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Pharmacology

Original Article

Antidepressant Potential of Diphenyl Dimethyl Bicarboxylate (DDB) in a Modified Forced Swimming Test

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ABSTRACT

Biphenyl Dimethyl Dicarboxylate (DDB); is an analogue of schisandrin C, a component isolated from Fructus Schisandrae Chinensis. It is now attracting growing attention for its polypharmacotherapeutic properties as an antiviral, hepatoprotective, and immunomodulator. It's been additionally possessed neurobehavioral impacts on experimental animals. Thus, the wide use of DDB, along with its neurobehavioral impacts; has encouraged us to study its antidepressant potential using the forced swimming test (FST). Furthermore, the protocol for FST was modified by combining it with the tail suspension test. The effect of DDB (100 mg/kg/day, p.o., 7 days) on combined technique was evaluated against fluoxetine (15 mg/kg, i.p., single dose). In the end, the brains were dissected; serotonin (5-HT), dopamine (DA), and norepinephrine (NE) levels were measured. The results demonstrated that DDB decreased immobility in FST time as fluoxetine. Furthermore, DDB elevated 5HT, NE, and DA. DDB may be useful as a supportive antidepressant drug in hepatic diseased patients. However, more clinical studies are needed to ensure its safety.

Keywords: Depression; fluoxetine; DDB; forced swimming test; tail suspension test.

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Citation | Mahfoz AM, 2021. Antidepressant potential of Diphenyl Dimethyl Bicarboxylate (DDB) in a modified forced swimming test. Arch Pharm Sci ASU 5(1): 63-70

DOI: [10.21608/aps.2021.76190.1060](https://doi.org/10.21608/aps.2021.76190.1060)

Print ISSN: 2356-8380. Online ISSN: 2356-8399.

Received 23 May 2021. Accepted 31 May 2021.

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Published by: Ain Shams University, Faculty of Pharmacy

1. INTRODUCTION

Depression is the most widespread neuropsychiatric disease. It is characterized by a persistent low mood that passes a heavy burden to patients' families and society. While there are many effective antidepressant drugs, many of them cause patient intolerance and severe adverse effects as sexual dysfunction, cardiotoxicity, and sleep disorder. The effort is invested to develop a novel antidepressant with fewer side effects and better efficacy. Fluoxetine; a popular selective serotonin reuptake inhibitor (SSRI), the most

prescribed class of antidepressants. Despite the extensive use of SSRIs; many depressed patients do not remit after initial mono-therapy [1-3]. Traditional Chinese Medicines have been reported to be more safe and effective in many circumstances [4]. Dimethyl-4,4'-dimethoxy-5,6,5',6'-dimethylene-dioxy-biphenyl-2,2'-dicarboxylate, usually abbreviated as DDB is an analogue of schisandrin C, a component isolated from Fructus Schisandrae Chinensis; a Chinese folk medicine traditionally used for its tonic, sedative, antitussive, antioxidants, and anticancer properties [5]. It is usually prescribed

as hepatoprotective in patients suffering from either viral or drug-induced hepatitis [6,7]. Recently, DDB was shown to be a behaviorally active agent, it was reported to be linked to significant effects on the behaviour of depressed partners in the sensory contact model, plus an immunomodulatory potential [8].

It is hard to develop an experimental model that accurately mimics the symptoms of depression in patients. The forced swim test (FST) was first developed by Porsolt *et al.* (1977) [9] for the rat and mouse [10]. FST is the easiest, reproducible, and commonly used test for preclinical experimental assessment of antidepressants [11, 12]. The original protocol of FST is dependent on the idea that rodents after the first escape movements have developed immobility if placed in a non-escapable cylinder of water (pretest). Then if they replaced it 24 hours later; they resumed this posture rapidly (test). The immobile position reflects the development of passive behavior (depression) that prevents the animal activity to handle stress [3]. Upon administration of an antidepressant agent in-between the 2 exposures, animals will swim (escape-directed behavior) for a longer time than after treatment by vehicle [13].

Tail suspension test (TST) is also a behavioral technique that allows fast evaluation of psychoactive drugs. It is a sensitive method, rapid (maximum 6 min), and is painless for the animal. Mice were suspended by the end of the tail; the presence of immobility (absence of limb movements) was measured over a 6-min session [14].

The present work aimed to study the antidepressant potential of DDB against fluoxetine using the FST. Furthermore, the protocol for FST was modified by combining it

with TST.

2. MATERIALS AND METHODS

2.1. Drugs and dosage

Fluoxetine (15 mg/kg) was obtained as a white powder from Sigma Company. It was prepared as a fresh solution in deionized water just before use. It was injected as a single i.p. dose, 1 h before behavioral tests.

DDB (100 mg/kg, p.o, 7 days) was obtained as a white crystalline powder from The Arab Company of Pharmaceutical and Medicinal Plants (MEPACO). It was suspended in distilled water using 1% Tween 80 as an emulsifying agent. Dosage preparation was performed just before administration.

The utilized dose level in the present work was in the range of the doses previously reported to result in behavioral changes or to produce immunomodulatory effects [8].

2.2. Animals

Adult male Swiss mice (25±5 g) were obtained from the animal house of the National Organization of Drug Control and Research (NODCAR). Animals were kept under standard laboratory animal housing conditions with free access to water & a conventional diet. The protocol of the current study is in line with the international guidelines for use of laboratory animals; published by the US NIH. It is also approved by the research ethics committee for experimental and clinical studies at the Faculty of Pharmacy, Modern University for Technology and Information (permit number: ES 884).

2.3. Experimental design

Animals were divided into 5 groups, each group consists of 7 animals.

Group 1: Animals were subjected to TST.

Group 2: Animals were subjected to FST.

Group 3: Animals were subjected to combination technique (TST+FST).

Group 4: Animals were treated with a single dose of fluoxetine (15 mg/kg, i.p.) and after 1 h they were subjected to the combination technique (TST+FST).

Group 5: Animals were treated with DDB (100 mg/kg, p.o.) daily for 7 consecutive days, and on the 8th day they were subjected to the combination technique (TST+FST).

DDB administration took place at 2-3 p.m., The testing sessions took place at 10-12 a.m.

2.4. Tests

2.4.1. The following behavioral tests were performed

2.4.1.1. The Tail Suspension Test (TST)

Mice were suspended by the tail with adhesive tape (40 cm above the table) for 6 minutes. The session was videotaped, and the duration of immobility in seconds was recorded. Mice were considered immobile only when hung passively and completely motionless [15].

2.4.1.2. The Forced Swimming Test (FST)

Each mouse was placed separately in a cylinder (12 x 24 cm) full with water to 12 cm height at 22-23 °C. The session was recorded and the period of immobility; swimming; and straggling through the latest 3 min were assessed. Immobility was demonstrated by stopping straggling and moving only to continue floating on the water surface. After the 6 min of the test, the animal was removed and allows drying [16]. The FST technique is widely used to study depression behavior in mice, rats, dogs [17], and also in kids disconnected from their parents [18].

2.4.1.3. Combination of TST&FST

For the sake of increasing the depressant potential of the FST, the effect of the

combination of two techniques of depression (TST&FST) was studied on the depressive state, and compared the influence of fluoxetine and DDB using this combination technique.

The animals were allowed to be suspended individually by their tail for 6 minutes (as the procedure mentioned above), then introduced to the FST apparatus for 6 min. The FST session was recorded and the period of immobility; swimming; and straggling through the last 3 min of the FST were measured.

2.4.2. Assay of brain neurotransmitters

Brain catecholamine and serotonin were determined spectrophotofluometrically according to Ciarlone (1978) [19] and Khalifa et al. (1997) [20] using Spectro-photofluometer RF-5000 Shimadzu, Japan. Animals were decapitated with the least disturbance using large scissors and the brain was rapidly dissected on ice, weighed, and homogenized with acidified butanol in a glass homogenizer submerged in ice. The homogenate was then centrifuged at 2000×g for 5 min, 2.5 mL of the supernatant was transferred to a test tube containing 1.6 ml of 0.2 N acetic acid and 5 mL n-heptane. Tubes were then vortexed for 30 sec, centrifuged at 2000×g for exactly 5 min. The supernatant organic layer was discarded and 0.2 mL of the aqueous phase was transferred to a test tube to measure serotonin and another 1 mL was transferred to another test tube to measure NE and DA. To the test tube containing 0.2 mL aqueous phase, 1.2 ml of 4% O-phthalaldehyde was added, mixed well then placed at boiling water bath for 10 minutes, cooled under tap water, and measured at 355 nm excitation and 470 emissions for the analysis of serotonin. To the 1 mL aqueous solution, 0.2 mL of 0.1 M EDTA was added and mixed well. Catecholamines were oxidized using 0.1 mL iodine and oxidation reaction was terminated after exactly 2 min by adding 0.2 mL alkaline sulfite. The tubes were allowed to stand

exactly 2 min then 0.2 mL of 5 N acetic acid was added and mixed. The mixture was placed in a boiling water bath for 2 min, cooled, and measured at 380 nm excitation and 480 emissions for NE. Test tubes were replaced with the boiling water bath for 5 min more, cooled, and measured at 320 excitations and 375 nm emissions for determination of DA. The assay depends upon estimating the norepinephrine and dopamine fluorphores formed after oxidation with iodine and measuring the serotonin fluorphores formed by derivatization using O-phthalaldehyde at different excitation and emission wavelengths.

2.5. Statistical analysis

All groups were statistically compared using one-way ANOVA [21] followed by the Tuckey post hoc test. The Difference between means was considered significant when $P < 0.05$. Statistical analysis was performed using SPSS 16 analysis pack.

3. RESULTS

3.1. Behavioral Analysis (Fig. 1): a. immobility, b. swimming, & c. straggling time

The results showed that the immobility time was significantly elevated in the group that passed by TST before FST (combination technique) more than the group passed FST directly (123.9%). However, both swimming and straggling time significantly decreased in combined technique as compared to FST (64.6%, 58.2%). There was a similar effect of both Fluoxetine & DDB on reducing the immobility time when compared to the combination technique.

3.2. Neurotransmitters levels (Fig. 2)

3.2.1. Serotonin

The results revealed that the whole brain's 5-HT concentration was significantly decreased following application of either TST (83%) or FST (67%). Moreover, after the combination of

the two techniques (TST+FST) the 5-HT level was significantly decreased less than the application of TST (55%) or FST (67%) alone. Treatment of the depressed mice with fluoxetine normalized 5-HT level of depressed mice. However, DDB treatment resulted in a significant elevation in 5-HT levels, as compared to the combined technique (184%).

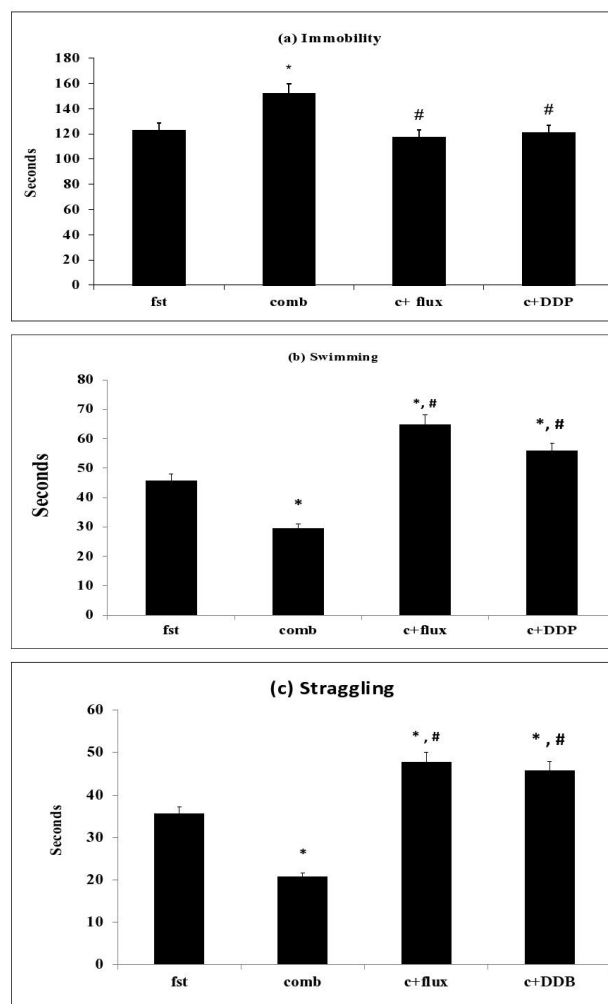


Fig. 1. Behavioral effects of the FST, combination technique, fluoxetine and DDB with combination technique (n= 6)

a. Immobility time, b. swimming time, c. straggling time
 FST: forced swimming test, comb: combination technique, c+flux: combination technique plus fluoxetine and c+DDB: combination technique plus DDB

* Significant difference, at $P < 0.05$, from FST

Significant difference, at $P < 0.05$, from combination technique

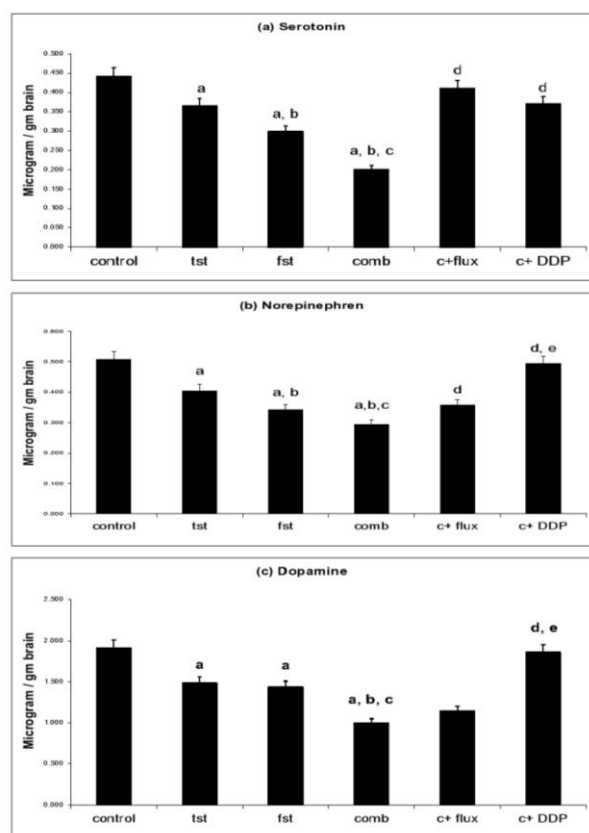


Fig. 2. Effects of the combination technique on neurotransmitters levels in the brain (a. serotonin, b. norepinephren and c. dopamine) with comparison to control and effects of fluoxetine and DDB in the combination technique on these neurotransmitters with comparison to the combined technique without treatments (n= 6)

tst: tail suspension test, fst: forced swimming test, comb: combination technique, c+flux: combination technique plus fluoxetine and c+DDP: combination technique plus DDB

a Significant difference, at $P < 0.05$, from normal control

b Significant difference, at $P < 0.05$, from tst.

c Significant difference, at $P < 0.05$, from fst.

d Significant difference, at $P < 0.05$, from comb

e Significant difference, at $P < 0.05$, from c+fst

3.3. Norepinephrine

The combined technique produced a significant lowering in brain NE when compared to TST (72.3%) or FST (85.7%) alone. Fluoxetine resulted in a significant elevation in NE level, compared to the combined technique (121.8%). In contrast, administration of DDB to animals subjected to combination technique

(TST+FST) nearly normalized brain NE level.

3.4. Dopamine

The obtained results declared that the DA level was significantly lowered in combination technique when compared to TST (67.3%) or FST (69.7%) alone. Administration of fluoxetine to animals subjected to the combination technique caused a non-significant increase in DA level. However, DDB treatment for 7 constitutive days induced a significant increase in DA level of mice exposed to combination technique (by 185.8%) nearly to the normal level.

4. DISCUSSION

The present study aimed to determine if the combination of two models of depression, TST and FST affects the immobility time (depressive state). In addition to, comparing the antidepressant potential of a standard antidepressant drug; fluoxetine with DDB. As shown previously the immobility time in both FST and TST is a reflection of the depressed state in mice [14, 22]. Concerning the combination of TST & FST, both are well-known models of depression; the present results found that there was an increase in the immobility time in the FST in animals passing TST before FST than animal passing FST directly. The increase in immobility time reflects the increase in the depressed state [22]. This increase was accompanied by a significant decrease in swimming, straggling time, and whole-brain neurotransmitters: 5HT, NE, and DA. These findings are similar to those obtained by several investigators [23, 24] who noticed that dysfunction in the 5HT concentration may causally related to major depressive disorder. The modified FST protocol in the current study allows so far the investigation of acute or chronic treatments and with lower doses of antidepressants or a lower number of animals. It may evaluate different types of antidepressant drugs acting by different mechanisms of action.

The present results showed that the increase in immobility time after subjected the animals to the combination of the two behavioral tests (TST, FST) is decreased by acute treatment with fluoxetine; however, swimming time increased. This finding is consistent with previous findings [25, 26]. This effect was accompanied by a significant increase in 5HT level in the whole brain of mice subjected to the combination technique and treated by fluoxetine; this finding is consistent with previous study which noticed that fluoxetine increased 5-HT in depressed animals. However, the results showed that injection of fluoxetine had little effect on NE or DA levels of depressed animals (mice subjected to the combination technique) [3].

Administration of DDB resulted in decreasing immobility time to a similar value as fluoxetine. This effect was accompanied by a significant increase in swimming, straggling time, and brain neurotransmitters. Dopamine is the major biogenic amine involved in the control of locomotor activity [27]. The clear effect of DDB on immobility time in the FST is correlated with a correspondent tendency to increase DA significantly in depressed mice. The utilized dose level of DDB in the present work (100 mg/kg given orally) was in the range of the doses previously reported to result in behavioral changes or to produce immunomodulatory and hepatoprotective effects [6]. The neurobehavioral impacts of DDB were previously reported in enhancing the aggressiveness of the winner partner in the sensory contact model and to increase motor activity in the open field test. Furthermore, it decreased anxiety and increased exploratory tendency and curiosity [8]. It is reported that the monoaminergic hypothesis is the most accepted mechanism of depression by major depletion of brain 5HT, NE, and DA [4]. Furthermore, the antioxidant and anti-inflammatory impact of DDB which is extracted from *Schisandra Chinensis* protects against

central neurodegeneration & neuroinflammation which have been reported in various CNS disorders [28-32]. So the antidepressant-like effect of DDB which was observed in the current study may be considered as a parallel to the observed changes in neurotransmitters and the increase in motor activity or to the decrease of anxiety as observed in the current study.

Conclusion

It could be concluded that the combination of TST and FST increases the sensitivity of FST for testing different antidepressant drugs. DDB is not an inert substance on the brain; it is maybe an antidepressant or antidepressant plus other neurobehavioral effects. So this study points to the need for other detailed complementary preclinical and clinical investigations to define the neuropharmacological potential of DDB.

Declarations

Ethics approval and consent to participate

Not applicable

Consent to publish

Not applicable

Availability of data and materials

The data generated or analyzed during this study all are included in the main manuscript.

Competing interests

The author declares that no competing interests exist.

Funding statement

No funding source was received

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