

## Efficacy and safety of the metformin-mazindol anorectic combination in rat

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The current study investigates the anorectic interaction and safety of the mazindol-metformin combination in rats. Isobologram and interaction index were used to determine anorectic interaction between mazindol and metformin in the sweetened milk model. The safety profile of the mazindol-metformin combination was determined by measuring anxiety, blood pressure, hematic biometry and blood chemistry. An acute dose of mazindol and metformin administered *per os*, individually or as a mixture, has reduced the milk consumption in rats in a dose-dependent manner. Theoretical effective dose 40 ( $ED_{40t}$ ) did not differ from the experimental effective dose 40 ( $ED_{40e}$ ) obtained with the mazindol-metformin mixture in the anorexia experiments, by Student's *t*-test. In addition, the interaction index confirmed the additive anorectic effect between both drugs. A single oral dose of  $ED_{40e}$  mazindol-metformin mixture induced anxiolysis in the elevated plus-maze test. Moreover, oral administration of mazindol-metformin combination for 3 months significantly decreased glycemia, but not blood pressure nor other parameters of hematic biometry and blood chemistry. Results suggest that mazindol-metformin combination exerts an additive anorectic effect, as well as anxiolytic and hypoglycemic properties. Mazindol-metformin combination might be useful in obese patients with anxiety disorders or diabetes risk factors.

**Keywords:** mazindol-metformin combination, anorectic interaction, anxiolysis, hypoglycemic effect

Obesity, defined as abnormal or excessive fat accumulation, represents health risks and is recognized as a global epidemic. Obese patients have a higher risk of developing metabolic disorders such as dysmetabolic syndrome, diabetes mellitus and dyslipidemia, among others (1). In addition, there is a significant increase in the mortality rate if the body mass index is above  $25 \text{ kg m}^{-2}$  (2). On the contrary, it is well-accepted that a weight-loss of more than 3 % produces metabolic benefits in obese patients including diabetes prevention, better insulin sensitivity and glucose control as well as improved dyslipidemia (1).

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Lifestyle interventions are considered first-line therapy for overweight and obese patients. They include a nutritional plan, increased physical activity and behavioral modification techniques; however, most of the intensive lifestyle programs are difficult to follow under everyday conditions (1, 3). In addition, obesity bears a higher aetiological risk factor for developing diabetes mellitus than genetic, sex, diabetes family history, physical activity or dietary habits (4).

Pharmacotherapy used as an adjunct to lifestyle intervention enhances weight loss and maintains any loss for a longer period (1, 3). Multimodal therapy is a strategy recently used to treat obesity. Clinical evidence demonstrates that the combination of different medications with complementary mechanisms of action provides a greater body mass reduction with a better safety profile (5).

Metformin is the most widely used first-line drug for diabetes mellitus (1). It is a biguanide that induces weight loss by anorectic effects as it inhibits the activity of AMP-activated protein kinase in the hypothalamic neurons (6). Metformin also improves sensitivity to insulin and leptin (6, 7) and increases levels of glucagon-like peptide 1 (8, 9). Despite the multiple mechanisms proposed to explain its anorectic effect, metformin prevents the onset of diabetes, but induces only a weak weight loss in patients with diabetes risk factors (10). Therefore, we suggest that the combination of metformin with other anorectic drugs could increase their anti-obesity effectiveness, which could have a positive impact on the reduction of diabetes risk.

Mazindol is an anorectic drug unrelated to  $\beta$ -phenylethylamines, so it does not share the side-effects, addiction potential and misuse of amphetamines. Its proposed mechanism of action used to explain the anorectic effect is related to the stimulation of the catecholaminergic system (11). Mazindol also inhibits the activity of glucose-responsive neurons located in the lateral hypothalamus, resulting in decreased appetite and lowered gastric acid secretion (12). Furthermore, mazindol improves cholesterol, triglycerides and insulin levels (13, 14).

Based on the mentioned concerns, the current study was performed to investigate the anorectic interaction, metabolic improvement and the safety profile of the mazindol-metformin combination in rats.

## EXPERIMENTAL

### *Animals*

Male Wistar rats, 160–200 g, were used in the current study. Animals were obtained from the bioterium of the National Institute of Respiratory Diseases (Ciudad de México, México) and they were kept in polysulfone cages with free access to food (Laboratory Autoclavable Rodent Diet 5010, Lab Diet, USA) and water in a 12-hour light/dark cycle and at temperature 22–25 °C. The total number of animals was 126 and they were randomized in experimental groups which consisted of 6 rats each.

All experiments were performed in agreement with the requirements published in the Technical Specifications for the Production, Care and Use of Laboratory Animals (NOM-062-ZOO-1999, SAGARPA; México) and by National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals (National Academies Press, 2011, Washington, DC). Moreover, the protocol was approved by the institutional ethics committee, INER/CI/397/18 (Number, B29-18).

### *Drugs and treatments*

Mazindol (purity of 99.8 %) and metformin (purity of 100 %) were provided by Productos Medix S.A. de C.V. (CDMX, Mexico). Mazindol, metformin, or the mazindol-metformin combination were freshly suspended in the vehicle (a mixture of 90 % physiological saline and 10 % Tween 80) used in a volume of 4 mL kg<sup>-1</sup> bm. Control group received only vehicle (4 mL kg<sup>-1</sup> bm). Oral dosing of the vehicle and the drugs was done using a metal feeding curved needle (Model 7914, Cadene Science, USA).

Experiments were divided into acute (a single oral dose) and chronic (a daily oral dose for 90 days) procedures. In the acute procedures, vehicle, metformin (30–300 mg kg<sup>-1</sup> bm), mazindol (1.7–30 mg kg<sup>-1</sup> bm) or the theoretical total doses of mazindol-metformin ( $ED_{5t} = 13.9$ ,  $ED_{10t} = 27.8$ ,  $ED_{20t} = 55.5$  and  $ED_{40t} = 111.0$  mg kg<sup>-1</sup> bm) were orally administered 30 minutes before the start of the anorexia test, whereas vehicle, metformin (208.9 mg kg<sup>-1</sup> bm), mazindol (13.2 mg kg<sup>-1</sup> bm), or mazindol-metformin mixture (128.7 mg kg<sup>-1</sup> bm) were orally administered just before the start of the anxiety test. In the chronic procedures, vehicle, metformin (208.9 mg kg<sup>-1</sup> bm), mazindol (13.2 mg kg<sup>-1</sup> bm), or the experimental total dose of mazindol-metformin ( $ED_{40e} = 128.7$  mg kg<sup>-1</sup> bm) was orally administered once a day, at 10:00 a.m., for 90 days. Anxiety, cardiovascular variables and blood parameters were measured on days 30, 60 and 90, before daily drug administration. Body mass was recorded weekly before the daily drug administration.

### *Anorectic effect evaluation*

To determine the anorectic effect of mazindol, metformin or their combination, the reduction of the sweetened milk intake was measured, as previously described by Cruz-Álvarez *et al.* (15). Rats were habituated for 7 days to drink sweetened milk for 90 min. On the 8<sup>th</sup> day, 12-h fasted rats were orally administered the vehicle or increasing doses of mazindol (1.7–30 mg kg<sup>-1</sup> bm), metformin (30–300 mg kg<sup>-1</sup> bm) or mazindol-metformin mixture. The sweetened milk was offered to the rats and the total milk intake was measured after 1.5 h.

To prepare the sweetened milk, 1 L of boiled water was mixed with 100 g of powdered milk and 100 g of sucrose.

### *Safety profile evaluation*

To compare the safety profile of mazindol, metformin or mazindol-metformin, the doses were fixed at 40 % of anorectic effect obtained experimentally ( $ED_{40e}$ ) for each drug treatment.

Thus, the anxiolytic effect of oral mazindol ( $ED_{40e} = 13.2$  mg kg<sup>-1</sup> bm), metformin ( $ED_{40e} = 208.9$  mg kg<sup>-1</sup> bm) or mazindol-metformin ( $ED_{40e} = 128.7$  mg kg<sup>-1</sup> bm), was evaluated using the elevated plus-maze model designated by Pellow and colleagues (16). The mazindol-metformin mixture was tested at a fixed-ratio of 1:1 in order to have a high probability of identifying the anorectic interaction, if any.

Briefly, the plus-maze had two closed arms and two open arms connected by a central platform. The four arms are elevated 50 cm from the floor and separated 90° each from the other. To test the anxiolytic effect, rats were administered orally with vehicle or with any of the treatments described above and placed in the center of the platform with their head

facing one of the open arms. Then, free exploration was allowed for 5 min. During this period, the percentage of open arm entries and the percentage of time spent on the open arms were measured. The increase in the percentage of open arm entries and the percentage of time spent on the open arms is considered as an anxiolytic effect. In acute experiments, anxiety was evaluated in 12-h fasted rats before the treatment and at 1, 2, 3, 4, 6, and 8 h after a single oral dose administration. In chronic experiments, the drugs were given once a day for 3 months and the anxiety was measured in 12-h fasted rats before the drug administration and at 1, 2 and 3 months, 24 h after the last administration.

In order to determine the chronic effect of the oral mazindol-metformin combination on body mass, the animals were administered daily with vehicle or mazindol-metformin ( $ED_{40e} = 128.7 \text{ mg kg}^{-1} \text{ bm}$ ), for 90 days. During this period, the body mass of the rats was recorded every week using an Ohaus Scout<sup>®</sup> balance H-7294 (Ohaus, Mexico).

In order to establish the cardiovascular effect of the chronic administration of the mazindol-metformin mixture, the blood pressure was measured in rats by a tail-cuff plethysmography blood pressure system (MRBP, IITC Life Sciences, USA). Animals were placed into an acrylic restrainer for 15 min during 3 consecutive days to allow them to adapt to the experimental conditions. The next day, rats were gently collocated inside the chamber for 15 min and baseline diastolic blood pressure and systolic blood pressure were acquired. After that, vehicle or mazindol ( $ED_{40e} = 13.2 \text{ mg kg}^{-1} \text{ bm}$ ), metformin ( $ED_{40e} = 208.9 \text{ mg kg}^{-1} \text{ bm}$ ) or mazindol-metformin combination ( $ED_{40e} = 128.7 \text{ mg kg}^{-1} \text{ bm}$ ) were orally administered daily during 3 months; diastolic and systolic blood pressure were recorded in 12-h fasted rats before the treatment and at 1, 2 and 3 months, 24 h after the last administration.

To check the health status of the rats after chronic administration of oral mazindol-metformin combination ( $ED_{40e} = 128.7 \text{ mg kg}^{-1} \text{ bm}$ ), 500  $\mu\text{L}$  of blood were taken from their lateral tail by a 23-gauge syringe needle, then the blood samples were tested in a BC7000 hematology analyzer (Kontrolab, Italy) and a dry chemistry analyzer HB1 (Skyla, China). Parameters were measured in 12-h fasted rats before the treatment and at 1, 2 and 3 months, 24 h after the last administration.

### Data analysis

$ED_{40e}$  for mazindol and metformin were obtained from their respective log dose-response curves by linear regression.  $ED_{40e}$  was chosen for the interaction analysis of mazindol-metformin due to the fact that metformin was not able to reach an anorectic effect of 50 % in the sweetened milk intake test (48.6 %).

To determine the type of interaction, theoretical effective doses of the combination were calculated using a fixed-dose ratio of 0.5: 0.5 and fractions of the  $ED_{40e}$  for mazindol and metformin by the following equation:

$$ED_{xt} = 0.5 (ED_{xe} \text{ of mazindol}) + 0.5 (ED_{xe} \text{ of metformin})$$

where,  $x = 5, 10, 20$  or  $40 \%$ . Thus,  $ED_{5t} = 0.8 + 13.1 \text{ mg kg}^{-1} \text{ bm}$ ,  $ED_{10t} = 1.7 + 26.1 \text{ mg kg}^{-1} \text{ bm}$ ,  $ED_{20t} = 3.3 + 52.2 \text{ mg kg}^{-1} \text{ bm}$ , and  $ED_{40t} = 6.6 + 104.4 \text{ mg kg}^{-1} \text{ bm}$ , resp. Then, theoretical doses were tested in the sweetened milk intake test, and  $ED_{40e}$  for mazindol-metformin ( $7.7 + 121.0 \text{ mg kg}^{-1} \text{ bm}$ , resp.) was interpolated from its log dose-response curve obtained experimentally. The fixed-dose ratio of 0.5:0.5 was established to increase the

probability of identifying any anorectic interaction. For the sake of clarity, the doses for the combination are shown as the sum of mazindol and metformin doses throughout the text.

In order to analyze the type of interaction of mazindol-metformin, isobologram was constructed plotting the  $ED_{40e}$  value of mazindol on the abscissa and the  $ED_{40e}$  value of metformin on the ordinate. Later, a diagonal line connecting both  $ED_{40e}$  values was traced to obtain the theoretical line of additivity; finally, either  $ED_{40e}$  or  $ED_{40t}$  for the combination were added within the isobologram.

To determine the type of interaction of mazindol-metformin theoretical effective dose 40 ( $ED_{40t}$ ) and  $ED_{40e}$  values were compared by Student's *t*-test, as well as by the interaction index ( $\gamma = ED_{40e}/ED_{40t}$ ), confidence intervals and isobologram plot (17, 18). The additivity between mazindol and metformin was established if  $ED_{40t}$  was not different from  $ED_{40e}$  by Student's *t*-test,  $ED_{40t}$  confidence interval overlapped the  $ED_{40e}$  confidence interval,  $\gamma$  was close to 1,  $\gamma$  confidence interval crossed through 1 and  $ED_{40e}$  was near to additivity line in the isobologram plot.

To determine the intensity and duration of anxiolytic effect with each treatment, the area under the curve (% open arm entries against time or % time spent on the open arms against time) was calculated by the trapezoidal rule.

All statistical differences between treatments were obtained by one-way analysis of variance (ANOVA), followed by Dunnett's test whereas differences with respect to the vehicle group, in time-course experiments, were analyzed by two-way ANOVA followed by Tukey's test. Experimental differences were statistically different if the *p*-value was less than 0.05.

All results are presented as the mean  $\pm$  standard error of the mean (SEM) of 6 rats.

## RESULTS AND DISCUSSION

### *Anorectic effects of mazindol and metformin alone*

The anorectic effect of mazindol, metformin and mazindol-metformin combination was assessed using the sweetened milk intake test. Increasing doses of acute oral administration of mazindol (1.7–30 mg kg<sup>-1</sup> bm) dose-dependently reduced milk intake (Fig. 1a). Thus,

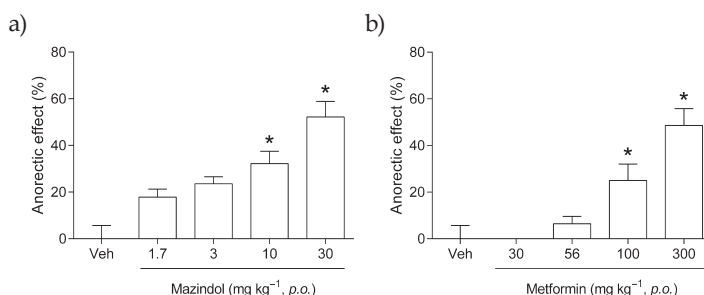


Fig. 1. Anorectic effect of individual treatment with: a) mazindol and b) metformin. Given are means and SEM bars,  $n = 6$ . \* Significant difference vs. vehicle group (Veh):  $p < 0.05$ .

Table I. Experimental effective doses 40 ( $ED_{40e}$ ) of mazindol or metformin

Drug	$ED_{40e} \pm \text{SEM}^a$	$R^2$
Mazindol (mg kg <sup>-1</sup> bm)	13.2 ± 2.8	0.953
Metformin (mg kg <sup>-1</sup> bm)	208.9 ± 27.0	0.979

<sup>a</sup> $n = 24$ .

mazindol had an anorectic efficacy of  $52.1 \pm 6.8\%$  at the dose of  $30 \text{ mg kg}^{-1} \text{ bm}$  and an  $ED_{40e} = 13.2 \pm 2.8 \text{ mg kg}^{-1} \text{ bm}$  (Table I). Previous studies have pointed out that the mazindol treatment was able to reduce the body mass increase and calorie intake in the models of ventromedial hypothalamic injured obese rats (14) and obese diabetic yellow KK mice (19). Concordantly, several double-blind, placebo-controlled clinical studies indicated that mazindol is more effective than a placebo in achieving weight loss (11, 13). Moreover, a recent meta-analysis confirmed that mazindol induced more weight loss than a placebo (20).

Also, metformin administered orally ( $30\text{--}300 \text{ mg kg}^{-1} \text{ bm}$ ) had decreased the sweetened milk consumption in a dose-dependent manner (Fig. 1b). According to the plot, metformin reached an anorectic effect of  $48.6 \pm 17.8\%$  at  $300 \text{ mg kg}^{-1} \text{ bm}$  and it had an  $ED_{40e} = 208.9 \pm 27.0 \text{ mg kg}^{-1} \text{ bm}$  (Table 1). In line with these results, metformin-induced weight loss in obese rats (21) and mice (22) and, decreased body mass in chickens (23). Moreover, a growing body of evidence demonstrates that metformin induces a modest weight loss in human beings (24–26). In this regard, the current study confirms the anorectic effect of mazindol and metformin individually in the sweetened milk intake test in rats.

#### Effect of mazindol-metformin combination on anorexia and body mass

To determine the anorectic interaction of mazindol and metformin in sweetened milk intake test, the  $ED_{5t} = 13.9 \text{ mg kg}^{-1} \text{ bm}$ ,  $ED_{10t} = 27.8 \text{ mg kg}^{-1} \text{ bm}$ ,  $ED_{20t} = 55.5 \text{ mg kg}^{-1} \text{ bm}$ , and  $ED_{40t} = 111.0 \text{ mg kg}^{-1} \text{ bm}$  were tested experimentally. The acute oral administration of mazindol-metformin reduced in a dose-dependent manner the sweetened milk consumption reaching experimentally an anorectic effect of  $40.0 \pm 6.1\%$  at the highest tested dose. In addition, the linear regression analysis for the dose-response curve of the combination showed  $ED_{40e} = 128.7 \pm 25.0 \text{ mg kg}^{-1} \text{ bm}$  (Fig. 2a). Contrary to our results, Iorio *et al.* (27) indicated that amobarbital, diazepam, thioridazine and prochlorperazine did not affect the decrease in food consumption induced by mazindol. This difference may be due to the mechanism of action of the drugs used in combination with mazindol (GABAA agonism or D2 antagonism *vs.* inhibition of AMP-kinase).

In the interaction analysis, the  $ED_{40e}$  ( $128.7 \pm 25.0 \text{ mg kg}^{-1} \text{ bm}$ ) of the mazindol-metformin combination was not statistically different from  $ED_{40t}$  ( $111.1 \pm 13.6 \text{ mg kg}^{-1}$ ) calculated for this combination (Table II), as is shown in the isobologram plot where the  $ED_{40e}$  is very near to  $ED_{40t}$  value (Fig. 2b). Concordantly, the  $ED_{40e}$  confidence interval of the mazindol-metformin overlapped with the theoretical  $ED_{40t}$  confidence interval of this combination, the interaction index ( $\gamma$ ) was around 1 and its confidence interval passed through 1 (Table II). In this regard, both, statistical and graphic analysis indicate that mazindol-metformin combination shows an additive anorectic effect in 12-h deprived rats.

Table II. Statistical analysis of mazindol-metformin combination

Mazindol-metformin combination (mg kg <sup>-1</sup> bm)		γ ± SEM (CI at 90 %) <sup>a</sup>
Theoretical <i>ED</i> <sub>40t</sub> ± SEM (CI at 90 %) <sup>a</sup>	Experimental <i>ED</i> <sub>40e</sub> ± SEM (CI at 90 %) <sup>a</sup>	
111.1 ± 13.6 (79.1–156.0)	128.7 ± 25.0 (55.8–296.9)	1.16 ± 0.27 (0.70–1.93)

<sup>a</sup> *n* = 24.

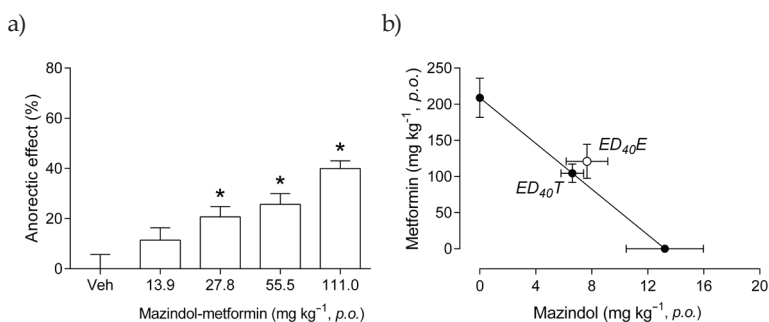


Fig. 2. a) Anorectic effect and b) isobolographic analysis of the combination mazindol + metformin in 12-h fasted rats. Given are means and SEM bars, *n* = 6. \* Significant difference *vs.* vehicle group (Veh): *p* < 0.05. *ED*<sub>40t</sub> (●) – theoretical effective dose for 40 % effect, *ED*<sub>40e</sub> (○) – experimental effective dose for 40 % effect.

The additive anorectic effect of the mazindol-metformin combination may be understood by the complementary mechanisms of both drugs. The mechanism of anorectic action of mazindol probably includes the stimulation of catecholaminergic systems in the central nervous system, including dopamine (28), serotonin (29) and noradrenaline (30). Additionally, mazindol also seems to reduce the food intake through suppression of the firing rate of glucose-sensitive neurons in the lateral hypothalamus by a dopamine-independent mechanism (12, 31). Moreover, it was suggested that mazindol may induce weight loss stimulating thermogenesis *via* increased noradrenaline turnover in the brown adipose tissue (19), inhibiting the gastric acid secretion (12) and absorption of glucose in the small intestine (32), increasing glucose uptake by the skeletal muscle (33) and reducing hypersecretion of insulin from the ventromedial hypothalamic area (14). In a complementary manner, metformin induces anorexia by inhibition of ghrelin-induced hypothalamic AMP-kinase/acetyl-CoA carboxylase/regulatory-associated protein of mTOR signaling (34). It also seems to down-regulate orexigenic peptides as agouti-related protein or neuropeptide Y and, increase anorexigenic peptides as pro-opiomelanocortin, in the hypothalamus, through STAT3 signaling pathway (35). Furthermore, it has been demonstrated that metformin improves the sensitivity to leptin and insulin, two hormones capable of modulating AMP-kinase and STAT3 in the hypothalamus, to produce anorexia (6, 34). Alike, metformin

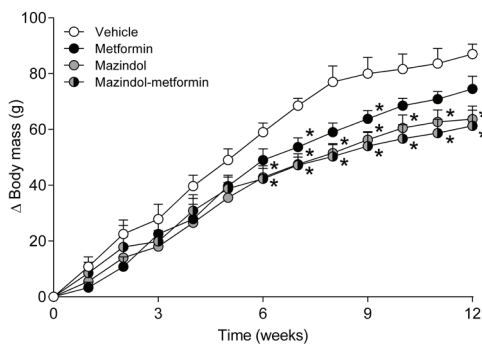


Fig. 3.  $ED_{40e}$  of mazindol ( $13.2 \text{ mg kg}^{-1} \text{ bm}$ ), metformin ( $208.9 \text{ mg kg}^{-1} \text{ bm}$ ) or mazindol-metformin combination ( $128.7 \text{ mg kg}^{-1} \text{ bm}$ ) on body mass. Given are means and SEM bars,  $n = 6$ . \* Significant difference *vs.* vehicle group,  $p < 0.05$ .

enhances glucagon-like peptide-1 level to induce satiation, through the inhibition of dipeptidyl peptidase-IV, an enzyme that degrades importantly to glucagon-like peptide-1 (8) and the activation of muscarinic and gastrin-releasing peptide pathways (9). The current data seems to indicate that mazindol-metformin combination may be achieved, due to their complementary mechanisms of action on the regulation of food intake, in lower doses of each drug, thus demonstrating additive action with a reduction of dose-related adverse events.

In addition, chronic administration of mazindol-metformin ( $ED_{40e} = 128.7 \text{ mg kg}^{-1} \text{ bm}$ ) for 90 days significantly decreased the body mass in rats from week 6 onwards in respect to the vehicle group, but not mazindol ( $ED_{40e} = 13.2 \text{ mg kg}^{-1} \text{ bm}$ ) or metformin ( $ED_{40e} = 208.9 \text{ mg kg}^{-1} \text{ bm}$ ) groups (Fig. 3). Thus, the current study demonstrates for the first time that mazindol-metformin combination has an additive anorectic effect, which in turn impacts the body mass.

#### *Safety profile of the mazindol-metformin combination*

Fig. 4 shows that a single dose of  $ED_{40e}$  of mazindol ( $13.2 \text{ mg kg}^{-1} \text{ bm}$ ), but not  $ED_{40e}$  of metformin ( $208.9 \text{ mg kg}^{-1} \text{ bm}$ ), enhanced significantly the time spent in the open arms (Figs. 4a,b) and open arm entries (Figs. 4c,d) in comparison to the vehicle group, in 12-h food-deprived rats. Interestingly, in the mazindol-metformin group ( $ED_{40e} = 128.7 \text{ mg kg}^{-1} \text{ bm}$ ), metformin was able to maintain the anxiolytic effect of mazindol with approximately the half of the mazindol dose used individually (Fig. 4). This finding seems to demonstrate for the first time the anxiolytic effect of mazindol, but this should be confirmed in other anxiolytic models since the plus-maze model loses predictive validity in the evaluation of drugs that act by mechanisms which do not include GABAergic system (36). Thus, the apparent anxiolytic effect could be also explained by an increase in motor activity induced by mazindol (37). The results of the current study allow us to suggest that mazindol-metformin combination may be directed to obese patients with anxiety disorders due to the fact that there is a positive association between obesity and anxiety (38) and, the prevalence of anxiety disorders as binge eating disorder in overweight patients is around 20 % (39).



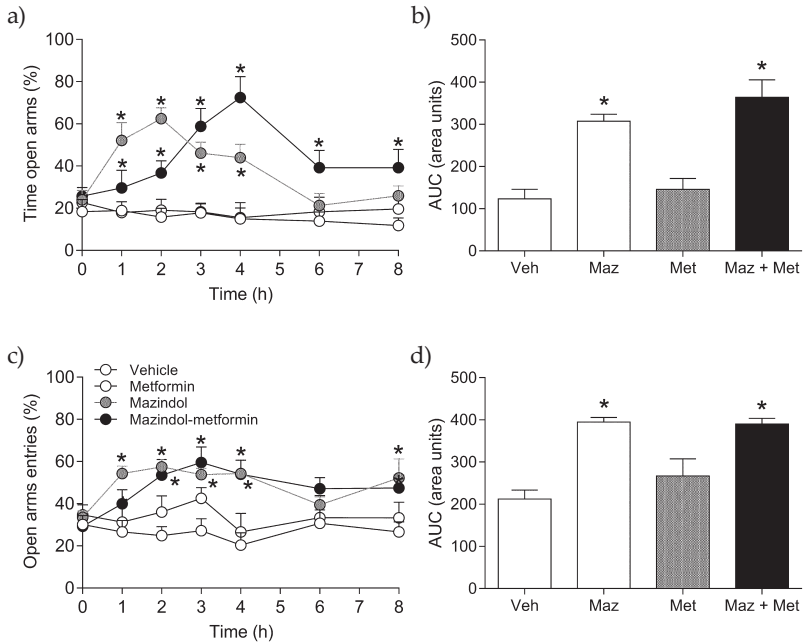


Fig. 4. The anxiolytic effect of  $ED_{40e}$  of mazindol ( $13.2 \text{ mg kg}^{-1} \text{ bm}$ ), metformin ( $208.9 \text{ mg kg}^{-1} \text{ bm}$ ) and mazindol-metformin mixture ( $128.7 \text{ mg kg}^{-1} \text{ bm}$ ) in the plus maze task. Given are means and SEM bars,  $n = 6$ . \* Significant difference vs. vehicle group,  $p < 0.05$ .

Oral administration for 90 days of mazindol, metformin or mazindol-metformin combination did not change significantly diastolic or systolic blood pressure. Likewise, blood cell and red blood cell count remained constant throughout the pharmacological treatment. On the contrary, glucose level, but not other blood chemistry parameters, was reduced from  $5.6 \pm 0.4$  to  $5.0 \pm 0.5 \text{ mmol L}^{-1}$ , and  $5.6 \pm 0.5$  to  $4.6 \pm 0.6 \text{ mmol L}^{-1}$  after 3 months of the chronic metformin alone (data not shown) or mazindol-metformin combination (Table III) treatment, resp. These data suggested a good tolerability profile of the combination and seems to show that mazindol enhances the glycemetic control induced by metformin. Taking in consideration that obesity is the strongest aetiological risk factor for developing type 2 diabetes (4) and that metformin is considered as a suitable candidate for diabetes prophylaxis, but with the disadvantage of a weak clinical weight-reduction (1), we suggest that mazindol-metformin combination may be especially useful as weight loss medication in obese patients with diabetes risk factors because of its anorectic and hypoglycemic effects. In line with the current results, the literature showed that metformin reduces the incidence of persons at high risk for diabetes mellitus (40) and mazindol reduces the blood glucose and insulin concentrations in mice (19).

Moreover, in the current study, acute administration of mazindol, but not metformin, showed mild piloerection, stereotyped movements of head and forepaws and walking in circles (data not shown), at the highest dose tested, indicating disturbances at the central nervous system level. These adverse events are in line with a previous study where the

Table III. Effect of ED<sub>40e</sub> of mazindol-metformin combination on blood chemistry, hematic biometry and blood pressure in rats

Parameter	Mazindol-metformin combination			
	Duration (month)			
	0	1	2	3
	Blood chemistry <sup>a</sup>			
Albumin (g L <sup>-1</sup> )	37.7 ± 4.6	37.3 ± 2.0	41.0 ± 2.0	41.4 ± 2.1
Globulins (g L <sup>-1</sup> )	23.2 ± 8.7	20.5 ± 4.6	21.7 ± 2.5	22.6 ± 2.2
Total protein (g L <sup>-1</sup> )	60.9 ± 12.7	57.8 ± 5.5	62.7 ± 4.1	64.0 ± 2.6
Glucose (mmol L <sup>-1</sup> )	5.6 ± 0.5	4.8 ± 0.6	4.7 ± 0.5*	4.6 ± 0.6*
Alkaline phosphatase (U L <sup>-1</sup> )	191.7 ± 55.6	211.3 ± 52.5	175.3 ± 55.8	189.4 ± 51.5
Alkaline aminotransferase (U L <sup>-1</sup> )	34.3 ± 3.4	40.3 ± 4.6	36.8 ± 5.7	36.4 ± 5.7
Amylase (U L <sup>-1</sup> )	166.5 ± 27.8	158.5 ± 19.5	184.5 ± 18.1	173.2 ± 21.7
Uremic nitrogen (mmol L <sup>-1</sup> )	6.2 ± 0.8	6.3 ± 0.5	6.5 ± 0.5	6.2 ± 0.5
Creatinine (µmol L <sup>-1</sup> )	31.7 ± 5.2	34.0 ± 8.3	36.2 ± 10.2	33.4 ± 7.4
Calcium (mmol L <sup>-1</sup> )	2.02 ± 0.22	2.07 ± 0.12	2.23 ± 0.13	2.16 ± 0.06
Phosphate (mmol L <sup>-1</sup> )	1.86 ± 0.10	1.90 ± 0.14	1.76 ± 0.21	1.80 ± 0.07
Sodium (mmol L <sup>-1</sup> )	153.7 ± 2.4	155.2 ± 1.9	151.8 ± 2.9	154.0 ± 2.0
Potassium (mmol L <sup>-1</sup> )	6.3 ± 0.2	6.7 ± 0.5	6.4 ± 0.3	6.4 ± 0.3
	Hematic biometry <sup>a</sup>			
White blood cells (×10 <sup>9</sup> cells L <sup>-1</sup> )	6.5 ± 1.5	6.6 ± 1.3	6.6 ± 0.9	6.7 ± 0.7
Lymphocytes (×10 <sup>9</sup> cells L <sup>-1</sup> )	1.0 ± 0.5	0.8 ± 0.2	0.8 ± 0.2	1.0 ± 0.2
Monocytes (×10 <sup>9</sup> cells L <sup>-1</sup> )	3.0 ± 0.5	3.3 ± 0.6	3.5 ± 0.4	3.3 ± 0.5
Granulocytes (×10 <sup>9</sup> cells L <sup>-1</sup> )	2.4 ± 0.7	2.6 ± 0.7	2.3 ± 0.5	2.4 ± 0.5
Red blood cells (×10 <sup>12</sup> cells L <sup>-1</sup> )	8.0 ± 0.7	8.1 ± 0.7	8.1 ± 0.6	8.0 ± 0.4
Hemoglobin (g L <sup>-1</sup> )	122.0 ± 8.3	125.5 ± 7.9	126.8 ± 10.0	124.8 ± 7.3
Hematocrit (%)	48.4 ± 4.2	51.9 ± 3.7	52.5 ± 4.6	51.9 ± 1.9
Platelets (×10 <sup>9</sup> cells L <sup>-1</sup> )	338.8 ± 57.2	279.5 ± 61.6	297.0 ± 61.1	296.2 ± 45.0
Mean platelet volume (fL)	15.00 ± 0.82	15.85 ± 0.80	15.70 ± 0.61	15.34 ± 0.54
Plateletcrit (%)	0.45 ± 0.08	0.43 ± 0.07	0.46 ± 0.08	0.42 ± 0.07
	Blood pressure <sup>a</sup>			
Systolic blood pressure (mm Hg)	119.4 ± 1.3	117.4 ± 1.3	120.2 ± 3.5	122.3 ± 2.1
Diastolic blood pressure (mm Hg)	91.0 ± 0.5	97.8 ± 3.5	90.8 ± 1.1	92.3 ± 1.2

<sup>a</sup> Mean ± SEM, n = 6.

\* Significant difference vs. basal value (time 0); p < 0.05.

authors showed that mazindol triggers stereotyped behaviors as sniffing, licking and false bites (37). Importantly, the mazindol-metformin combination did not show such effects confirming that metformin improves the mazindol safety profile.

## CONCLUSIONS

The results suggest that mazindol-metformin combination shows an additive anorectic effect, as well as hypoglycemic and anxiolytic properties, with a good safety profile. Thus, mazindol-metformin may be a therapeutic option as initial treatment for obesity in patients with diabetes risk factors and/or anxiety disorders, which deserves to be further studied in clinical trials.

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