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RESEARCH ARTICLE

Development and *In vivo* Evaluation of Atomoxetine Hydrochloride ODMTs in a Nicotine-induced Attention Deficit Hyperactivity Disorder (ADHD) Model in Rats

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Abstract

The current study aimed to evaluate the efficacy of orally administered rapid mini-tablets containing atomoxetine hydrochloride (ODMT) relative to the conventional capsule formulation of atomoxetine hydrochloride (ATO). To mask the bitter taste of ATO and render it more palatable for pediatric administration in individuals with Attention Deficit Hyperactivity Disorder (ADHD), an inclusion complex of ATO with β-cyclodextrin (β-CD) was synthesized. The ODMT and conventional capsule ATO formulations were administered orally to a cohort of ADHD rat pups born to nicotine-exposed dams, facilitating an *in vivo* efficacy assessment. Behavioral assays, including the open field test, novel object recognition test, and Barnes maze test, were conducted pre- and post-administration of the therapeutics. The outcomes suggested that the ODMT formulation, incorporating ATO-β-CD inclusion complexes, shows promise as a viable alternative to the capsule form of ATO. Conclusively, the preparation of the ATO-β-CD complexes and ODMTs leveraged a factorial experimental design, with the animal model being subjected to nicotine-induced hyperactivity to provide a unique evaluative framework for the ODMT formulation under development.

Keywords atomoxetine hydrochloride · behavioral tests · experimental design · nicotine · pediatric orally disintegrating mini tablets

Introduction

Attention Deficit Hyperactivity Disorder (ADHD) is a neurobehavioral disorder with two distinct types: inattention and hyperactivity. The hallmark features of ADHD include a short attention span, restlessness, and agitation in behavior or cognition. Children with ADHD may display behaviors of inattention or hyperactivity that are not appropriate for their developmental age. Typically, symptoms of the disorder begin to manifest early in life, with onset often occurring around age 3 [1, 2]. ADHD is one of the most commonly diagnosed psychiatric disorders in children, with a prevalence rate of 5–10% in childhood and 4% in adulthood. ADHD symptoms can have negative effects on a child's mental performance, productivity, and motivation, as well as difficulties in expressing emotions, speech, and reasoning. Often, accompanying psychiatric conditions such as behavior, mood, and anxiety disorders are present with ADHD symptoms, which can lead to decreased social adjustment and contribute to the child's academic struggles [3]. Individuals with ADHD are much more prone to personality disorders and psychiatric problems such as alcohol

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and/or substance use problems, academic problems such as school-related problems, and social problems such as frequent accidents, causing accidents, pregnancy at a young age, and failure in working life, more often than normal individuals. Therefore, the diagnosis and treatment methods of ADHD are of great importance [4]. Moreover, as the incidence has been increasing and its consequences have been intervening in the quality of life of individuals, treatment options have gained increasing importance in recent years. Although some studies have suggested that complementary therapies, such as homeopathy, aromatherapy, and food supplements, can improve the symptoms of ADHD, there is a general consensus that drug therapy plays a crucial role in the management of this neurobiological disorder. An effective treatment for ADHD should address both the disorder itself and any co-occurring psychiatric problems in childhood and adulthood. While the underlying cause of ADHD remains unclear, studies suggest that disturbances in serotonin, noradrenaline, and dopamine metabolism characterize the neurobiology of the disorder [5].

Accordingly, current drug therapies are classified into two main categories: central nervous system stimulants (e.g., methylphenidate, dexmethylphenidate, amphetamine, and dextroamphetamine) and selective noradrenaline reuptake inhibitors (e.g., atomoxetine and venlafaxine) [6, 7].

Atomoxetine (ATO) is an important alternative in the treatment of ADHD and is the first non-stimulant active substance approved by the Food and Drug Administration (FDA). It is often preferred as a first-line treatment, especially in cases where the patient has accompanying substance use, tic, and anxiety disorders [8]. Moreover, it has been reported that ATO is more effective and safer in patients with a history of previous stimulant use. Another study conducted in 2008 showed that ATO was beneficial in 43% of the patients who were unresponsive to methylphenidate treatment (78%). Additionally, ATO's low abuse potential, as demonstrated in various studies underscores its importance as a treatment option for ADHD patients with alcohol and substance use disorders [8, 9]. However, because ATO has a very bitter taste, currently, only a capsule form is available in the pharmaceutical market, and there is no dosage form specifically designed for pediatric use. Pharmacotherapy for pediatric patients differs significantly from that for adult patients, including the route of drug administration, drug-induced toxicity, and taste characteristics. Therefore, selecting drugs for pediatric patients based on their age and physiological condition is critical in treatment.

Orally disintegrating mini tablets (ODMTs) are small-sized solid dosage forms that rapidly dissolve or disintegrate in the oral cavity without the need for extra water [10; 11]. Different pharmacopoeias and references have varying definitions for ODMTs. For example, the Food and Drug Administration (FDA) defines ODMTs as solid dosage forms that disintegrate with saliva on the tongue within a few seconds, while the European Pharmacopoeia (EP) refers to uncoated tablets that disintegrate rapidly in the oral cavity within three minutes, classifying them as orodispersible tablets [12, 13]. Studies suggest that even one-month-old infants can easily accept and use 2 mm diameter ODMTs as opposed to syrups. ODMTs combine the advantages of orodispersible tablets and mini-tablets for pediatric therapy [14–16]. Thus, ODMTs can serve as an alternative to traditional solid dosage forms, offering dose flexibility and alleviating swallowing difficulties, especially in pediatric use [17]. On the contrary, while the EMA generally defines mini-tablets as up to 5 mm in diameter, research has observed that there is some flexibility for tablets slightly larger, around 6–7 mm in diameter, particularly when these tablets are formulated for easy swallowing or rapid dispersal. This attribute is seen as advantageous, especially for tablets that disintegrate quickly in the mouth [18–21].

Building a design space and implementing risk management are essential steps in the development of ODMT formulations with stable properties that meet patient acceptance criteria [22]. Quality by design involves understanding how process and formulation parameters impact product properties and optimizing the final specifications of the product [23]. In the case of ODMTs, it is crucial to develop formulations with appropriate disintegration times, acceptable taste, resistance to packaging and transportation conditions, and optimized process parameters due to their low hardness. In our study, we employed the quality by design approach to investigate the effects of formulation components, such as the quantity and type of disintegrant and lubricant, on critical quality attributes of the tablet [24, 25]. These attributes include hardness, friability, taste acceptability, disintegration time, and dissolution rate. By adopting this approach, we have found that time and cost savings can be achieved in the formulation development process [26, 27].

There are various animal models for ADHD in rodents. In order for the ADHD model used to be considered successful, animals in the disease group should exhibit impulsivity, hyperactivity, and/or inattention-related behaviors similar to ADHD symptoms in humans [28]. However; like the heterogeneity of the disease itself, animal models of ADHD vary depending on the employed method, such as substance depletion or genetic disorder. For instance, hyperdopaminergic transmission plays a crucial role in the behavioral consequences of all models [29]. In addition, the foretold behavioral outputs like impulsivity, hyperactivity, or inattention do not affect patients with ADHD in an equal manner, which means that each animal model in use can have different behavioral outputs regarding ADHD symptoms [30]. Furthermore, in regard to hyperactivity, some rodent models show hyperactive behavior in a novel environment, while others may show hyperactivity in a familiar environment, which is characteristic of ADHD [31]. Among the various

animal models, one of the most widely used and easy methods to achieve an ADHD model in rodents is prenatal nicotine exposure, which successfully mimics maternal cigarette smoking during pregnancy [32, 33].

To prepare pediatric ODMT formulations with a 25 mg ATO dose, the direct compression method was employed using inclusion complexes with beta-cyclodextrin (β-CD) to mask the bitter taste of the active substance [34]. The study used the experimental design method to optimize both ATO-β-CD inclusion complexes and ODMTs, with the selection of ODMT components considering pediatric use. The optimized ODMTs demonstrated faster dissolution and disintegration times than the capsule form, successfully suppressing the bitter taste of the active substance, as confirmed by taste tests with an electronic tongue [35]. These results indicate that the ATO-β-CD complex is a viable option for pediatric use in ODMT formulation. The therapeutic efficacy of the optimized ODMT formulation was evaluated *in vivo* using the novel object recognition test (NORT), Barnes maze test (BMT), and open field test (OFT) in rats with hyperactivity induced by nicotine application for 30 days. The animals' hyperactivity levels before and after treatment with the ODMT formulation were evaluated based on the results of these tests.

On this basis, we aimed to develop an ODMT ATO formulation and test its efficacy in a nicotine-induced ADHD rat model using sensorimotor behavioral tests.

Materials and Methods

Materials

Atomoxetine hydrochloride was provided by Abdi İbrahim İlaç A.Ş. (Istanbul, Turkey). β- Cyclodextrin, sucrose, Avicel PH 101®, magnesium stearate, nicotine were purchased from Sigma Aldrich (USA), Parteck ODT® and mannitol were supplied from Merck (USA), Ac-Di-Sol® was purchased from IMCD (Holland). In addition, all solvents used, were used as HPLC grades.

Methods

Preparation and experimental design of ATO-β-CD inclusion complexes

The grinding method was used in the preparation of the solid state inclusion complex, which is widely preferred due to its ease of processing and they were optimized with factorial experimental design. The preparation of inclusion complexes and the experimental design and optimization steps have been reported in our previous paper [36].

Preparation of ATO-β-CD ODMTs

ODMT formulations containing Parteck ODT® and Ac-Di-Sol® were prepared by direct compression with an Erweka® single punch tablet machine (Erweka, Germany), containing the ATO-β-CD (7:3) inclusion complex and weighing 120 mg, 6.5 mm in diameter [36].

Physicochemical Controls on ODMTs

Disintegration time, weight change, diameter and thickness, hardness and friability tests were performed on ODMTs. In addition to these tests, water absorption time and absorption capacity of ODMTs, which play an important role in the evaluation of the rapid disintegration of tablets, were also determined. The amount of active substance in the optimized ODMTs containing active substance was determined by the method specified in USP XXIV for atomoxetine capsules [37].

Animals and Experimental Protocol

The animal experiments were approved by the Hacettepe University Institutional Ethics Committee for the Care and Use of Experimental Animals (Issued 2021/06–06) and conducted in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals. Young adult (5–7 weeks old) virgin female Sprague–Dawley rats (n = 12) and male Sprague–Dawley rats (n = 3) proved to be fertile were housed in separated cages, under standard conditions at 22 ± 2 °C and 40–70% relative humidity with a 12-h dark/12-h light cycle (lights were on between 08:00 and 20:00 h) with free access to standard rat chow and water. After one week of the adaptation period, female rats were randomly divided into two groups: control (n = 6) and nicotine (n = 6) by simple randomization. Control group female rats received tap water only, whereas nicotine group female rats received nicotine-added (200 µg/mL) tap water to induce ADHD model during pre-fertilization period for 21 days, mating and pregnancy, and nursing period until the pups were separated from the mothers at the postnatal 21st day. Pregnancy was confirmed with the observation of a vaginal plug or detection of motile sperm in vaginal lavage specimens, and the pregnant female rats were housed separately. At the fifth postnatal day, gender discrimination of the pups was performed by measuring the anogenital distance and female pups were euthanized by carbon dioxide inhalation due to both the potential interference of ovarian hormones on pharmacological effects and interactions of the administered medication and the higher incidence of disease in boys [38–40]. All the experiments were performed/conducted on male pups exclusively. The male

pups were randomly allocated into four experimental groups: negative control (C-) group; male pups of mothers who received tap water and received placebo (PLA) (n = 7); positive control (C +) group; male pups from nicotine-exposed mothers and received PLA (n = 7); ATO capsule group (Capsule), male pups from nicotine-exposed mothers treated with ATO in capsule form (n = 6); ATO ODMT group (ODMT): male pups from nicotine-exposed mothers treated with ATO in ODMT form (n = 7). The capsule group was treated with the conventional pharmaceutical capsule form (1 mg/kg/day, p.o.), and animals in the ATO-ODMT group received ATO as ODMT formulation (1 mg/kg/day, p.o.). C + and C- groups were given no-drug loaded ODMT formulation.

The animals were subjected to behavioral tests twice: on postnatal 30th-37th days (pre-treatment) and 70th-77th days (post-treatment). After the first round of behavioral tests, treatment procedures were initiated accordingly for 38–40 days for each animal. All the substances/formulations were administered orally in the morning at 9:00–10:00 am. Swallowing/licking behavior was observed, and examination of the oral cavity was performed to confirm proper administration. The animals were anesthetized by ketamine (90 mg/kg, ip)-xylazine (10 mg/kg, i.p.) approximately 90 min after drug administration on the day following the completion of the experimental protocol, and blood samples were collected. The samples were centrifuged at 10,000 g for 10 min to separate the serum fractions, which were then stored at -80 °C until serum atomoxetine quantification was performed. The blood atomoxetine concentration was determined in Üsküdar University Clinical Pharmacogenetics Laboratory by the high-performance liquid chromatography mass spectrometry (HPLC–MS/MS) method, specifically utilizing the Agilent 6470 HPLC–MS/MS equipment (IET Co., based in Illinois, USA). The timeline of experimental protocol is given in Fig. 1.

Animal Behavioral Tests

As ADHD is a spectrum disorder, symptoms vary and are generally classified into three main categories: impulsivity or inattention, hyperactivity, and learning difficulties [37]. To evaluate these symptoms, we utilized the Open Field Test (OFT), Novel Object Recognition Test (NORT), and Barnes Maze Test (BMT). A camera (Camersa; Bandicam®, USA) was positioned approximately 1.5 m above the entire test areas, and test sessions were recorded and analyzed afterward by two blinded researchers, separately.

Open Field Test Animals were placed on the center of an open-top box (100 × 100 × 40 cm) which has a central area of 50 × 50 cm and a peripheral zone measuring 25 cm on each side and spontaneous activity was recorded for five minutes [41, 42]. Then the duration of grooming periods (seconds), time spent in the central area (seconds), the number of entries into the central zone, and the number of rearing was determined.

Novel Object Recognition Test Based on the behavioral observation that rodents display greater interest and spend more time with newly encountered objects compared to familiar objects, the NORT was developed to evaluate recognition memory and attention. The test consists of the habituation, first, and second phases, conducted in an open-top area (50 × 50x30 cm). The habituation phase encapsulates the familiarization of the rat with the field where no objects are present. In the first phase, two identical objects were placed in predetermined locations inside the field, and the rat was allowed free in the field for 10 min. In the second phase, after proper disinfection (to remove any leftover scents), one of the objects was replaced by a different object in the exact spot while the other object remains completely stationary, and the rat got reintroduced into the field for 3 min. Each rat spent 60 min as a retention interval between the first and second phases [43]. We assessed

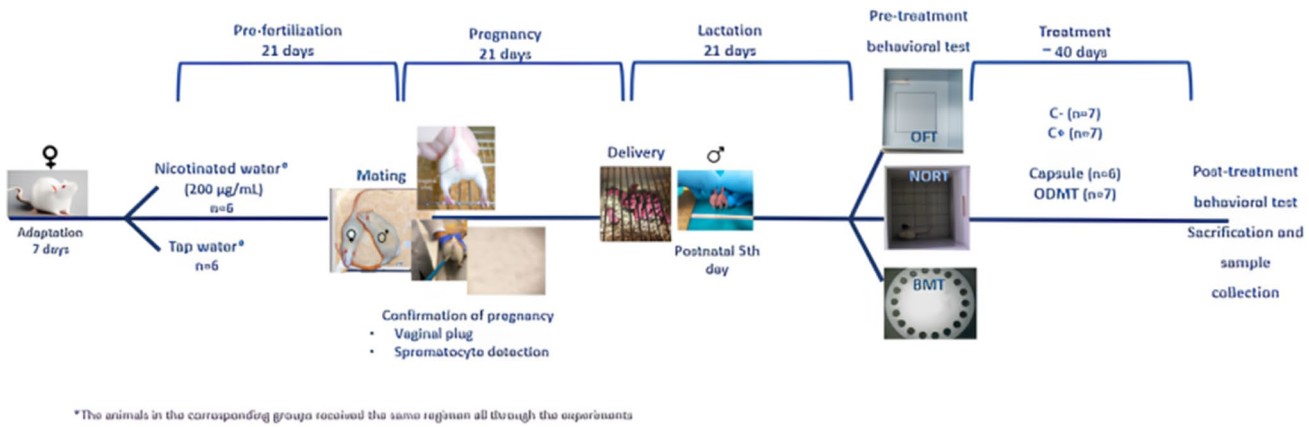

Fig. 1 The timeline of experimental protocol

the animals' preferences and behaviors towards the objects, by evaluating the time spent and the number of interactions with both new and old objects in the second phase of the test by calculating the discrimination index, which is the ratio of the time spent near the novel object to the total time spent around both objects, and the preference index, which is the ratio of the number of reaches/touches towards the novel and old objects [44].

Barnes Maze Test This test is performed on a rotating circular platform 122 cm in diameter with 18 holes of 10 cm in diameter placed at the circumference at equal intervals, is used to assess spatial learning and memory functions in rodents. The platform is one meter elevated off the ground. Distinct visual hints were positioned on the same spots throughout the experiment. An escape box was placed beneath one of the 18 holes (target hole) where the animals could feel safe. The test protocol consisted of 4 days of trial, followed by the recording day, for a total of 5 days. During the trial days, the rats were placed at the center of the Barnes maze with the escape box in place. In each trial day the tests were performed twice for 3 min with a 15-min intervals in-between. If the rats couldn't find the escape hole during this time, they were manually placed to the box through the hole, and kept there for 30 s to recognize and learn location of the escape box. On the day of the recording, the escape box was removed from the maze, followed by a recording of 3 min after the rat was placed on the center of the platform [45]. The parameters noted was the time spent in the quadrant where the escape box was previously located, latency (the time to the first visit of the animal to the target hole from the center of the maze), errors (the number of times the animal touches with its nose or front paws to the boundary of the other holes until it finds the target hole), and total errors (the number of times the animal touches the boundary of the other holes throughout the recording).

Statistical Evaluations

IBM SPSS 23.0 statistical software was used in the analysis of the results. The data was not distributed normally when tested for normality by Shapiro–Wilk test. Kruskal–Wallis test was used for multiple comparison of groups [46]. For post hoc analyses Dunn test was applied. Wilcoxon test was used for within group evaluations of pre- and post-treatment results. All graphics were given as bars.

Results

Preparation and Characterization of ATO-β-CD Inclusion Complexes

Inclusion complexes prepared using different ATO and Beta β-CD molar ratios were characterized by DSC and FTIR techniques. The same complexes were optimized taking into account the mixing time (independent variable) in the preparation procedure. As dependent variables, ATO % concentration and dissolution rate parameters in the inclusion complexes were determined. As a result of the statistical evaluations, it was determined that the ATO-β-CD inclusion complex with optimum properties should be prepared in a molar ratio of 7:3. The results of this part of the study were reported in your previous paper [36].

Preparation and Optimization of ATO-β-CD ODMTs

In our study, we employed two types of superdisintegrants, namely Parteck ODT® and Ac-Di-Sol®, alongside magnesium stearate as a lubricant. Each group of components was utilized in three distinct ratios, specifically 5, 7.5, and 10 for superdisintegrants, and 0.5, 1, and 1.5 for magnesium stearate. The percentage of superdisintegrant and lubricant content was designated as the independent variables, while the friability, hardness, and disintegration time of the orally disintegrating tablets (ODMTs) served as the dependent variables [47]. Our study findings, which were conducted with two replications, underwent rigorous statistical evaluation through ANOVA. The formulation of ODMTs was meticulously designed based on the independent variables within the experimental design, incorporating the most suitable formulation components. These formulations were then prepared using the direct compression method. Furthermore, a series of comprehensive physicochemical assessments were carried out on all 18 ODMT formulations that were prepared as part of this study.

Conventional quality control studies (hardness, friability, active ingredient determination, disintegration time, dissolution rate determination) were performed on all ODMT formulations. In addition to these studies, wetting time, wetting percentage and taste tests using electronic tongue were performed on the optimized ODMT formulations. It was determined that the taste characteristics of ODMTs containing ATO-β-CD complex were more acceptable than ODMT tablets containing free ATO. In our previous publication, the optimization of ODMTs was carried out by considering the hardness and friability values of ODMTs as dependent variables, which would be negatively affected during the determination of the most important characteristic of ODMTs, disintegration time. The results were evaluated using ANOVA and the effect of the preparation parameters of ODMTs on the dependent variables were analyzed and interpreted [36].

In vivo ADHD Model and Treatments

Body weights of the mothers before fertilization and at the time of delivery as well as the pups at the time of separation

from both control and nicotine-exposed mothers were similar. Caregivers observed that the offspring of nicotine-exposed (200 µg/mL) mothers displayed higher activity levels and restlessness compared to the offsprings of control rats, which was further corroborated by behavioral tests. The basal results of the OFT, obtained before any treatment were similar between control and nicotine-exposed animals. However, values obtained at the end of protocol indicated significantly higher numbers of rearing in C + group, representing the higher impulsivity in non-treated ADHD modeled animals compared to the control animals. The administration of ATO both in the conventional capsule and tested-ODMT formulations improved OFT parameters, significantly decreased numbers of rearing in both treatment groups compared to positive control group (p C + *vs* Capsule = 0.005, p C + *vs* ODMT = 0.015).

In the BMT, nicotine-exposed animals showed impaired learning and memory functions documented by significantly increased total errors and errors in ADHD-modeled animals (Fig. 2a and b). Other parameters measured in BMT, such as latency and time spent in the target quadrant, supported our model, although statistical significance was not reached.

The ATO-treated animals, regardless of ODMT or capsule form, performed better in the BMT, showing significant improvements in error numbers and time spent in the target quadrant compared to their pretreatment values as well as to the positive control animals (Fig. 2a, b, d). The latency parameter of the BMT improved in all groups; however, the significance between pre- and post-treatment values was valid only for the ODMT-ATO group (Fig. 2c).

NORT primarily assessed attention, through discrimination and preference indices, time spent around the new object, and the number of touches to the new object.. Pretreatment values indicated decreased attention in nicotine-exposed animals compared to control animals, as evidenced by lower discrimination and preference indices (Fig. 3a and b). Post-treatment trials showed improvement in both indices, although group differences were not significant.Serum ATO levels were measured using the HPLC method from blood samples collected at the end of the protocol in both ATO-treated (Capsule and ODMT groups) and control animals; both Control + and Control- groups). The serum drug levels were similar in commercial pharmaceutical capsule formulation and developed ODMT formulation.(p > 0.005). However, significantly higher serum drug levels were measured in all of the ATO-treated animals in comparison to control animals (Fig. 4).

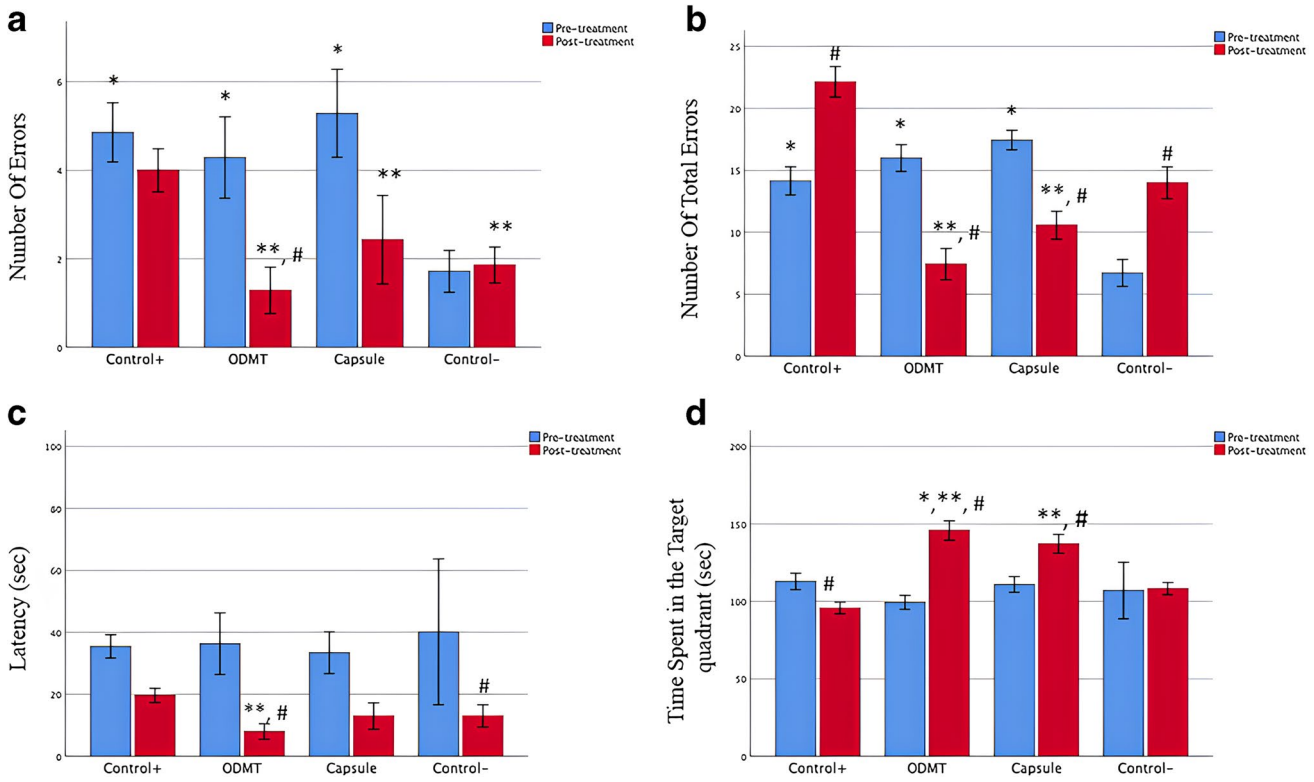

Fig. 2 The results of the Barnes Maze Test. (**a** Number of Errors, **b**Number of Total Errors, **c** Latency, **d** Time Spent in the Target Quadrant. Blue bars indicate pre-treatment results. Red bars indicate post-treatment results. (*) p < 0.05 *vs* Negative Control of the same color, (**) $p < 0.05$ *vs* Positive Control of the same color. (#) $p < 0.05$ Post-treatment *vs* Pre-treatment within the same group.Control + (n = 7), ODMT(n = 7), Capsule (n = 6), Control- (n = 7)

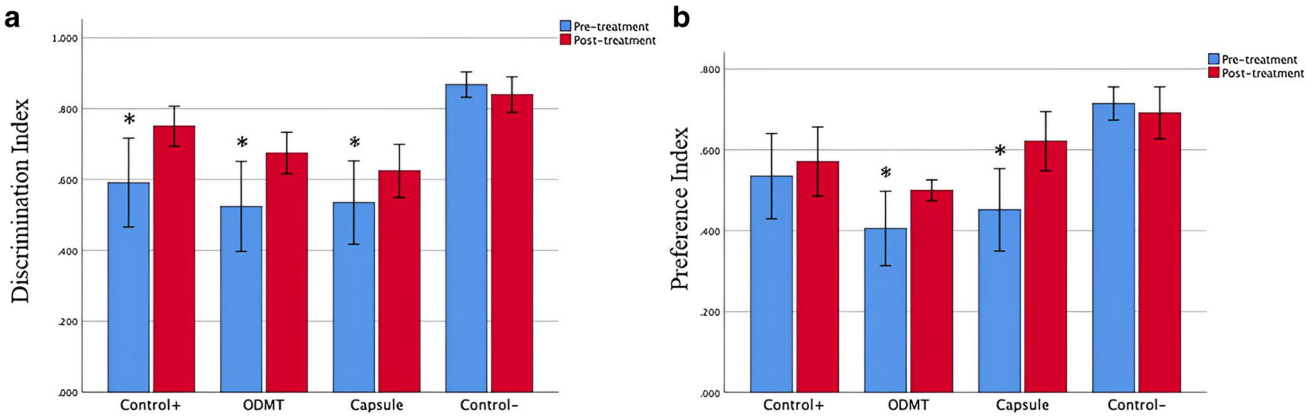

Fig. 3 The results of the Novel Object Recognition Test. **a** Discrimination Index, **b** Preference Index. Blue bars indicate pre-treatment results. Red bars indicate post-treatment results. (*) $p < 0.05$ *vs* Negative Control of the same color. Control + (n = 7), ODMT(n = 7), Capsule (n = 6), Control- (n = 7)

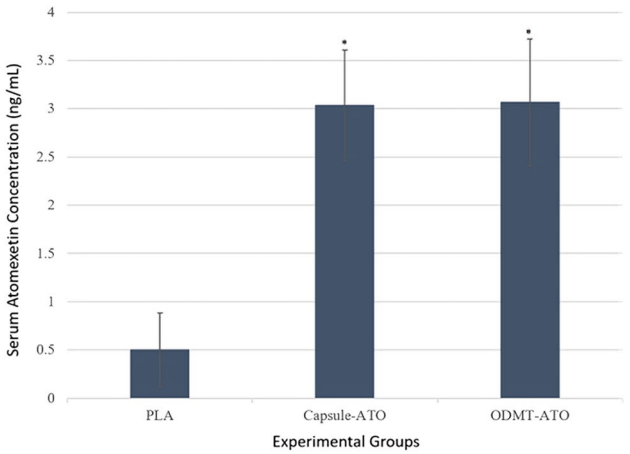

Fig. 4 Serum atomoxetine levels of the animals given ATO in the capsule (Capsule-ATO), ODMT (ODMT-ATO) forms and only PLA with no atomoxetine loaded (PLA)

Discussion

After a thorough evaluation of *in vitro* testing of ATO-β-CD inclusion complexes, such as dissolution rate and amount of ATO loaded, it was determined that the most favorable formulations in terms of cumulative amounts of dissolved and loaded ATO per unit time could be obtained with a 2:3 ATO:β-CD ratio and a mixing time of 20 min, as well as a 7:3 ratio and a mixing time of 40 min. Considering the intended ATO content of 25 mg in the target ODMT and an average total tablet weight of 120 mg, it was concluded that the 7:3 molar ratio and a mixing duration of 40 min would be the most appropriate choice. Extensive analyses including Infrared Spectroscopy (IR), Scanning Electron Microscopy (SEM), and Differential Scanning Calorimetry (DSC) confirmed the formation of the inclusion complexes.

The hardness, thickness-diameter, friability (< 1%), and weight deviation values of the nine different ODMT formulations, each prepared in duplicate, fell within the acceptable limit range. Based on this data, it can be confidently stated that the prepared bulk masses are suitable for the preparation of ODMTs using the direct compression method, as per USP 32 standards.

Optimization of the disintegration time, friability, and hardness values of the prepared ODMTs was achieved through ANOVA analysis. According to the results, formulations F1 (containing 10% Parteck ODT® and 0.5% lubricant), F2 (comprising 10% Parteck ODT® and 1% lubricant), and F10 (containing 10% Ac-Di-Sol® and 1% lubricant) were identified as the optimized formulations. Subsequently, ODMTs were prepared using the optimized ATO-β-CD inclusion complexes and subjected to *in-vitro* taste tests and dissolution rate assessments. Following these evaluations, animal experiments were initiated to determine the *in-vivo* efficacy of the F2 formulation, which exhibited optimal results.

The study employed a prenatal nicotine exposure-induced ADHD model, based on the well-established link between maternal nicotine exposure in smoking mothers and the increased risk of ADHD in their children [46]. This model employs the physiological mechanism involving the competition between nicotine and acetylcholine for nicotinic receptors, resulting in compromised learning and memory abilities mediated by acetylcholine. Nicotine also induces muscle contractions, indirectly leading to hyperactivity, similar to acetylcholine, thus mimicking the hyperactivity, impulsivity, attention deficits, and learning difficulties observed in ADHD patients [48, 49].

The behavioral battery was chosen to encompass the wide range of ADHD symptoms and successfully validated our model of prenatal nicotine exposure. OFT, frequently used in ADHD models to evaluate spontaneous activity and impulsivity [13, 26, 49]. The inborn characteristic of rats is to prefer areas closer to the walls and hesitate in moving to the center of the open fields. As a sign of increased impulsivity time in the center, number of entries to the central area as well as increased grooming and rearing behaviors are observed. However, in this study OFT findings were not conclusive to test the efficiency of developed formulation possibly due to anxiety associated with open field and should be considered in future studies. BMT assessed spatial learning and attention, relying on the animals' use of spatial cues to locate an escape hole [50]. The results of BMT and selected findings of OFT indicated increased activity and impulsivity, alongwith decreased attention and disturbed spatial learning in the animals with ADHD- before treatment regimens were initiated.The ATO treatment, regardless of pharmaceutic formulation, reduced rearing numbers and grooming times and eliminated differences between control and ADHD animals. However, no significant improvements were observed in the time spent in the central zone and the number of entrances neither between groups nor the treatments. This could potentially be attributed to anxiety experienced by animals in open areas [51], as well as dosage of the ATO, since we applied a single dose for both formulations.

The results of BMT as a spatial-learning and attention testing task further substantiated our findings. The statistically significant differences between control and nicotine-exposed animals in the number of errors and total errors in the pre-treatment round of tests, indicated that the ADHD model in the rat through prenatal maternal nicotine exposure was successfully established. On the other hand, the lack of difference for latency in pre-treatment tests pointed out similar ability of learning in control and ADHD-modeled animals. On the other hand ADHD-modeled animals benefited from ATO treatment as, latency i.e. time to find the target hole and number of errors and time spent in the target quadrant significantly improved in post- treatment tested. ATO treated animals spent more time in the target quadrant in comparison to their own pretreatment values. Additionally, ATO treated animals spent more time in this area compared to both control groups, as a reflection of the efficacy of the administered drug.

NORT results indicated improved selective attention and short-term memory in the ATO-treated ADHD animals, aligning with the findings of previous animal models of ADHD and ATO treatment [52].

There were some limitations in the study. Especially the low number of animals in behavioral experiments caused our observations not to be adequately reflected in statistics. However, the results show that the ADHD model created with pre-natal nicotine exposure is confirmed, as a relatively easy and cheaper method for disease modelling. Most importantly, our results confirmed our hypothesis of the produced formulation of ODMT. The ODMT formulation has a similar effect with the conventional capsule form, in accordance with the main purpose of the study.

Conclusion

Orally Disintegrating Mini Tablets (ODMTs) incorporating ATO-β-CD inclusion complexes were meticulously optimized through the application of experimental design techniques. This optimization process involved the consideration of varying ratios of two superdisintegrants, namely Parteck ODT® and Ac-Di-Sol®, along with different ratios of a lubricant.

The study's *in vivo* findings revealed the manifestation of ADHD-related symptoms in rats exposed to nicotine during both pregnancy and lactation. Remarkably, the developed ODMT formulation, alongside the commercially available ATO capsule, demonstrated an ability to ameliorate various ADHD symptoms. Behavioral tests administered in the study indicated improvements in attention and learning, coupled with reductions in rearing and grooming behaviors, particularly pronounced with the ODMT formulation. These beneficial effects were found to be *on par* with those observed with the capsule formulation currently available in the pharmaceutical market.

In summation, the results suggest that the developed ODMT formulation possesses significant potential and advantages. Notably, it exhibits rapid oral disintegration and a palatable taste profile, making it a promising alternative to the conventional capsule form of ATO. This is particularly pertinent in cases involving younger children, where ease of administration and acceptability can be crucial factors in treatment compliance and effectiveness.

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Author Contributions OA: Data collection, writing, visualization and data curation.
EDO: Data collection, editing and assessment.
DO: Data curation, assessment, conceptualization, reviewing and assessment.
BP: Data curation, editing and assessment.
TC: Data collection, editing and assessment.

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Data Availability The datasets used and analyzed during the present study are available from the corresponding author on reasonable request.

Declarations

Conflict of Interest No authors declare no conflict of interest.

Ethical Approval All procedures involving animals were in accordance with official guidelines and regulations regarding the care and the use of animals for the experimental procedures.

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