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BEHAVIOURAL PATTERNS AND GROWTH PERFORMANCE OF MALE WISTAR RATS EXPOSED TO CIGARETTE SMOKE: EFFECTS OF CURCUMIN AND HESPERIDIN

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ABSTRACT

This study evaluated the behavioural responses of male Wistar rats to normal air or cigarette smoke (CS) and compared the effects of curcumin and hesperidin on growth performance. In experiment 1, male rats were randomised into two groups (n = 10): control and CS-exposed groups. During exposure (four weeks), the dietary and behavioural patterns were monitored. In experiment 2, forty-eight rats were distributed across eight groups (n = 6): normal control, CS control, CS + curcumin (10 mg.kg^{-1}), CS + curcumin (20 mg.kg^{-1}), CS + hesperidin (10 mg.kg^{-1}), CS + hesperidin (20 mg.kg^{-1}), curcumin (20 mg.kg^{-1}), and hesperidin (20 mg.kg^{-1}) for 6 weeks. Growth performance (feed intake, weight gain, and feed conversion ratio FCR) were assessed. In the first experiment, there was no significant difference (P > 0.05) in the body weight of the CS-exposed group compared to the normal control, whereas feed intake was significantly (P > 0.05) lower in the CS-group. The time to access feed and water was higher in the CS-group, while other behavioural responses (locomotion, stand upright, climbing, stand and stare, sniffing, sitting, and digging) were significantly reduced (P < 0.05) compared with normal control, especially after two weeks. In the second experiment, weight gain, feed intake, and FCR were significantly lower in the CS-exposed group compared to the control group, whereas treatment with curcumin and hesperidin, especially at the higher dose (20 mg.kg^{-1} b. wt.), significantly improved the growth performance of the CS-exposed groups. This study submits that CS exposure negatively impacts on the growth performance and behavioural patterns and demonstrates the potentials of curcumin and hesperidin in addressing these CS-provoked changes.

Key words: behavioural patterns; cigarette smoke; curcumin; growth performance; hesperidin; rats

INTRODUCTION

Cigarette smoking – a major risk factor for the development of cancer, respiratory, cardiovascular and other systemic diseases – is still a common social habit globally [14]. Cigarette smoke contain several substances, such as nicotine and carbon monoxide, that can elicit chemo-mod-

ulatory effects in nerve endings, which can alter pulse rate, reaction time, awareness, etc. [24, 25]. Besides the addictive effect of nicotine, smokers tend to form a behavioural or psychosocial reliance on cigarette smoking, where they resort to smoking to deal with pressure. These behavioural patterns quickly become ingrained, coupled with negative reinforcement and the fear of withdrawal symptoms, continue to compel one to keep smoking [24]. It is therefore not surprising that a randomised clinical study revealed that only about 20 % of people that quitted smoking sustained abstinence after 12 months [17].

Emerging evidence from experimental studies suggests that the impacts of cigarette exposure extends beyond physical health. In a murine study, C a r d o s o and colleagues revealed changes in anti-predatory response, following exposure to cigarette residue [5], while dependence behaviour has been reported in rats exposed to tobacco smoke [22]. Another study highlighted the adverse effects of aqueous extract of tobacco on feed intake and growth performance [1]. Other studies, both *ex vivo* [9] and *in vitro* [2], also indicated that cigarette smoke can inhibit growth.

Utilising natural products to combat disease conditions is now a common theme in research. The potentials of some phytochemicals against cigarette smoke-associated diseases have been reviewed elsewhere [28]. Curcumin, the major curcuminoid present in *Curcuma longa*, has been shown to demonstrate relevant pharmacological properties, including anti-inflammatory, antioxidant and neuroprotective activities [3, 7]. Hesperidin – a bioflavonoid present in citruses – demonstrates similar properties [3]. Interestingly, separate studies have demonstrated the roles of these phytochemicals against cigarette-induced pathologies [10, 26].

Most studies on cigarette smoke that utilised murine models focus mainly on the metabolic responses of the animals, making information on the effects of exploratory and consummatory behaviour, as well as on growth performance, of subjects exposed to cigarette smoke sparse in literature.

This study was designed with two objectives: 1) to elucidate some behavioural responses of male Wistar rats to normal air or cigarette smoke; 2) to compare any resulting effect of treatment with nutritional supplements (curcumin and hesperidin).

MATERIALS AND METHODS

Test substance

The cigarettes used in the experiments (Benson and Hedges) were products of Philip Morris British American Tobacco (Lausanne, Switzerland). Curcumin and hesperidin (both > 98 % purity) were purchased from Solarbio Life Science and Co. Ltd. (Tongzhou District, Beijing, China).

Experimental animals

A total of fifty-eight adult male Wistar rats (average weight: 170—180 g) were used in this study and procured from the small animals' breeding unit of the College of Veterinary Medicine, Federal University of Agriculture, Abeokuta (FUNAAB). They were acclimatized for two weeks under ambient conditions (25 °C, 85 % relative humidity, 12 hours day-night cycles) in the animal house of the Department of Biochemistry, (FUNAAB).

Ethical statement

The experiment was endorsed by the Ethical Committee of the Department, and assigned with ethical approval number FUNAABBCH-PG17/0226-01. All animals were handled according to the ARRIVE guidelines for animal care [15].

Experimental design

For the first experiment, twenty animals were distributed, by simple randomisation, into two groups (n = 10), the control group and cigarette smoke (CS) exposed group. The animals were housed in separate propylene cages and observed physically when exposed to CS (CS-exposed group) and normal air (normal control). Whole-body exposure to cigarette smoke occurred for 90 minutes per day, every day, for a total of 4 weeks with a smoking chamber. The smoking chamber consisted of an animal containment system and a cigarette smoking control system [23]. The cigarette smoke was generated from double-filtered Benson and Hedges cigarettes (16 sticks daily). The exposure was monitored for particulate matter size, volatile organic compounds (VOC), and carbon monoxide levels using an aerosol gas analyser (Horiba PG-300; HePing, Shenzen, China). The control animals were housed in their respective cages 150 metres away from the CS-exposed group. Feed was rationed to the animals in both experimental

groups, while drinking water was provided *ad libitum*. The feed intake and body weight data were monitored throughout the experimental period.

For the second experiment, forty-eight rats were distributed into eight groups (n=6): normal control, CS control, CS+curcumin (10 mg.kg^{-1} b.wt.), CS+ curcumin (20 mg.kg^{-1} b.wt.), CS + hesperidin (10 mg.kg^{-1} b.wt.), CS+hesperidin (20 mg.kg^{-1} b.wt.), curcumin (20 mg.kg^{-1} b.wt.), and hesperidin (20 mg.kg^{-1} b.wt.). Exposure to CS was the same as in the first experiment, but the duration was for 6 weeks. Olive oil, which was also given to the normal and CS-control groups, was used to dissolve curcumin and hesperidin. Treatments were administered via oral gavage.

Data collection

For the first experiment, behavioural patterns of the animals (normal control and CS-exposed) were taken simultaneously daily, for four weeks at ten minutes intervals for the duration of exposure – ninety minutes – (Table 1). The first time to access drinking water and feed was taken ten minutes before exposure to CS, during the exposure period, and ten minutes post-exposure for the CS-exposed rats.

For the second experiment, growth performance parameters (feed intake, weight gain, and feed conversion ratio FCR) were assessed [16].

Statistical analyses

Quantitative variables were expressed as mean±standard error mean (S.E.M) of ten rats. The normality of data distribution and homogeneity of variance was tested using the Shapiro-Wilk test and Levine's test, respectively. The significance of difference in particulate matter, body weight, feed intake, and FCR (parametric tests) were analysed with independent sample t-test, while other behavioural pattern evaluations were analysed with Kruskal Wallis non-parametric test. Data from experiment 2 were analysed using one-way analysis of variance and the Tukey test. All analyses were done with the Statistical Package for Social Sciences (IBM SPSS, version 20.0) and graphs were plotted using GraphPad Prism (version 8.02).

RESULTS AND DISCUSSION

Particulate matter evaluation

We begin our investigation by evaluating the composition of the air exposed to both the control and the CS-groups. The control groups were exposed to normal air, which contained significantly ($P<0.001$) lower levels of both PM 2.5 and PM 10 matter, compared to CS (Table 1). Airborne PM were often a mixture of solid and liquid particles and occurred in several sizes. It is a common practice to classify these PMs on the basis of their sizes. PM 2.5 and PM 10 are fine breathable particles of sizes $\leq$2.5 and $\leq$10 µm, respectively [4]. The lesser the particulate size, the easier for them to infiltrate the respiratory system and the broader are the adverse outcomes [4, 29]. The PM levels vary within diverse cigarette products, as the contents of various additives, nicotine, and tar may contribute to the PM released. Nevertheless, the PM 2.5 and PM 10 levels we observed are similar to those reported by L o f f r e d o

Table 1. Ethogram showing behavioural patterns monitored in the first experiment

Behaviour	Description
Locomotion	When rat moves around their cages or smoking chamber
Climbing	When rat climbs the wall of the cages or smoking chamber
Sitting	When rat crouches in one corner of the cages or smoking chamber
Stand and stare	When rat stands still with the feet and forehand all on the floor of the cages or smoking chamber
Feeding	When rat takes food from the feeder and take it to the mouth to eat
Sniffing	When rat draws up air audibly through the nose to detect a smell
Stand-upright	When rat stands on their hind leg in the centre of the cage
Digging	When rat scratches or dig in the sawdust
Drinking	When rat licks the water from the drinker
Self-grooming	When rat uses paws to scratch their body, including mouth

Table 2. Particulate matter (PM) evaluation

Groups	PM2.5 [μg.m^{-3}]	PM10 [μg.m^{-3}]	CO [ppb]	VOC [ppb]
Normal air	35.67 ± 9.68	9.20 ± 15.93	92.67 ± 1.33	250.00 ± 28.87
Cigarette smoke	618.33 ± 35.24	855.00 ± 60.73	117 ± 2.19	5508.33 ± 92.61
P value	0.000	0.000	0.002	0.006

Data are means ± SEM (n = 10); CO = carbon monoxide; VOC = volatile organic compounds; ppm = parts per million; ppb = parts per billion. Data were analysed with independent sample t-test

et al. [11] and B r a u n et al. [4], although these values for the normal air where lower that those reported by L o f f r e d o et al. [11] for venues where tobacco smoking was banned (PM 2.5 levels of 72—81 μg.m^{-3} compared to 35.67 μg.m^{-3} reported here), probably owing to the controlled conditions of the animal house where the control groups were situated. Other gaseous and volatile components were also significantly higher in the CS than the normal air. Cigarettes contain several hydrocarbons that when partially combusted (as is the case during smoking) release CO and other volatile organic compounds. Inhaling high levels of CO have been said to increase heartbeat, induce headaches, nausea, and tiredness [19], which may in turn influence appetite and other habits.

Exposure to cigarette smoke alters behavioural and dietary patterns in rats (Experiment 1)

The reductions in body weight of the CS-exposed rats did not attain statistical significance compared to the control group, after four weeks (Table 2). However, by the second week, the feed intake began to be significantly ($P < 0.05$) lower in the CS-exposed rats, which was even more pronounced by the fourth week of CS exposure (Fig. 1). The feed intake of the CS-group was fairly consistent throughout the four weeks, whereas the control rats continued to consume more. Constituents present in CS, with nicotine being the chief suspect, are known to possess inhibitory effects on the hypothalamic-appetite regulating axis in the brain [8, 27]. By binding to the nicotinic acetylcholine receptors, nicotine stimulates the release of neurotransmitters, such as dopamine and serotonin, that decreases hunger and thereby, affecting the appetite [18]. Interestingly, we observed that the CS-exposed rats took longer time to access feed or drink, particularly after the CS-exposure, as early as the first week of exposure (Fig. 2 and 3). This delayed craving to drink or eat was sustained

Table 2. Body weights of control and cigarette smoke (CS)-exposed groups

Weeks	Control group	CS-exposed group
1	189.80 ± 9.17	209.20 ± 15.93ns
2	195.40 ± 9.22	209.60 ± 12.93ns
3	201.80 ± 8.97	185.20 ± 12.67ns
4	205.80 ± 8.28	193.60 ± 12.24ns

Data are means ± SEM (n = 10); ns = not significantly different from the respective control, i.e. $P > 0.05$. Data were analysed with independent sample t-test

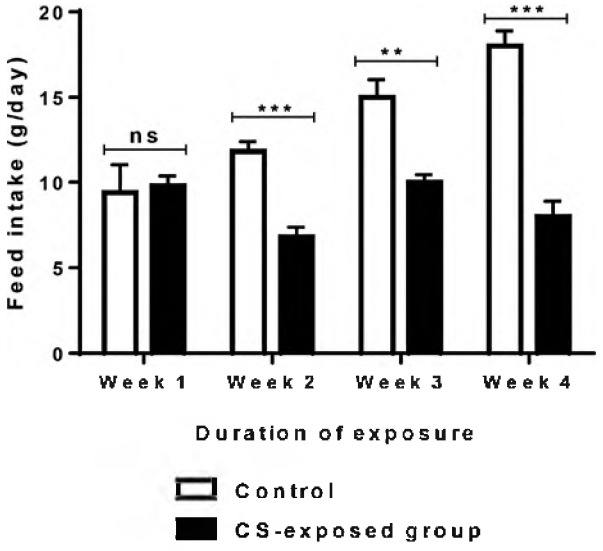

Fig. 1. Weekly feed intake of control and cigarette smoke (CS)-exposed groups
Bars are means ± SEM of seven days. Significance: ns – not significant (i. e., $P > 0.05$); ** – $P < 0.01$; *** – $P < 0.001$. Data were analysed with independent sample t-test

at the fourth weeks and further affirms that CS alters the dietary behaviour of exposed subjects.

The behavioural patterns examined during exposure to CS, in the first experiment, were locomotion, climbing, sitting, stand and stare, feeding, sniffing, stand upright, dig-

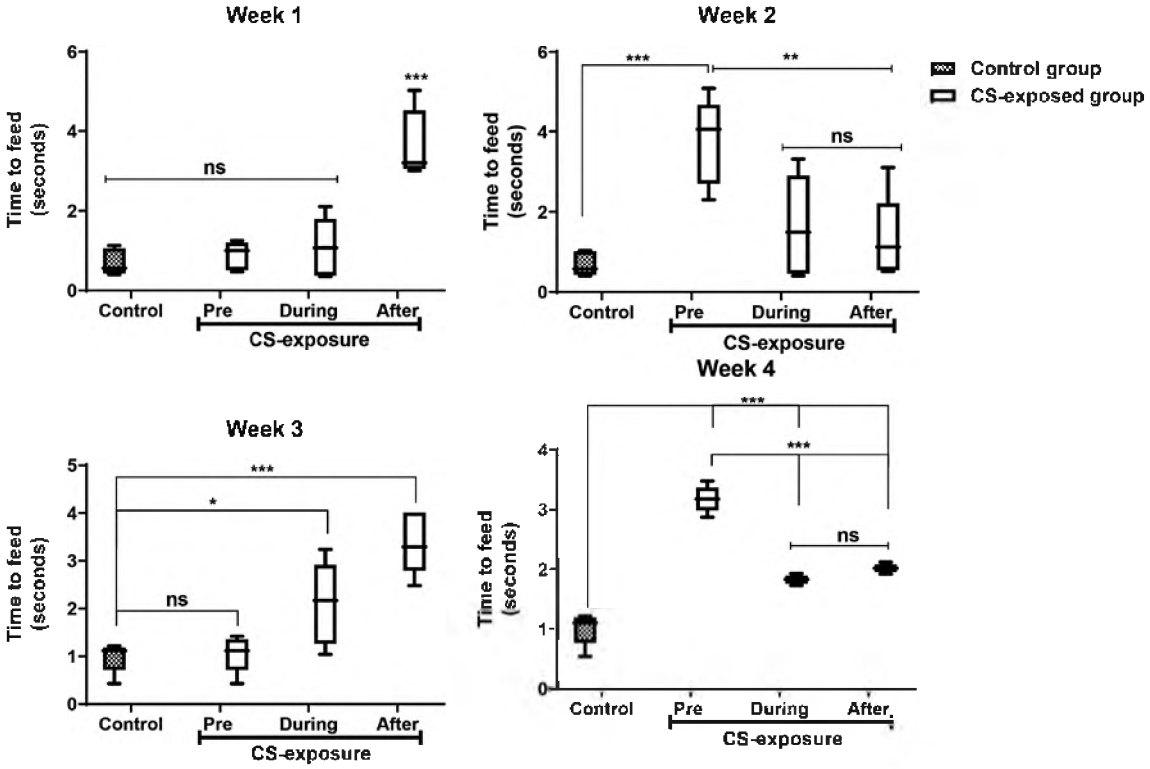

Fig. 2. Box and whisker plots of time to feed (seconds) in control and cigarette smoke (CS)-exposed groups
Data were collected for the seven days of each week. Significance: ns – not significant (i. e., $P > 0.05$); * – $P < 0.05$; ** – $P < 0.01$; *** – $P < 0.001$. Data were analysed with Kruskal Wallis non-parametric test

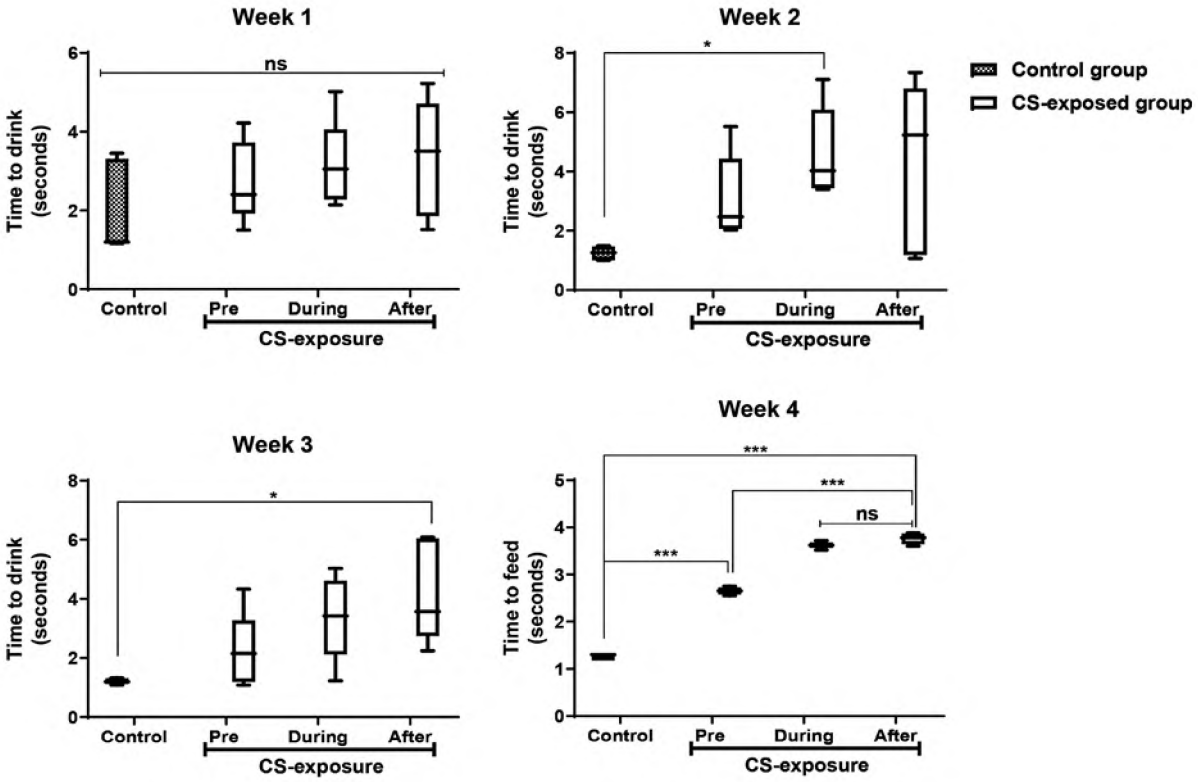

Fig. 3. Box and whisker plots of time to drink (seconds) in control and cigarette smoke (CS)-exposed groups
Data were collected for the seven days of each week. Significance: ns—not significant (i. e., $P > 0.05$); * – $P < 0.05$; ** – $P < 0.01$; *** – $P < 0.001$. Data were analysed with Kruskal Wallis non-parametric test

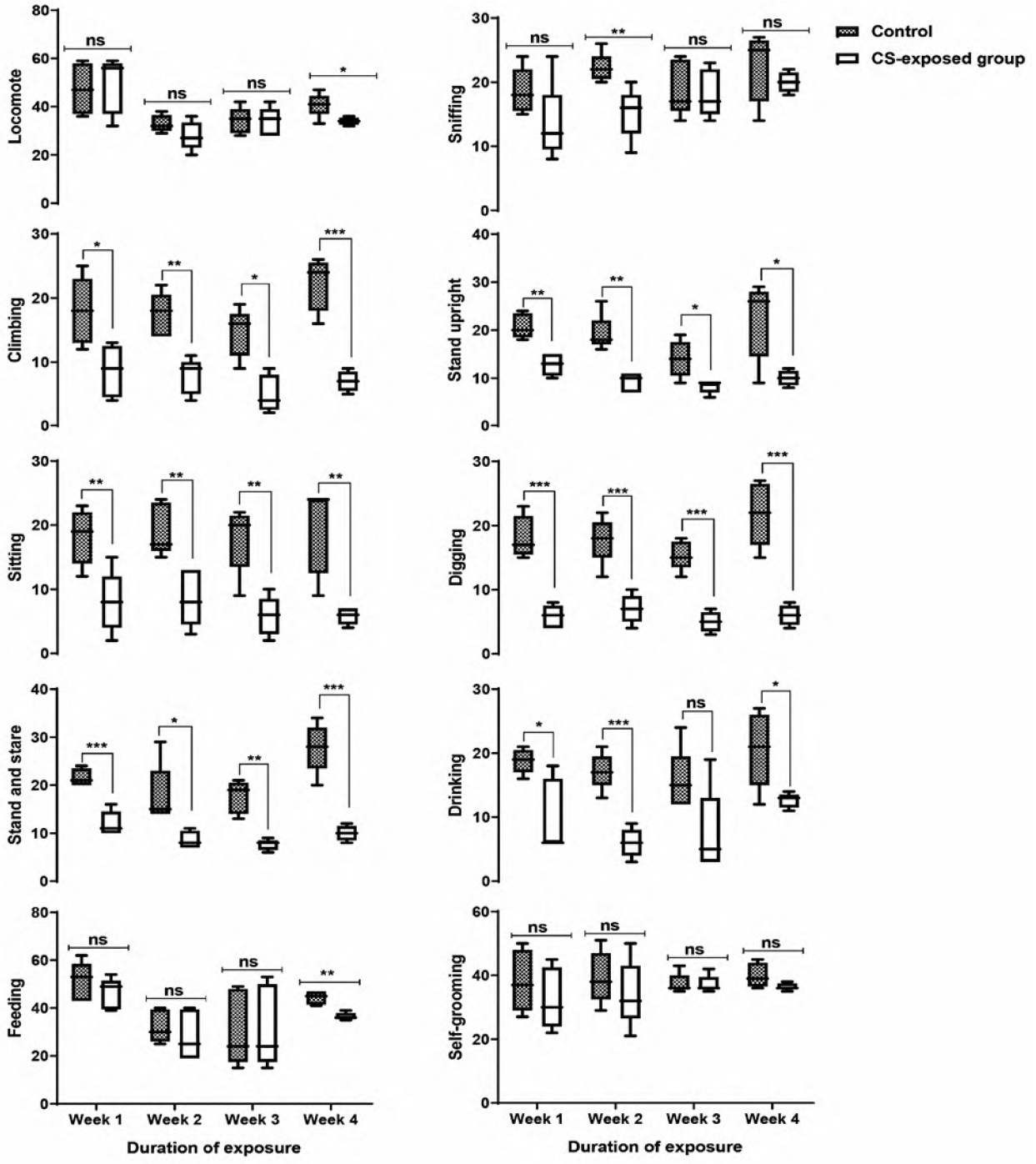

Fig. 4. Box and whisker plots of behavioural patterns in the control and cigarette smoke (CS)-exposed groups
Data were collected for the seven days of each week. Significance: ns – not significant (i. e., $P > 0.05$); * – $P < 0.05$; ** – $P < 0.01$; *** – $P < 0.001$. Data were analysed with Kruskal Wallis non-parametric test

ging, drinking, and self-grooming (Fig. 4). We report that CS distorted the behavioural patterns in exposed rats, characterized by reduced willingness to locomote or explore their environment (by climbing, stand and stare, digging). This may suggest reduced locomotor activity or anxiety (due to unwillingness to explore). Besides, reduced activity, anxiety, and panic-related behaviours have been reported in rats exposed to tobacco-free CS [6]. Smokers often report a sense of calm during and immediately after smoking, though transient [24]. This false sense of calm, attributed to satisfying one's nicotine craving, may be involved in the reduced behavioural activity, as observed in the CS-exposed rats.

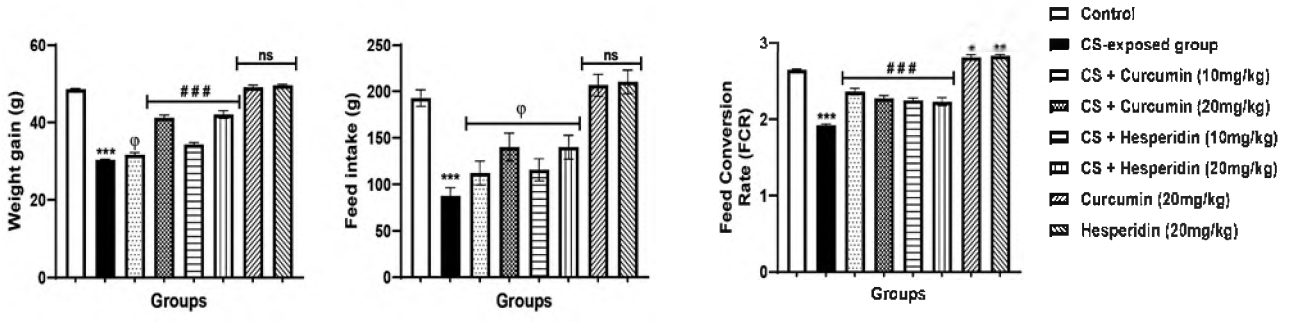

Fig. 5. Growth performance parameters of control and cigarette smoke (CS)-exposed groups treated with(out) curcumin and hesperidin
(A) Weight gain (g); (B) Feed intake (g); and (C) Feed conversion ratio (FCR). Bars are means ± SEM of six rats. Significance: ns—not significant compared to control; *, **, ***—significant compared to control at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively; φ – not significant compared to the CS-exposed group; # # # – significant compared to control at $P < 0.05$. Data from experiment 2 were analysed using one-way analysis of variance and Tukey test

ExCurcumin and hesperidin improves growth performance in CS-exposed rats (Experiment 2)

In the second experiment, we compared the effects of some nutritional supplements (curcumin and hesperidin) on growth performance parameters in the control and CS-exposed rats, while using a longer duration (6 weeks) than the first experiment. After 6 weeks of exposure, weight gain, feed intake, and FCR were significantly lower in the CS-exposed group compared to the control group (Fig. 5), which is in line with the works of A n d o n g et al [1] and Y p s i l a n t i s et al [27]. Nicotine, in the short term at least, upregulates energy expenditure, which impairing appetite, leading to reduced body weight gain [8], and may explain the reduced growth performance in rats exposed to CS. Treatment with curcumin and hesperidin, especially at the higher dose (20 mg.kg^{-1} b. wt.), significantly improved the weight gain and feed intake of the CS-exposed groups. Separate studies have reported similar beneficial effects on weight gain, feed intake, and FCR, in different animal models and under different conditions [12, 13, 20, 21]. Also, although the weight gain and feed intake in the normal rats treated with curcumin and hesperidin (at the dose of 20 mg.kg^{-1} b. wt.) were not significantly different ($P > 0.05$) compared to the control group, the FCR was significantly higher in both treatments compared to the control. This further corroborate the benefits of these nutritional supplements.

CONCLUSIONS

Exposure to cigarette smoke impairs exploratory and consummatory behaviour in rats, characterized by reduced feed and water intake as the duration of exposure is prolonged. Furthermore, behaviour patterns, such as standing, climbing, and locomotion, were impaired, suggesting that CS smoke might cause a reduction in the physical fitness and willingness to explore in the animals. More so, attributes such as digging, and sitting which might be taken as signs of comfort and wellbeing decreased significantly, suggesting an inherent discomfort to the animals due to CS exposure. Treatments of CS-exposed rats with curcumin and hesperidin demonstrated improvements in the growth performance, particularly at the higher dose (20 mg.kg^{-1} b. wt.).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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