (Figure 2A-C). One-way analysis of variance with the Newman-Keuls post hoc test showed significant variation within the means. Fluoxetine, on the other hand, decreased the immobility score while increasing the swimming score without affecting the climbing score (Figure 2A-C). The potencies of these drugs are shown in Table 1.

Dose-response relationships

Figure 3 shows a log dose-response relationship between BFT and FLX doses and the percentage decline in immobility scores in TST and FST, used to determine their respective ED_{50} values. The log dose-response relationship of BFT and IMI doses with percentage decline in immobility score in TST and FST for the determination of their respective ED_{50} values are shown in Figure 4. Figure 5 shows the sub-effective doses of BFT/FLX and BFT/IMI combinations on the behaviour of mice in the FST. The sub-effective doses of the BFT/FLX combination produced slightly significant diminutions in immobility behaviour in mice ($p = 0.025$). In contrast, BFT/IMI combination in the forced swimming test resulted in a significant reduction in the mean immobility score ($p = 0.0002$).

Isobolographic analysis

From the isoboles plotted, the experimental ED_{50} (Z_{mix}) of BFT/IMI combinations was lower than the theoretical ED_{50} (Z_{add}). Thus, the $Z_{mix(BFT/IMI)}$ plot was below the line of additivity (Figure 6). The interaction index was 0.31, suggestive of a synergistic antidepressant effect (Table 2). Similarly, the isoboles of BFT/FLX combinations showed a lower experimental ED_{50} (Z_{mix}) compared to those of the theoretical ED_{50} (Z_{add}). Thus, the plot of the $Z_{mix(BFT/IMI)}$ was below the line of additivity (Figure 7). The calculated interaction index of 0.41 (Table 2) indicates a synergistic antidepressant effect because it is significantly < 1.

Contribution of serotoninergic and noradrenergic systems to the antidepressant effect

Figure 8 shows the possible contribution of serotoninergic systems to the antidepressant effect of sub-effective dose combinations of BFT/FLX or IMI. The mean swimming score, which is highly predictive of the enhancement of central serotoninergic activity was not significantly increased by combinations of BFT/FLX or BFT/IMI ($p > 0.05$). Figure 9 shows the possible contribution of central noradrenergic systems to the antidepressant effect of sub-effective dose combinations of BFT/FLX and FLX/IMI. The mean climbing score, a highly predictive parameter for drugs that enhance central noradrenergic activity, was significantly increased by BFT/FLX ($p = 0.0037$) and BFT/IMI ($p < 0.05$) combinations.

DISCUSSION

This present study reports that acute administration of low-dose BFT possesses antidepressant-like effects in FST and TST using murine models. Moreover, sub-effective dose combinations of BFT/FLX and BFT/IMI showed a synergistic antidepressant effect. As science continues to make great strides in discovery, the pharmacological scope of many drugs keeps widening. Thus, some drugs exhibit pharmacological and therapeutic effects that are different from their traditional indications. The discovery that tramadol, an opioid analgesic has an antidepressant effect and ketamine, a general anaesthetic, possesses rapid-onset antidepressant effects are just a few of the instances. These discoveries have the potential to change the way certain drugs are used clinically and also alter our knowledge of drug-drug interactions. Our investigation revealed that BFT, a thiazide diuretic used in managing hypertension, reduced the mean immobility score (indicative of antidepressant-like activity) in both FST and TST. The FST and TST are widely used tests for identifying compounds with antidepressant-like effects. The tests exhibit high predictive validity and are reliable [39]. Thus, the observation of antidepressant-like activity in murine models by BFT is unlikely to be a false positive. This is because changes in monoaminergic pathways are known to underlie the antidepressant activity of most current antidepressants, and the study aimed to investigate whether these pathways also contribute to the activity of BFT [14,15,40].

In the murine FST and TST, changes in swimming, climbing, and swinging scores are suggestive of monoaminergic activity. The elevated swinging score observed in the murine TST is considered an indicator of increased monoaminergic activity, and this finding is supported by numerous previous studies [32, 41, 42]. Since the swinging results from the TST did not show exactly which monoamines are elevated, the results from the FST provide substantial support in this regard. This is because drugs that activate serotoninergic neurotransmission increase the swimming score without affecting the climbing score, while drugs that increase the climbing score without any effect on swimming are known to increase the noradrenergic neurotransmission score [43-44]. Considering that BFT increased swinging and swimming scores, it is plausible that the enhancement of serotoninergic activity underlies the behavioural activity observed. Moreover, increased curling scores in the TST connote increased opioidergic activity as a possible mechanism of antidepressant action [35]. The BFT did not increase the curling score, suggesting that opioidergic activity may not contribute to its antidepressant activity. Scientific evidence indicates that thiazide diureticns inhibit

carbonic anhydrase, an enzyme implicated in the pathophysiology of depression. Since reports on carbonic anhydrase and its role in depression are contrasting, further studies need to be conducted to ascertain its role in contributing to the observed behavioural effects [21,45]. It is worth noting that the inhibition of sodium, potassium, and chlorine ion (Na^+K^+-$2Cl^-$) transporters have been shown to prevent various conditions, including addictive and compulsive disorders, alcoholism, drug addiction, and smoking addiction. It can also help with neuropathic pain, bipolar disorders, anxiety, panic attacks, depression, schizophrenia, post-traumatic stress syndrome, and epilepsy [46]. Perhaps the contribution of inhibition Na^+K^+-$2Cl^-$ cotransporters in the antidepressant effect of BFT would be worth investigating given that BFT inhibits these transporters [47]. An isobolographic analysis was conducted to investigate the pharmacodynamic interaction that would occur when sub-effective doses of BFT are combined with equipotent sub-effective doses of FLX or IMI. The analysis suggests that the combinations confer a synergistic antidepressant effect in the mice, though the synergy between BFT/IMI combination was greater. Again, we sought to provide mechanistic explanations for the behavioural effects of BFT/FLX or BFT/IMI combinations. To achieve this, we examined two specific active behaviours in the FST. Though these drug combinations failed to increase swimming scores, mean climbing scores were significantly increased. These results seem to suggest that the enhancement of serotoninergic neurotransmission may not contribute to the acute antidepressant effect of the BFT/IMI combination. In contrast, BFT/IMI combinations increased the climbing score, indicating a potential enhancement of noradrenergic neurotransmission. Interestingly FLX, which acutely increases synaptic serotonin instead of noradrenaline, increased the mean climbing score which is suggestive of a noradrenergic-dependent behaviour.

Tricyclic antidepressants like IMI act by preventing the reuptake of both serotonin and noradrenaline, leading to increases in synaptic levels of these neurotransmitters. The values from the interaction index seem to suggest that combining bendroflumethiazide with a drug that enhances noradrenergic activity offers better antidepressant activity than those that enhance serotoninergic activity only. These results certainly raise interesting questions that must be further investigated. We did not assess the potential neurotoxic effects of compounds in experimental mice used in the study. The study did not investigate how the pharmacokinetic drug-drug interactions between BFT/IMI or BFT/FLX may have contributed to the observed synergistic antidepressant effects of these combinations.

Conclusion

Our study has demonstrated that low doses of BFT have an antidepressant-like effect and that concomitant administration of sub-effective doses of BFT and IMI show a greater synergistic antidepressant-like effect, compared to sub-effective does of BFT and FLX, in mice.

DECLARATIONS

Ethical considerations

We complied with ethical standards. All animals used in these studies were treated according to the Guide for the Care and Use of Laboratory Animals (NRC, 1996).

Consent to publish

All authors agreed to the content of the final paper.

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Competing Interests

No potential conflict of interest was reported by the authors.

Author contributions

JAM, KKEK conceived the study. KKEK, JAM, AEK, SA-A, PA, TAT, BKNB, EBD, EOA, SKA developed the methods for investigation. SKA, EOA, JAM, PA, AEK, SA-A, EBD were involved in data collection and analysis. All authors were involved in developing the manuscript for submission.

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Availability of data

Data for this work is available upon reasonable request from the corresponding author.

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