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Original Article

Effect of *Theobroma cocoa* Extract on Olanzapine-Induced Metabolic Syndrome in Rats

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ABSTRACT

Objectives: This study examined the effects of *Theobroma cocoa* hydro-alcoholic Extract (TCE) on the metabolic syndrome that rats developed because of olanzapine. **Materials and Methods:** Dry cocoa powder was macerated in 80% ethanol for seven days to create the TCE. Filtration and concentration were then carried out under lower pressure. Rats (*n*=6) were divided into five groups. Three dosages of TCE (100, 200, and 400 mg/kg) were administered orally for 21 days along with olanzapine (2 mg/kg/day, intraperitoneally). Body weight was measured every three days, and food intake was recorded every day. An Oral Glucose Tolerance Test (OGTT) was conducted last week. The lipid profiles that were assessed on the final day included Triglycerides (TG), Total Cholesterol (TC), HDL, LDL, and VLDL levels. The oxidative stress indicators Glutathione (GSH), Catalase (CAT), Superoxide Dismutase (SOD), and Malondialdehyde (MDA) were measured in liver tissue. **Results:** While olanzapine dosing decreased GSH, SOD, and HDL levels in the liver, it markedly increased weight gain, food consumption, TG, LDL, TC, VLDL, and MDA. The weight of adipose and hepatic tissue was also increased, while locomotor activity was decreased. Treatment with TCE at different doses significantly decreased food intake, TG, LDL, TC, VLDL, MDA, and weight gain. In addition, TCE decreased liver and adipose tissue weight, increased locomotor activity, and improved GSH, SOD, and HDL levels in comparison to the olanzapine group. According to these results, TCE reduces the oxidative and metabolic stress brought on by olanzapine. **Conclusion:** According to the study, olanzapine-induced weight gain, oxidative stress, abnormalities in lipid metabolism, and decreased locomotor activity are all mitigated by *Theobroma cocoa* extract.

Keywords: Olanzepine, Locomotor activity, Lipid metabolism, Weight gain, *Theobroma cocoa*.

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INTRODUCTION

Olanzapine, a second-generation antipsychotic that was approved by the FDA in 1996 for the treatment of schizophrenia, has been available in generic form since 2011. Additionally, it is utilized as a long-acting injectable for schizophrenia and in conjunction with fluoxetine to treat treatment-resistant depression and bipolar depression.[1-3] Olanzapine, however, has serious side effects, especially weight gain[4,5] and a higher risk of metabolic syndrome, which includes diabetes, insulin resistance, and dyslipidemia.[6-8] Several strategies have been investigated to treat olanzapine-induced weight gain, including mixing olanzapine with betahistine, metformin, and curcumin, zingiber officinale,[9-11] Despite these attempts, weight gain remains a polygenic condition involving several proteins that contribute to metabolic syndrome and obesity. There is still a need for therapeutic approaches that can reduce these adverse effects without compromising olanzapine's therapeutic benefits.

Potential remedies offered by Ayurvedic medicine include the use of herbal remedies such as Theobroma cacao, a plant that is abundant in secondary metabolites and comes from the Amazon and Central and South America. Criollo, Forastero, and Trinitario are the three primary cultivar groupings that are used to identify Theobroma cacao, which is a member of the sterculiaceae family.[12,13] Previous studies have shown that cocoa can positively impact diabetes,[14] obesity,[15,16] dyslipidemia,[17-19] hypertension.[20,21] In previous A recent study describes dyslipidemia as a lipoprotein metabolism disorder characterized by excesses and deficits of total cholesterol, Low-Density Lipoprotein (LDL) and triglycerides. High-Density Lipoprotein (HDL) increases lipid peroxidation and lowers antioxidant enzyme levels, aggravating oxidative stress. The blood metabolites of Nitric Oxide (NO), blood nitrate and Nitrite (NOx), are reduced by oxidative stress. Flavanol antioxidants such as procyanidin, epicatechin, and catechin are abundant in extract. The disorder dyslipidemia, which is marked by elevated HDL and decreased LDL, triglyceride, and total cholesterol levels,[18,22]

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The effects of a hydroalcoholic extract of *Theobroma cocoa* on weight growth, food intake, lipid profile, oxidative stress markers, and organ weight will be examined in a model of olanzapine-induced weight gain using female Sprague-Dawley rats. The goal is to ascertain whether cocoa can lessen olanzapine's metabolic side effects without compromising the drug's therapeutic efficacy.

MATERIALS AND METHODS

Animals

National Lacsmi Bio Farm, Pune, provided healthy female Sprague-Dawley rats weighing 180±10 g (CPCSEA Reg. No: 1277/PO/RcBt/S/09/CPCSEA). The rats were given a regular pellet meal and unlimited water while living in suboptimal settings (22-25ºC, 65-70% humidity, and a 12:12 light/dark cycle). Pravara Rural College of Pharmacy's IAEC accepted the study protocol (CPCSEA Reg. No: 1942/PO/Re/S/17/CPCSEA/2023/01/05/01). Prior to the trial, the animals were acclimated to a 12:12 light/dark cycle for 7 days.

Chemicals

Olanzapine was bought in Mumbai, India, from Yarrow Chem Products. 0.1 N hydrochloride acid was used to dissolve olanzapine, 0.1 N NaOH was used to bring the pH down to 5.5 and adjust the final volume by using distilled water.[10]

Collection and preparation of hydroalcoholic extraction of *Theobroma cocoa* (TCE)

We bought cocoa seeds from VALLEYSPICE, which is based in Kerala, India's Idukki District. The gathered pods were cleaned, sliced, let to dry in the shade, and then ground into a coarse powder in a pulverized. Petroleum ether (bp 40-60ºC) was used to defat the powder, and it was then macerated for a week in 80% ethanol. After that, the extract was lyophilized, concentrated, filtered, and kept for later use in an airtight container.[23]

Total Phenol Content

The total phenolic content of the cocoa seed extracts was determined using the Folin-Ciocalteu technique. 100 µL of each of the extract's various strengths was mixed with 1.5 mL of 2% (w/v) $NaCO_3$ and 0.5 mL of Folin-Ciocalteu reagent (1:10 dilution). The mixture was incubated at room temperature for 15 min. Absorbance was measured at 765 nm. The total phenolic content was evaluated in milligram of gallic acid equivalents (µg GAE/mg extract).[24]

Total Flavonoid Content

The Dowd method was used to ascertain the cocoa seed extracts' total flavonoid content. After diluting aliquots (1 mL) of the extract with 200 µL of distilled H_2O, 150 µL of 5% sodium nitrite was added. Following mixing, 150 µL of 10% Aluminum Chloride was added, and the mixture was left to stand for 10 min at room temperature. After adding 10 mL of distilled H_2O and 2 mL of 4% sodium hydroxide, the mixture was left at RT for 2 hr in the dark. Measure the total flavonoid content at 510 nm by using UV-vis spectrophotometer.[25]

Study Design

Female SD rats were chosen as the animals, and they were divided into five groups of six using random numbers. The medications were given twice a day for 21 days. (1) NORMAL: Receives Vehicle (0.1 N HCl adjusted pH with 0.1 N NaOH) 2 mL/kg divided in two dose at 12 hr interval. (2) OLZ: Receives olanzapine injected by 2 mg/kg/day, divided in 2 doseat 12 hr interval i.p administration for 21 days Served as control.[26] (3) TCE100+OLZ :Receives olanzapine injected by 2 mg/kg/day, i.p. divided in 2 dose at 12 hr interval+TCE extract 100 mg/kg one dose orally administration for 21 days.[18,24] (4) TCE 200+OLZ: Receives olanzapine injected by 2 mg/kg/day, i.p. divided in 2 dose at 12 hr interval+TCE extract 200 mg/kg one dose orally administration for 21 days. (5) TCE 400+OLZ: Receives olanzapine injected by 2 mg/kg/day, i.p. divided in 2 dose at 12 hr interval+TCE extract 400 mg/kg 1 dose orally administration for 21 days. The animals in each of the five groups' three cages held two each, and they were all fed the same pellet diet.

Measurement of body weight and food intake

Food consumption was monitored daily during the trial were measured every day for 24 hr. The following formula was used to determine the food intake for that day.[27]

Food intake=amount of food added-remaining amount of food

Measuring % gain in body weight

Body weight and food intake were recorded from the first to the 21st day. The percentage rise in body weight for the relevant day was calculated using the following formula.

$$\%\text{gain in body weight} = \frac{\text{Final weight} - \text{Initial weight}}{\textit{Initial weight}} \times 100$$

Locomotor activity

The Y-Maze apparatus was used to analyse the behaviour of the animals. For a brief period, each animal was placed in a Y-Maze arm for 2 min to record the animals' basal locomotor activity. After 30 min, the drug was administered, and the locomotor activity was measured as the distance travelled in millimeters.[28]

Biochemical analysis

Blood was drawn into Eppendorf tubes using the retro-orbital method at the conclusion of the experiment. Samples for following coagulation were centrifuged at 4000 rpm for 5 min at 4ºC. To biochemical analysis, the serum was isolated and kept at -20ºC. A glucometer (Accu-Chek Instant, India) was used to measure

the levels of glucose. Kits from Transasia Biomedicals Ltd., AUTOSPAN, and PBHDL were used to quantify Triglycerides (TG), Total Cholesterol (TC), and High-Density Lipoprotein (HDL), respectively. Low-Density Lipoprotein (LDL) and Very-Low-Density Lipoprotein (VLDL) were calculated using standard formulae.

$$\text{VLDL (mg/dL)} = \frac{\text{TG}}{5} \text{LDL (mg/dL)} = \text{TC} - (\text{HDL} + \text{VLDL})$$

Oral Glucose Tolerance Test (OGTT)

Normal rats that had been fasting for the whole night (18 hr) were used for the oral glucose tolerance test. Five groups of six rats were created. The vehicle's volume was the same for the control animals. Groups II-V rats were given oral. An intraperitoneal glucose meal (2 g/kg) was given 30 min after the extract. As expected, after 30, 60, 90, and 120 min, blood was drawn from the tail vein.[29]

Tissue collection

Weigh an animal's organ. Quickly remove the necessary organ and wash the ice-cold tris buffer twice. Accurately weigh the tissue and then prepare 10% w/v of it with ice-cold tris buffer (10 mM, pH 7.4). Slice the tissue into small pieces, use a homogeniser to create a clear homogeneous mixture, and then place the tissue in a plastic centrifuge set on high speed cooling (6000 rpm for 20 min). Maintain the homogenate in a frozen state.

In liver tissue Measuring the amount of Malondialdehyde (MDA)

Malondialdehyde (MDA) levels in liver tissue were measured using the Thiobarbituric Acid (TBA) reaction. 200 mg of liver samples were homogenized in 1.15% KCl to create a 10% tissue homogenate. 500 µL of this homogenate were mixed with 3 mm of 1% phosphoric acid and 1 mL of 0.6% TBA. The combination was heated for 45 min in boiling water, then cooled and vortexed with 4 mL of butanol. After 15 to 20 min of centrifugation, the top pink layer was seen at 532 nm. The MDA content of the tissue was expressed as nmol/g.[30]

In liver tissue Measuring the amount of Glutathione (GSH)

This experiment measures sulfhydryl group reactions with 5,5'-Dithiobis-(2-Nitrobenzoic acid) (DTNB), which generates a yellow complex with maximum absorbance at 412 nm. 200 mg of liver samples were homogenized in phosphate buffer (pH 7.4) to create a 10% tissue homogenate. 500 µL of homogenate and 500 µL of 10% Trichloroacetic Acid (TCA) were combined, and the mixture was centrifuged at 10,000 rpm for 6 min. Next, 500 µL of the supernatant was combined with 2.5 mL of phosphate buffer (pH 8) and 500 µL of DTNB reagent. After measuring absorbance at 412 nm, GSH levels were calculated using a standard curve and expressed as nmol/g tissue.[31]

In liver tissue Measuring the amount of Catalase (CAT) in liver tissue

To test for catalase activity, 50 µL of tissue supernatant was mixed with 1.0 mL of 50 mM phosphate buffer (pH 7) and 0.1 mL of 30 mM hydrogen peroxide. For 30 sec, absorbance was measured at 5-sec intervals using a spectrophotometer set to 240 nm, focusing on the reaction's decreasing slope. The catalase activity was expressed in units (U) per gram of tissue.[32]

Measuring the amount of Superoxide Dismutase (SOD) in liver tissue

640 µL of distilled water, 10 µL of 0.3% Triton X-100, 100 µL of 1 mM EDTA, 100 µL of 240 µM NBT, 25 µL of tissue supernatant, and 1 mM hydroxylamine were mixed to create a mixture for the enzyme activity test. To track the increase in the reaction's slope, spectrophotometric measurements were taken in kinetic mode for 3 min at a wavelength of 560 nm at 1 min intervals. The enzyme activity was measured in Units (U) per gram of tissue.[33]

Statistical analysis

All of the data are analyzed using Graph-Pad Prism version 10, and the results are displayed as Mean±SEM. Body weight, food and water intake, blood glucose levels during the OGTT, and locomotor activity were all investigated using two-way ANOVA and Tukey's *post-hoc* test. Mean±SEM is used to show oxidative stress in addition to the lipid profile. One-way ANOVA using Graph-Pad Prism software (version 10) and Bonferroni *post hoc* tests.

RESULTS

Preliminary Phytochemical Analysis and Extraction Yield

A TCE yield of 3.025% w/w per 100 g of dried powder was determined. Phenols and flavonoids were found in COE, according to preliminary phytochemical study. Using the regression equation of the calibration curve (y=0.0192x+0.0257; R2=0.992), the total phenolic content was calculated and reported as 72.5 µg Gallic Acid Equivalents (GAE) per milligram of extract. Furthermore, a calibration curve using the regression equation (y=0.0009x+0.0237; R2=0.9801) was used to calculate the total flavonoid content of COE. The result was 165 µg Quercetin Equivalents (QE) per milligram of extract.

Effect on body weight

Rats treated with olanzapine had a considerably larger percentage rise in body weight at the conclusion of the study ($p<0.0001$, F=56.93) than the control group. From day 6 to day 21, there was a significant rise in body weight gain ($p<0.0001$). The olanzapine-induced body weight gain from day 6 to day 21 was considerably decreased ($p<0.0001$) when TCE at doses of

100, 200, and 400 mg/kg was administered concurrently with olanzapine (Figure 1).

Effect on cumulative food intake

There was observed that co-administration of TCE 200,400 mg/kg with olanzapine 2mg/kg significantly decreased the cumulative food intake from 6th day till the 21st day. (p<0.0001, F=9.087) and the Cumulative food intake during 15th, 18th and 21st day were significantly higher (p<0.0001, p<0.0001, p<0.0001) in olanzapine treated rats compared to normal (Figure 2).

Effect of TCE on lipid profile

There were significant differences in Triglycerides (TG) (p<0.0001, F=64.20, R^2=0.9277), Total Cholesterol (TC) (p<0.0001, F=34.09, R^2=0.8721), HDL (p<0.0001, F=50.85, R^2=0.9105), LDL (p<0.0001, F=36.04, R^2=0.8782) and VLDL (p<0.0001, F=64.16, R^2=0.9277) among the groups. In the olanzapine group, there was a significant increase in triglycerides (p<0.001), total cholesterol (p<0.01), LDL (p<0.0001), and VLDL (p<0.0001), along with a decrease in HDL (p<0.01) compared to the normal group. Co-administration of TCE with olanzapine at doses of 200 and 400 mg/kg led to a significant increase in HDL levels (p<0.05, p<0.01) and a decrease in TG (p<0.05, p<0.01, p<0.001) and VLDL (p<0.05, p<0.01, p<0.001) compared to olanzapine alone. Additionally, TCE co-administration significantly decreased LDL levels (p<0.0001) and total cholesterol (p<0.001 for both doses) at 100, 200, and 400 mg/kg compared to the olanzapine group (Table 1).

Effect on locomotor activity

Following the co-administration of TCE and Olanzapine, we found a significant difference in locomotor activity (p<0.0001 time dependent Travelled distance, F=11.94). On the 21st day, the olanzapine group's locomotor activity was significantly lower (p<0.0001) than normal. The reduction of locomotor activity brought on by olanzapine was significantly exacerbated (p<0.0001, p<0.0001) when TCE (200, 400 mg/kg) and olanzapine were administered together (Table 2).

Effect on organ weight

A notable variation in the weight of the organs was noted. When TCE (100, 200, 400 mg/kg) and olanzapine were administered together, there was a substantial decrease in the weight of the liver (p<0.0001), heart (p<0.05), and adipose tissue when compared to rats treated with olanzapine. However, as compared to the olanzapine group, there was no discernible difference in kidney weight. Compared to the typical and non-significant difference in heart and kidney weight, the olanzapine-treated group had significantly greater liver and adipose tissue organ weights (Table 3).

Effect on TCE on oral glucose tolerance test

A non-significant difference in blood glucose levels during OGTT, which was both time-dependent and group-dependent, was observed at the conclusion of the 3rd week of the trial (p=0.0032 for both time-dependent glucose levels, F=7.288 vs. 17.43 as glucose level vs. time). The groups' total area under the glucose curve also showed a significant difference (P=0.1152, F=2.889, R2=0.3662). Likewise, the olanzapine-treated rats' OGTT area under the curve and fasting blood glucose level did not differ substantially from the control. Fasting blood glucose levels were not significantly affected by co-administration of TCE. However, rats treated with olanzapine showed significant total area under the curve and glucose levels at all three measurement intervals (30, 60, and 120 min) when TCE (100, 200, and 400 mg/kg) was administered in conjunction with olanzapine (Figure 3).

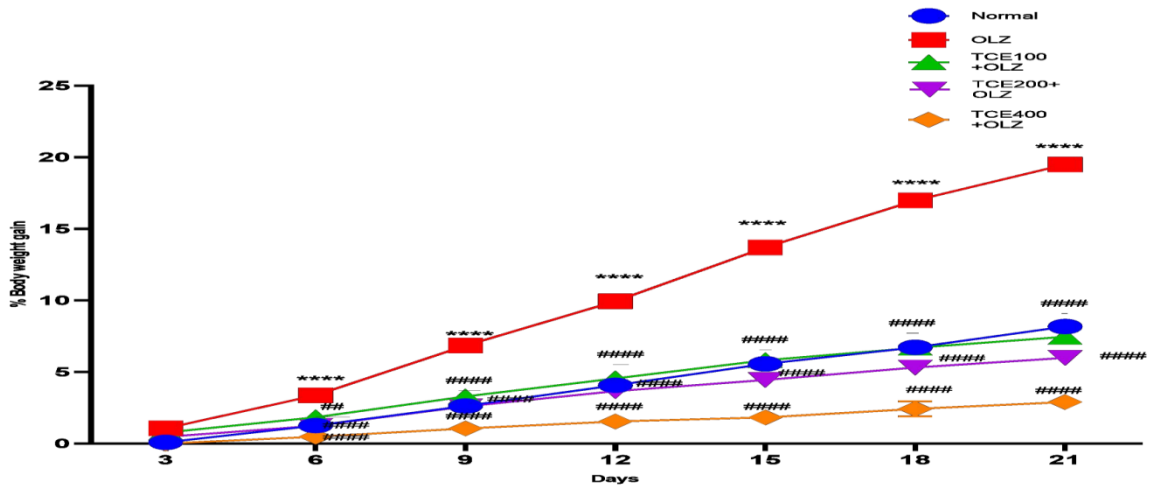

Figure 1: Body weight gain (%) at different intervals (Days) in rats treated with vehicle olanzapine (2 mg/kg/day),OLZ plus TCE (100, 200, 400 mg/kg/day). **** p<0.0001 compared to normal, ## p<0.001, #### p<0.0001 compared to olanzapine.

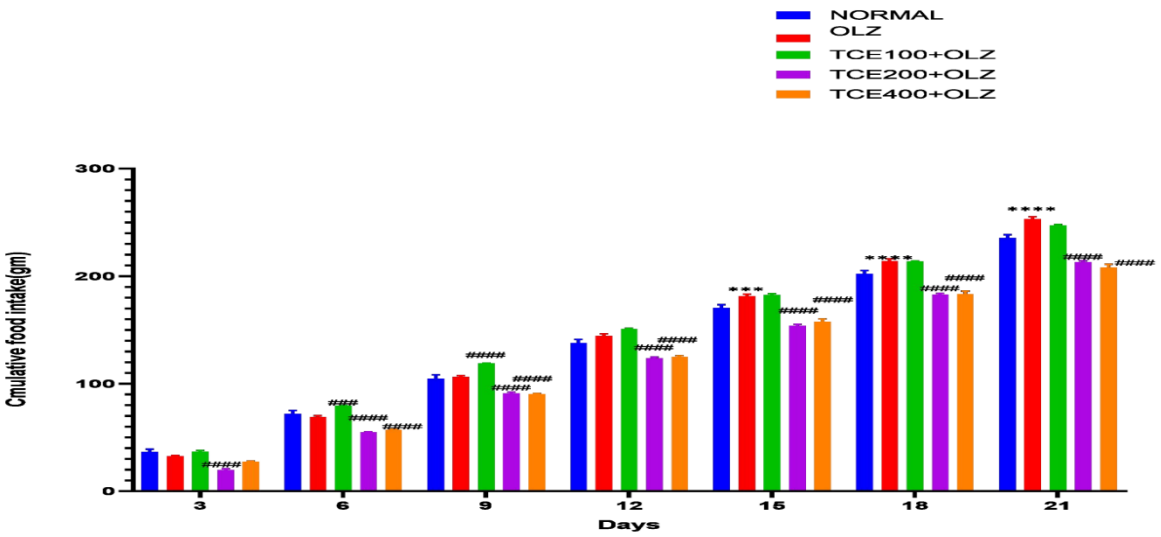

Figure 2: Cumulative food intake (g) at different intervals (Days) in rats treated with vehicle olanzapine (2 mg/kg/day), OLZ plusTCE (100, 200, 400 mg/kg/day) **** p<0.0001 compared to normal, ### p<0.001, #### p<0.0001 compared to olanzapine.

Table 1: Lipid profile in rats treated with vehicle olanzapine (2 mg/kg/day), olanzapine plus TCE (100, 200, 400 mg/kg/day).

Groups	TG (mg/dL)	TC (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)
Normal	40.58±1.82	45.21±1.72	41.61±0.67	-3.135±2.10	8.117±0.36
OLZ	88.19±3.43 ****	62.21±0.98 **	25.32±1.12 ***	19.54±1.47 ****	17.64±0.68 ****
TCE100+ OLZ	61.17±1.56 #	48.93±1.04 ##	30.18±1.14	3.620±1.79 ####	12.20±0.30 #
TCE200+ OLZ	54.98±1.39 ##	47.12±0.51 ##	32.92±0.48 #	3.577±0.66 ####	11.00±0.27 ##
TCE400+ OLZ	47.44±3.22 ###	36.59±2.72 ####	38.25±0.81 ##	-11.15±2.54 ####	9.472±0.65 ###

All data are represented in Mean±SEM (n=6), **** p<0.0001, *** p<0.001, ** p<0.01 compared to normal, #p<0.05, ##p<0.001, ###p<0.001, ####p<0.0001 compared to olanzapine.

Effect of on MDA, GSH, CAT and SOD levels of liver tissue

The level of CAT in the liver tissue of rats treated with olanzapine did not change significantly from that of the normal group, as shown in Figure 4. When compared to the olanzapine group, co-administration of TCE at dosages of 100, 200, and 400 mg/kg did not significantly alter CAT levels.

When compared to the normal group, olanzapine treatment dramatically raised MDA levels in liver tissue (p<0.05), as seen in Figure 5. However, the groups getting TCE plus olanzapine had considerably reduced MDA levels (p<0.01). While TCE co-administration at different doses had no discernible effect on SOD levels when compared to olanzapine alone, Figure 6 shows a significant drop in SOD levels in the liver tissue of rats treated with olanzapine when compared to the normal group. Olanzapine treatment dramatically reduced GSH levels in liver tissue (p<0.05), as shown in Figure 7. On the other hand, GSH levels were considerably elevated by co-administration of TCE at 400 mg/kg in comparison to the olanzapine group (p<0.01).

DISCUSSION

Previous research has shown that olanzapine and other atypical antipsychotic medications induce metabolic problems such weight gain, hyperglycemia, hypertension, hyperlipidemia, diabetes, and "cardiovascular disorders."[34] Rats treated with olanzapine (2 mg/kg/day) for 21 days experienced significant weight gain linked to hyperphagia, which is consistent with the earlier report.[35,36] Body weight increase, obesity, and serum lipid profile levels in LDL, VLDL, TG, HDL, and TC were observed to decrease when olanzapine was treated with TCE or its active ingredients.[36,37] According to our findings, TCE significantly reduces the weight increase that olanzapine causes in this rat model. Throughout the course of the trial, rats who received combination therapy had shown a tendency to gain less weight

at every body weight measurement interval. Furthermore, compared to rats treated with olanzapine, these rats consumed less food. These findings imply that TCE's capacity to reduce body weight gain brought on by olanzapine may result from its ability to lessen olanzapine-induced hyperphagia.[9] In a rat model of olanzapine-induced weight gain, earlier research revealed decreased locomotor activity and hypothesized that olanzapine-induced weight gain could be largely caused by lower energy expenditure.[38,39] Rats treated with olanzapine in the current investigation had decreased locomotor activity in the third week of the trial, which is consistent with these results.

In the third week of our investigation, TCE treatment improves olanzapine-induced hyper loco-motor activity partial movement at the highest doses (200, 400 mg/kg). This is probably one of the causes of the lack of weight growth in rats given olanzapine and TCE together. Increased expression of pck1, a gene controlling hepatic gluconeogenesis, and decreased expression of GLUT4 in skeletal muscle and adipose tissue are thought to be the causes of insulin resistance leading to hyperglycemia in a rat model of olanzapine-induced weight gain.[30] Olanzapine-treated rats in the current investigation did not exhibit glucose tolerance, which is in line with prior results. The current investigation found no discernible impact of TCE treatment on rats' glucose tolerance. Rats given olanzapine for three weeks in the current study displayed elevated plasma levels of triglycerides and total cholesterol as well as decreased HDL levels, which is in line with other findings.[6-21,23-40,47] In a rat model of weight gain, olanzapine has been shown to down-regulate the expression of CPT1A, which is involved in lipolysis through fatty acid β-oxidation, and up-regulate the expression of the hepatic SREBP-1 gene, which is involved in lipogenesis.[41] Administration of Cocoa The suppression of FA synthesis and the promotion of FA oxidation

Table 2: Locomotor activity in rats treated with vehicle olanzapine (2 mg/kg/day), OLZ Plus TCE (100, 200, 400 mg/kg/day).

Sl. No.	Groups	Day 21 (travelled distance (mm))
I	Normal	48680.8±5533.9
II	Control	5588.16±796.9****
III	TCE 100	16253.7±2138.5
IV	TCE 200	25699.6±2848.3####
V	TCE 400	25619.8±570.5####

All data are represented in mean±SEM (n=6), ****p<0.0001 compared to normal, #### p<0.0001 compared to olanzapine.

Table 3: Organ weight rats treated with vehicle olanzapine (2 mg/kg/day), OLZ plus TCE (100, 200, 400 mg/kg/day) plus TCE (100, 200, 400 mg/kg/day).

Groups	Heart(g)	Adipose tissue (g)	Kidney (g)	Liver (g)
Normal	0.61±0.03	5.03±0.22	0.64±0.01	6.31±0.25
OLZ	0.67±0.03	8.14±0.38 ****	0.76±0.05	7.44±0.12 **
TCE100+OLZ	0.58 ±0.01	5.15±0.26 ####	0.68±0.02	6.60±0.25
TCE200+OLZ	0.59±0.02	4.23±0.18 ####	0.63±0.00	5.74±0.18 ####
TCE400+OLZ	0.52 ±0.02#	3.75±0.42 ####	0.61±0.01	5.55±0.19 ####

All data are represented in Mean±SEM (n=6), **p<0.01, ****p<0.0001 compared to normal, #p<0.05, #### p<0.0001 compared to olanzapine.

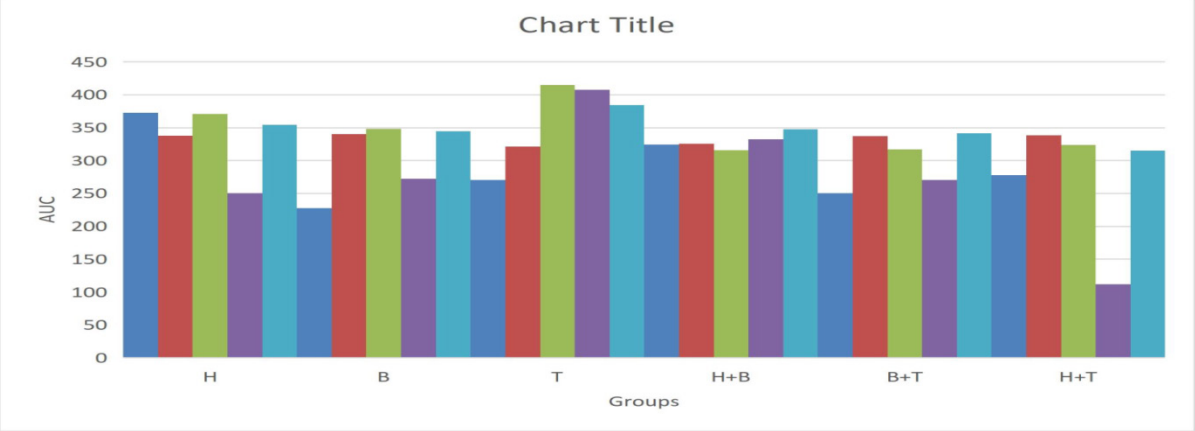

Figure 3: OGTT IN AUC in rats treated with vehicle olanzapine (2 mg/kg/day), OLZ plus TCE (100, 200, 400 mg/kg).

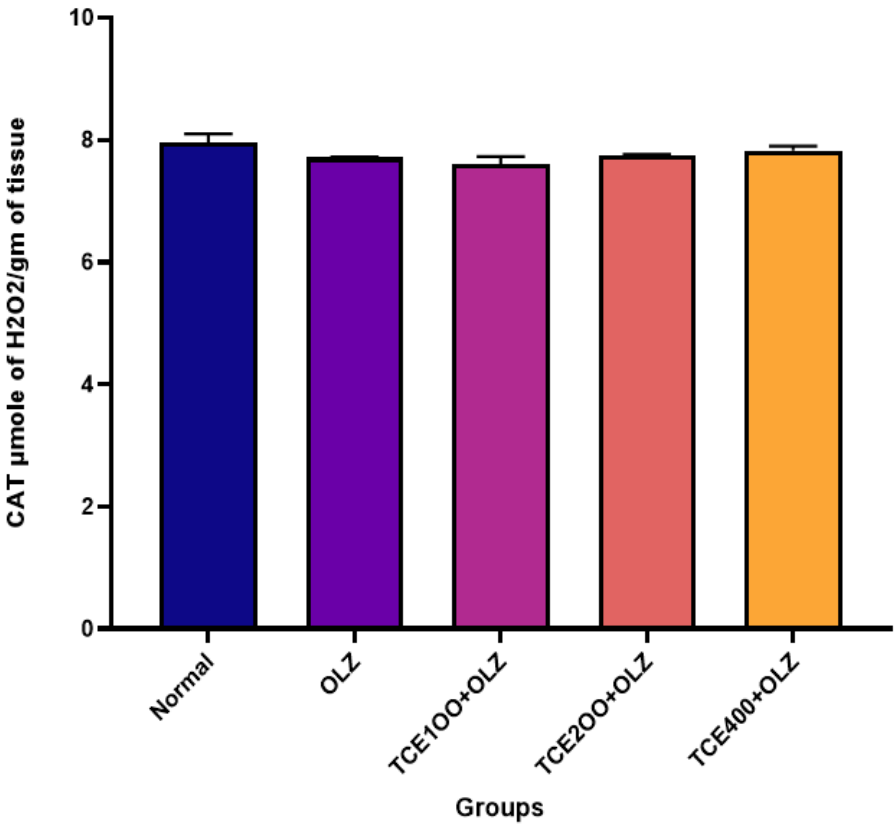

Figure 4: Catalase (micro-mole of H_2O_2/g of tissue) in rats treated with vehicle olanzapine (2 mg/kg/day), OLZ plus TCE (100, 200, 400 mg/kg/day).

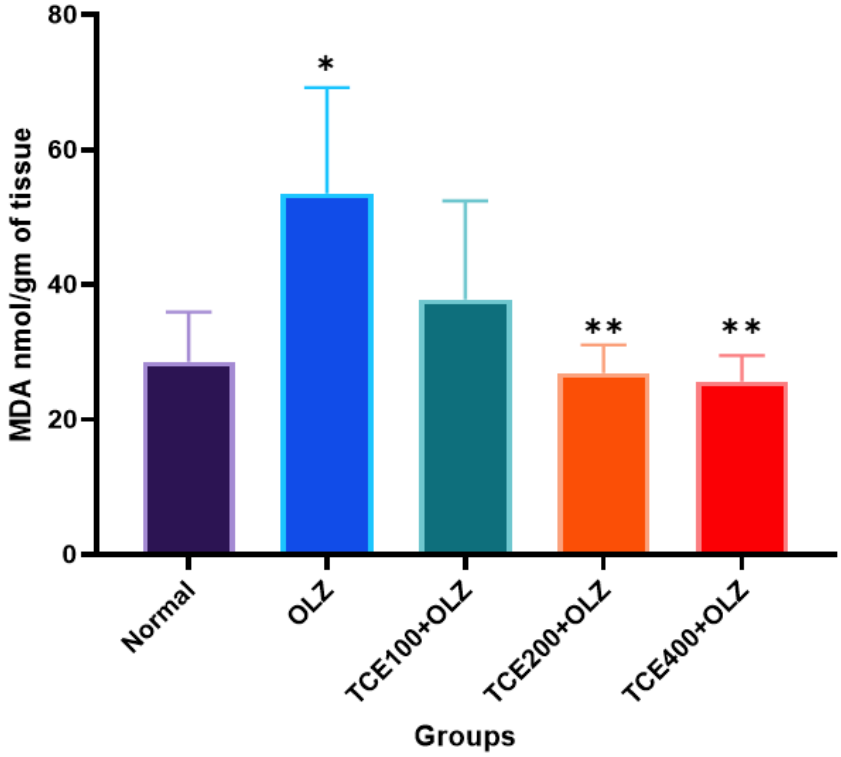

Figure 5: Malondialdehyde (nmole/g of tissue) in rats treated with vehicle olanzapine (2 mg/kg/day), OLZ plus TCE (100, 200, 400 mg/kg/day). * p<0.05 compared to normal, ** p<0.01 compared to olanzapine.

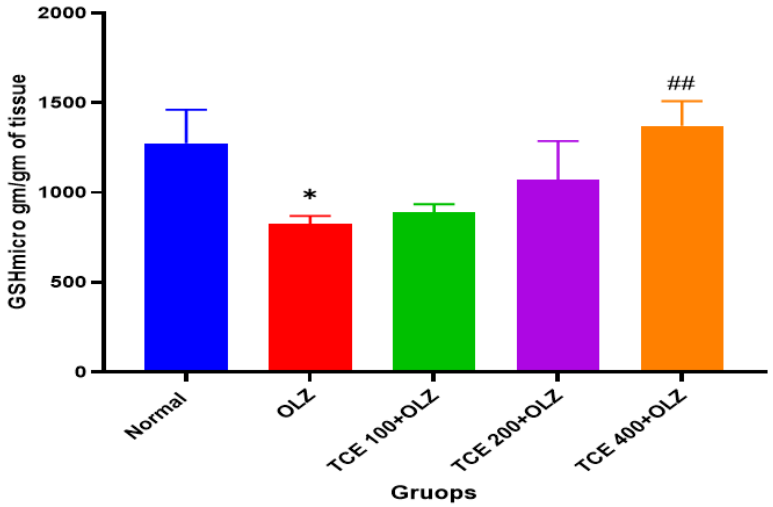

Figure 7: Glutathione (mg/g of tissue) in rats treated with vehicle olanzapine (2 mg/kg/day), OLZ plus TCE (100, 200, 400 mg/kg/day).*p<0.05 compared to normal, ##p<0.01. compared to olanzapine.

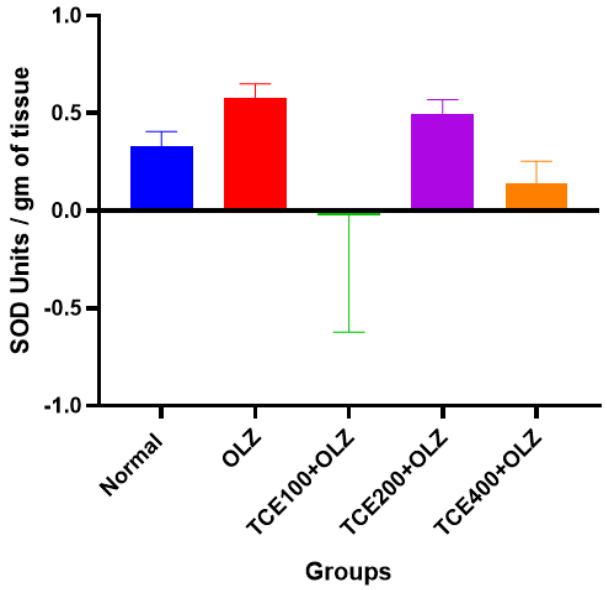

Figure 6: SOD (Unit/g of tissue) in rats treated with vehicle olanzapine (2 mg/kg/day), OLZ plus TCE (100, 200, 400 mg/kg/day).

are two of the main mechanisms of hepatic antiobesity. On the other hand, we discovered that whereas cocoa consumption increased the expression of genes for FA oxidation, it lowered the expression of genes for FA synthesis in the liver.[42] In our study, TCE co-treatment prevented olanzapine-induced elevations in total cholesterol, triglycerides, and low-density lipoprotein levels. Cocoa protein is claimed to prevent dyslipidaemia in rats by decreasing the expression of SREP1-C; these effects of TCE could be the reason for the results of the current investigation.[43] Cocoa flavonols such as catechin and epicatechin are highlighted as potential bioactive components that could influence metabolic responses. These compounds may interact with receptors or enzymes involved in glucose metabolism and appetite regulation.[36]

Oxidative stress is one process that exemplifies the negative effects of disrupting the balance between pro-oxidative and antioxidant chemicals.[44] Lipid peroxidation is assumed to be the cause of MDA, a marker of oxidative stress in tissues and cells.[45] Obesity can result in oxidative stress due to an imbalance between pro-oxidants and antioxidants in the body.[46] In individuals with schizophrenia, antipsychotic drug therapy has also been shown to increase serum levels of oxidative stress markers, including MDA.[47] While GSH, CAT, and SOD were determined to be non-significant, olanzapine treatment was found to dramatically increase MDA in liver tissue. Administration of TCE was reported to reduce MDA.

CONCLUSION

The current study found that olanzapine treatment increases food intake and weight gain, and raises blood serum levels of Triglycerides (TG), Total Cholesterol (TC), Low-Density Lipoprotein (LDL), and Very Low-Density Lipoprotein (VLDL). In addition, the liver and fatty tissues now weigh more. Olanzapine also induces oxidative stress, which leads to metabolic disease, by increasing Malondialdehyde (MDA) and locomotor activity and decreasing Glutathione (GSH) and Superoxide Dismutase (SOD) levels. Conversely, co-administration of TCE and olanzapine results in weight reduction, reduced food and water intake, and decreased levels of TC, TG, LDL, and VLDL. By decreasing MDA and increasing GSH and SOD levels, it also combats oxidative stress and lowers the weight of the liver and fatty tissue.

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ABBREVIATIONS

TCE: *Theobroma cocoa* hydroalcoholic extract; **OLZ:** Olanzapine; **TG:** Triglycerides; **TC:** Total cholesterol; **HDL:** High-density lipoprotein; **LDL:** Low-density lipoprotein; **VLDL:** Very-low-density lipoprotein; **GSH:** Glutathione; **CAT:** Catalase; **SOD:** Superoxide dismutase; **MDA:** Malondialdehyde; **OGTT:** Oral glucose tolerance test.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICAL STATEMENTS

The study adhered to ethical guidelines as approved by the Institutional Animal Ethics Committee (IAEC) of Pravara Rural College of Pharmacy. The animals were provided with standard care conditions, including a regular diet and proper housing environment. All experimental procedures were conducted in compliance with CPCSEA regulations to ensure humane treatment of the animals throughout the research process.

SUMMARY

The study investigates the effects of *Theobroma cocoa* hydroalcoholic Extract (TCE) on metabolic syndrome induced by olanzapine in female Sprague-Dawley rats. Olanzapine, a second-generation antipsychotic, is known to cause significant weight gain and metabolic disturbances. The research involved administering TCE at three different doses (100, 200, and 400 mg/kg) alongside olanzapine (2 mg/kg) over a 21-day period.

KEY FINDINGS INCLUDE

Olanzapine treatment led to increased body weight, food intake, and adverse changes in lipid profiles (increased TG, TC, LDL, VLDL; decreased HDL).

TCE treatment significantly reduced these negative effects, improving lipid profiles and reducing body weight gain.

Biochemical analyses showed that TCE improved oxidative stress markers (GSH, SOD, CAT) while decreasing MDA levels in liver tissue.

The study concludes that TCE may mitigate the metabolic side effects of olanzapine without compromising its therapeutic efficacy.

REFERENCES

1. Thomas K, Saadabadi A. Olanzapine. In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2023.
2. Leucht S, Komossa K, Rummel-Kluge C, *et al.* A meta-analysis of head-to-head comparisons of second-generation antipsychotics in the treatment of schizophrenia. Am J Psychiatry. 2009; 166(2): 152-63. doi:10.1176/appi.ajp.2008.08030368
3. Meftah AM, Deckler E, Citrome L, Kantrowitz JT. New discoveries for an old drug: a review of recent olanzapine research. Postgrad Med. 2020; 132(1): 80-90. doi:10.1080/00325481.2019.1701823
4. Svensson CK, Larsen JR, Vedtofte L, *et al.* One-year follow-up on liraglutide treatment for prediabetes and overweight/obesity in clozapine- or olanzapine-treated patients. Acta Psychiatr Scand. 2019; 139(1): 26-36. doi:10.1111/acps.12982
5. Lin CH, Lin SC, Huang YH, Wang FC, Huang CJ. Early prediction of olanzapine-induced weight gain for schizophrenia patients. Psychiatry Res. 2018; 263: 207-11. doi:10.1016/j.psychres.2018.02.058
6. Hardy TA, Henry RR, Forrester TD, *et al.* Impact of olanzapine or risperidone treatment on insulin sensitivity in schizophrenia or schizoaffective disorder.*Diabetes Obes Metab*. 2011; 13(8): 726-35. doi:10.1111/j.1463-1326.2011.01398.x
7. Lanktree, Matthew B Genomic and Precision Medicine, Metabolic Syndrome., Third edition, London, academic press,2018, 47-63. doi:10.1016/B978-0-12-801812-5.00015-9
8. Huhn M, Nikolakopoulou A, Schneider-Thoma J, Krause M, Samara M, Peter N, *et al.* Comparative efficacy and tolerability of 32 oral antipsychotics for the acute treatment of adults with multi-episode schizophrenia: a systematic review and network meta-analysis. Lancet. 2019,394(10202): 939-51. doi: 10.1016/S0140-6736(19)31135-3
9. Deng C, Lian J, Pai N, Huang X-F. Reducing olanzapine-induced weight gain side effect by using betahistine: a study in the rat model.*Journal of Psychopharmacology*. 2012; 26(9): 1271-9 doi:10.1177/0269881112449396
10. Parasuraman S, Zhen KM, Banik U, Christapher PV. Ameliorative Effect of Curcumin on Olanzapine-induced Obesity in Sprague-Dawley Rats.*Pharmacognosy Res*. 2017; 9(3): 247-52. doi:10.4103/pr.pr_8_17
11. Ullagaddi MB, Patil BM, Khanal P. Beneficial effect of*Zingiber officinale* on olanzapine-induced weight gain and metabolic changes.*J Diabetes Metab Disord*. 2021; 20(1): 41-4 doi:10.1007/s40200-020-00695-x
12. Swamy MK, editor. Benefits of Theobroma cacao and its phytocompound as cosmeceuticals. In: Plant-derived bioactives. 2020. doi:10.1007/978-981-15-1761-7.
13. Jean-Marie E, Jiang W, Bereau D, Robinson JC.*Theobroma cacao* and*Theobroma grandiflorum*: Botany, Composition and Pharmacological Activities of Pods and Seeds.*Foods*. 2022; 11(24): 3966. doi:10.3390/foods11243966

14. Ramos S, Martín MA, Goya L. Effects of Cocoa Antioxidants in Type 2 Diabetes Mellitus.*Antioxidants (Basel)*. 2017; 6(4): 84. doi:10.3390/antiox6040084
15. Halib H, Ismail A, Mohd Yusof BN, Osakabe N, Mat Daud ZA. Effects of Cocoa Polyphenols and Dark Chocolate on Obese Adults: A Scoping Review.*Nutrients*. 2020; 12(12): 3695. doi:10.3390/nu12123695
16. Rodríguez-Pérez C, Segura-Carretero A, Contreras MM. Phenolic compounds as natural and multifunctional anti-obesity agents: A review. Crit Rev Food Sci Nutr. 2017; 57(11): 1212-29. doi:10.1080/10408398.2017.1399859.
17. Guan H, Lin Y, Bai L, *et al.* Dietary Cocoa Powder Improves Hyperlipidemia and Reduces Atherosclerosis in apoE Deficient Mice through the Inhibition of Hepatic Endoplasmic Reticulum Stress.*Mediators Inflamm*. 2016; 2016: 1937572. doi:10.1155/2016/1937572
18. Wiryanthini IA, Sutadarma IWG, Yuliana. *Theobroma cocoa* and lipid profile: Cocoa beans extract (*Theobroma cacao* L.) improves lipid profile but has no effect on blood NOx concentration in dyslipidemia white male rats (*Rattus norvegicus*). Indonesia J Biomed Sci. 2017; 11(2): 1-5. ISSN: 2085-4773, 2302-2906. doi.org/10.15562/ijbs.v11i2.140
19. Davis DW, O'Sullivan TA, Bellissimo N, Camfield DA, Fitzgerald V, Annunziata G, *et al.* Effects of acute cocoa supplementation on postprandial apolipoproteins, lipoprotein subclasses, and inflammatory biomarkers in adults with type 2 diabetes after a high-fat meal. Nutrients. 2020; 12(7): 1902. doi:10.3390/nu12071902.
20. Desch S, Schmidt J, Kobler D, Sonnabend M, Eitel I, Sareban M, *et al.* Effect of cocoa products on blood pressure: systematic review and meta-analysis. Am J Hypertens. 2010; 23(1): 97-103. doi:10.1038/ajh.2009.213.
21. Ried K, Fakler P, Stocks NP. Effect of cocoa on blood pressure. *Cochrane Database Syst Rev*. 2017; 4(4). doi:10.1002/14651858.CD008893.
22. Osakabe, N., and Yamagishi, M. Procyanidins in Theobroma cacao reduced plasma cholesterol levels in high cholesterol fed rats. Journal of Clinical Biochemistry and Nutrition, 2009; 45: 131-6
23. Patil PP, Khanal P, Patil VS, Charla R, Harish DR, Patil BM, Roy S. Effect of *Theobroma cacao* L. on the efficacy and toxicity of doxorubicin in mice bearing Ehrlich ascites carcinoma. *Antioxidants*. 2022; 11(6): 1094. doi:10.3390/antiox11061094.
24. Gudina LCM, Munoz MNM, Aguilor UP. Phytochemical screening and evaluation of antihyperglycemic effects of a local variety of *Theobroma cacao* L. seed extract in streptozotocin-induced hyperglycemic mice. Int J Biosci. 2022; 20(2): 368-77. DOI:10.12692/ijb/20.2.368-377
25. Senguttuvan J, Paulsamy S, Karthika K. Phytochemical analysis and evaluation of leaf and root parts of the medicinal herb, *Hypochaeris radicata* L. for *in vitro* antioxidant activities. *Asian Pac J Trop Biomed*. 2014; 4(Suppl 1): 359-67.. doi:10.12980/APJTB.4.2014C1030.
26. Dixit MP, Sammeta SS, Dhokne MD, *et al.* Chronic agmatine treatment prevents olanzapine-induced obesity and metabolic dysregulation in female rats.*Brain Res Bull*. 2022; 191: 69-77. doi:10.1016/j.brainresbull.2022.10.013
27. Kanagali SN, Patil BM, Khanal P, Unger BS. Cyperus rotundus L. reverses the olanzapine-induced weight gain and metabolic changes-outcomes from network and experimental pharmacology.*Comput Biol Med*. 2022; 141: 105035. doi:10.1016/j.compbiomed.2021.105035
28. Karunakaran S. Unraveling Early Signs of Navigational Impairment in APPswe/PS1dE9 Mice Using Morris Water Maze.*Front Neurosci*. 2020; 14: 568200. Published 2020 Dec 15. doi:10.3389/fnins.2020.568200
29. Kumar R, Patel D, Prasad S, Sairam K, Hemalatha S. Antidiabetic activity of alcoholic root extract of *Caesalpinia digyna* in streptozotocin-nicotinamide induced diabetic rats. Asian Pac J Trop Biomed. 2012; 2(2)-S940. doi:10.1016/S2221-1691(12)60340-2.
30. Mihara M, Uchiyama M. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test.*Anal Biochem*. 1978; 86(1): 271-278. doi:10.1016/0003-2697(78)90342-1
31. ELLMAN GL. Tissue sulfhydryl groups.*Arch Biochem Biophys*. 1959; 82(1): 70-7. doi:10.1016/0003-9861(59)90090-6
32. Aebi H. Catalase *in vitro*.*Methods Enzymol*. 1984; 105: 121-6. doi:10.1016/s0076-6879(84)05016-3
33. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase.*J Biol Chem*. 1972; 247(10): 3170-5.
34. He M, Zhang Q, Deng C, Wang H, Lian J, Huang XF. Hypothalamic histamine H1 receptor-AMPK signaling time-dependently mediates olanzapine-induced hyperphagia and weight gain in female rats.*Psychoneuroendocrinology*. 2014; 42: 153-64. doi:10.1016/j.psyneuen.2014.01.018
35. Ferno J, Varela L, Skrede S, *et al.* Olanzapine-induced hyperphagia and weight gain associate with orexigenic hypothalamic neuropeptide signaling without concomitant AMPK phosphorylation.*PLoS One*. 2011; 6(6): e20571. doi:10.1371/journal.pone.0020571
36. Ruzaidi A, Amin I, Nawalyah AG, Hamid M, Faizul HA. The effect of Malaysian cocoa extract on glucose levels and lipid profiles in diabetic rats.*J Ethnopharmacol*. 2005; 98(1-2): 55-60. doi:10.1016/j.jep.2004.12.018
37. Coronado-Cáceres LJ, Rabadán-Chávez G, Mojica L, Hernández-Ledesma B, Quevedo-Corona L, Lugo Cervantes E. Cocoa (*Theobroma cacao* L.) Seed Proteins' Anti-Obesity Potential through Lipase Inhibition Using *in silico, in vitro* and *in vivo* Models.*Foods*. 2020; 9(10): 1359. Published 2020 Sep 25. doi:10.3390/foods9101359
38. Weston-Green K, Huang XF, Deng C. Olanzapine treatment and metabolic dysfunction: a dose response study in female Sprague Dawley rats.*Behav Brain Res*. 2011; 217(2): 337-46. doi:10.1016/j.bbr.2010.10.039
39. van der Zwaal EM, Janhunen SK, la Fleur SE, Adan RA. Modelling olanzapine-induced weight gain in rats.*Int J Neuropsychopharmacol*. 2014; 17(1): 169-86. doi:10.1017/S146114571300093X
40. Patel JK, Buckley PF, Woolson S, *et al.* Metabolic profiles of second-generation antipsychotics in early psychosis: findings from the CAFE study.*Schizophr Res*. 2009; 111(1-3): 9-16. doi:10.1016/j.schres.2009.03.025
41. Liu X, Lian J, Hu CH, Deng C. Betahistine co-treatment ameliorates dyslipidemiainduced by chronic olanzapine treatment in rats through modulation of hepatic AMPKα-SREBP-1 and PPARα-dependent pathways.*Pharmacol Res*. 2015; 100: 36-46. doi:10.1016/j.phrs.2015.07.023
42. Matsui N, Ito R, Nishimura E, *et al.* Ingested cocoa can prevent high-fat diet-induced obesity by regulating the expression of genes for fatty acid metabolism.*Nutrition*. 2005; 21(5): 594-601. doi:10.1016/j.nut.2004.10.008
43. Coronado-Caceres LJ, Hernández-Ledesma B, Mojica L, Quevedo L. Cocoa (*Theobroma cacao* L.) seed-derived peptides reduce blood pressure by interacting with the catalytic site of the angiotensin-converting enzyme. Foods. 2021 Sep; 10(10): 2340. doi: 10.3390/foods10102340.
44. Kunwar A, Priyadarsini I. Free radicals, oxidative stress and importance of antioxidants in human health. J Med Allied Sci. 2011; 1(2): 53-60.
45. Koltas IS, Yucebilgic G, Bilgin R, Parsak CK, Sakman G. Serum malondialdehyde level in patients with cystic echinococcosis.*Saudi Med J*. 2006; 27(11): 1703-5.
46. Fernández-Sánchez A, Madrigal-Santillán E, Bautista M, *et al.* Inflammation, oxidative stress, and obesity.*Int J Mol Sci*. 2011; 12(5): 3117-32. doi:10.3390/ijms12053117
47. An H, Du X, Huang X, *et al.* Obesity, altered oxidative stress, and clinical correlates in chronic schizophrenia patients.*Transl Psychiatry*. 2018; 8(1): 258. Published 2018 Nov 29. doi:10.1038/s41398-018-0303-7

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