Ameliorative Effects of Alchornea cordifolia Extract on Bisphenol A-Induced Obesity in Wistar rats

Abdulhakeem Rotimi Agboola
agboolar06@gmail.com
University of Calabar
Ako Hogan Itam
University of Calabar
Chinedum Martins Ekeleme
University of Calabar
Eyuwa Ignatius Agwupuye
University of Calabar
Zuwairatu Oyiza Ahmed
Federal University of Technology Minna
Godswill Peter Igiakong
University of Calabar
Gideon Bassey Agbor
University of Calabar
Asmau Niwoye Abubakar
Federal University of Technology Minna
Zainab Adamu
Federal University of Technology Minna
Esien David-Oku
University of Calabar

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Abstract

Background

Bisphenol A (BPA) is a chemical found in plastic bottles and has been linked to obesity and its associated health problems. Due to the cost and possible side effects that are associated with the orthodox medications employed for weight management, there is a shift in attention to local medicinal plants as they are readily available and are believed to be free from negative side effects. One plant that has found application for weight loss amongst the local population in Nigeria is *Alchornea cordifolia* which commonly grows in Africa and has been confirmed to possess numerous medicinal properties which include; anti-inflammatory, anti-diarrheal, hepaprotective, antiviral, and anti-diabetic effects.

Objective:

To investigate the effects of *Alchornea cordifolia* ethanol extract (ACEA) against the harmful impacts caused by BPA-induced obesity in Wistar rats.

Methods

ACEA was prepared and tested on BPA-induced Wistar rats. Thirty (30) young male Wistar rats were divided into five groups with six rats per group where Group 1 (Normal control) received commercial feed and water only, Group 2 (BPA) received commercial feed and 50 mg/kg of BPA, Group 3 (BPA + Orlistat (30 mg/kg), Group 4 (BPA + *A.cordifolia* (500 mg/kg)) ethanol extract of *A.cordifolia*, Group 5 (BPA + *A.cordifolia*(1000 mg/kg) ethanol extract of *A.cordifolia*. The parameters studied after obesity was induced and after treatments are; Anthropometric parameters; body weight, waist circumference, lees index and biochemical and kidney function parameters; fasting blood glucose, serum glucose, total protein, relative weight of the kidneys, serum levels of urea, creatinine, uric acid, concentrations of sodium, chloride, and potassium, serum levels of total cholesterol (TC), triacylglycerides (TAG), high-density lipoprotein cholesterol (HDL-c) lipase, low-density lipoprotein cholesterol (LDL-c) and HMG-CoA reductase activity.

Results

Results showed that Bisphenol-A caused obesity and related complications after 4 weeks of administration as evidenced by the elevation of the anthropometric, biochemical and kidney function parameters. However, treatment with low (500 mg/kg) and high dose (1000 mg/kg) of *A.cordifolia* reduced the elevated levels of the anthropometric, biochemical and kidney function parameters in the Wistar rats.

Conclusion

The results obtained from this study demonstrates the potential of ACEA in the management of obesity and its related complications

Background

Obesity and the illnesses associated with it have become significant global health concerns. It is now recognized as the fifth most common cause of death {1}. According to the World Health Organization (WHO), obesity is an "abnormal or excessive fat accumulation that may impair health. The fundamental cause of obesity and overweight is an energy imbalance between calories consumed and calories expended {2}. Obesity is regarded as an epidemic that ravages virtually every parts of the world contributing to the increased prevalence of conditions like hypertension,

cardiovascular disease, type 2 diabetes etc {3}. Globally, the prevalence of overweight or obese children and adolescents aged 5 to 19 more than quadrupled from 4–18% between 1975 and 2016. {4}. In Africa, between 1992 and 2005, the prevalence of obesity increased significantly with women having the highest occurrence than men {5}. In Nigeria, as at 2020, the South western region especially Lagos that is highly industrialized, had the highest prevalence of obesity while the North eastern region such as Maiduguri had the lowest prevalence {6}. The major causes of obesity include diet, lack of physical exercise, genetics, illnesses, lack of sleep and exposure to endocrine disruptor chemicals {7}. One of the major Endocrine disruptor chemicals is Bisphenol A (BPA) which is a widely produced industrial chemicals in the world. It is mainly utilized in the production of water bottles, food cans, food storage containers, dental sealants, and thermal papers {8}. BPA has been found to interact with a diverse range of hormone receptors. It can imitate, block, alter, or otherwise affect hormonal activities controlled by the brain. These activities may be associated with regulation of Hunger and satiety {9, 10}. Specifically, BPA is an estrogenic substance that binds to estrogen receptors, ERα and ERβ, to prevent the release of adiponectin. Also, BPA increases inflammatory cytokines and decreases adiponectin via interacting with adipocytes and invading macrophages [11]. In this case, inflammatory cytokines like TNFα and IL-6 cause inflammation in the fat cells, which prevents lipolysis. Under these circumstances, lipid overflow towards oxidative tissues such as the liver and skeletal muscles would encourage ectopic fat distribution, which will result in belly obesity {12}. In addition, elevated levels of BPA have been reported in people with renal diseases. This is characterized by decreased glomeruli filtration rate {13, 14}. Several experimental studies correlated BPA accumulation, and its plasma levels with impaired renal function {15}. The exposure of animals to BPA causes deleterious effects on kidney function in various capacities, such as increased expression of proinflammatory mediators, the induction of oxidative stress, and the incapacitation of antioxidants response in renal tissues {16}. BPA can increase the expression and enzyme activity of caspase-3 in renal tissues, which can lead to cell apoptosis {16}. Confirmatory tests for Obesity include body weight, waist circumference, lees index, fasting blood glucose, serum glucose, total protein and albumin {17, 18, and 19}. Orlistat, Phentermine, Sibutramine and Rimonabant are conventional drugs used for the treatment of obesity. They have associated side effects such as oily stools, increased defecation, headaches, dizziness, constipation and diarrhoea {20}.

Alchornea cordifolia is also called Christmas bush. In Nigeria, it is called *Bambani* (Hausa), *Ubobo* (Igbo) and *Esinyin* (Yoruba). *Alchornea cordifolia* is a plant found along the coastal areas of West Africa and has been used traditionally for curing various diseases {21, 22, 23}. *A. cordifolia* has been demonstrated to have biological actions that include anti-inflammatory, anti-diarrheal, hepaprotective, antiviral, and anti-diabetic effects. {24, 25, 26, 27, 28}. The efficiency of *A. cordifolia* against a variety of pathogenic microorganisms, including as gastrointestinal, cutaneous, respiratory, and urinary tract pathogens, has also been demonstrated by numerous antimicrobial screenings, corroborating the plant's traditional use for treating such conditions {29, 30, 31, and 32}. A previous *in vitro* study has revealed that methanolic extract of *Alchornea cordifolia* posseses antilipase activity {33} which shows this plant might have anti-obesity potential. However, sparse information is available on the *in vivo* antihyperlipidemic effect of *A. cordifolia.* In addition, studies on Bisphenol-A induced obesity are limited. Consequently, the present study is aimed at investigating the effect of *A. cordifolia* on anthropometric and biochemical parameters in Bisphenol-A induced obese rats.

Materials and Methods

Chemicals and reagents

The Bisphenol A ([2,2-bis(4-hydroxyphenyl)propane] in the form of pure pellets and the reagents used are of analytical grades, majorly obtained from Sigma Aldrich Company, UK through Bristol Scientific.

Collection plant material

A.cordifolia leaves were obtained from a garden in Uyo, Akwa Ibom State, south-south Nigeria. The leaves were authenticated in the Department of Plant and Ecological Studies, Faculty of Biological Sciences, University of Calabar, Calabar and given a voucher number: Bot/Herb/UCC/611 and was preserved. After drying at room temperature in the shade, was ground into a coarse powder using an electric grinder.

Preparation of extract

The ethanolic extract leaves were then produced following the method used by Hogan *et al*. {34} Briefly, the leaves were washed, air-dried and then milled into powder using a manual blender. Powdered leaves were extracted with ethanol. Using a Soxhlet extractor, 540 grams of coarsely powdered *A. cordifolia* leaves were extracted with 90% ethanol. The extraction process maintained a solvent ratio of 1:10 in the Soxhlet apparatus. During each cycle, about 100 grams of dried *A. cordifolia* leaf powder was utilized, and the heating mantle was adjusted to approximately 80°C. The extraction duration ranged from 12 to 24 hours, as detailed in the study by Walbi *et al*. {35}. Following extraction, the resulting extract was collected, concentrated through a rotary evaporator, and stored in desiccators. The extract exhibited a distinct sweet fragrance, possessed a dark green colouration and crystalline in consistency.

Induction of obesity

To induce obesity through exposure to BPA, the experimental rats were orally administered BPA (50 mg/kg/day) dissolved in olive oil (GOYA® Extra Virgin Olive Oil, Spain) via oral gavage. The selected BPA dosage was determined based on prior research conducted by Sharma *et al*. {36} and Meli *et al.* {37}. After 28 days of exposure to BPA, the rats were re-grouped based on the administered extract doses.

Experimental design

Young male Wistar rats (80–100 g) were obtained from the Animal House, Department of Biochemistry, Faculty of Basic Medical Sciences, University of Calabar, Calabar, Cross River State. The study animals were fed with commercial rat pellets for one (1) week and water was given *ad libitum.* The procedures were approved by the Faculty of Basic Medical Sciences Animal Ethics Committee, University of Calabar, Calabar. Obesity was induced by orally exposing all the test animals except the normal control to 50 µg/kg BPA for four consecutive weeks (28 days). During this time, the animals also received water freely. The rats having Lee's indexes of 0.3 and above {38}, and/or rats with a BMI of 20% greater than controls were considered obese {39}. The experimental animals were subsequently randomly divided into five groups, each comprising six rats (n = 6). Group 1 served as the normal control and received only commercial feed and water. Group 2 consisted of BPA-induced obese rats exposed to 50 mg/kg of BPA, serving as the BPA untreated obese control. Groups 3, 4, and 5 received 50 mg/kg of BPA along with 30 mg/kg of Orlistat (Orlistat/standard drug control), 500 mg/kg of ethanol extract of A.C (Treatment 1), and 1000 mg/kg of ethanol extract of A.C (Treatment 2), respectively. This treatment regimen continued for 28 days. The justification for selecting precise dosages of 500 mg/kg and 1000 mg/kg of *A. cordifolia* in this study stems from a prior investigation. In this earlier research, the LD50 has already been determined as above 5000mg/kg, but the findings are currently pending review and await publication in a separate study. Induction of obesity and treatment lasted for a period of 56 days (28 days for induction and another 28 days for treatment).

Feed intake measurement

Feed intake was measured daily from each cage. The animals were provided with a freshly prepared diet and feed consumption was established by weighing leftover feed after consumption and subtracting its value from the initial

weight of the diet. The sum of the average weekly caloric intake and the calorie content of the diet were used to compute the weekly caloric intake.

Feed intake (g/day) = Difference between the initial and leftover weight of the diet consumed

Anthropometric measurements

The following formulas were used to determine the Lee's Index and BMI, which were used as indications of obesity:

Weight difference = Initial weight (g) – final weight (g)

% weight difference compared with normal control = (weight – normal control/ weight) * 100

Lee's Index = (Body weights [g]) 1/3 / Nasal – anal length (cm)

BMI = Body weight (g) / Nasal – anal length 2 (cm2)

The rats having Lee's indexes of 0.3 and above were considered obese {38}, and/or with a BMI of 20% greater than controls were considered obese {39}

Fasting blood glucose (FBG) and Serum glucose level

Animals were fasted once weekly and FBG was measured weekly for 8 weeks with Glucometer (Accu-chek Performa, Roche, Germany). For serum glucose level, the rats were fasted for 12 hours before the tail vein was punctured and blood from the tail was dropped on the tip of glucose strip placed in a glucometer, and blood glucose concentration was recorded in mg/dl {40}.

Collection and preparation of blood, serum, and tissue homogenates

Diethyl ether was used to anaesthetize the animals at the end of the treatment, after which they were slaughtered. Jugular vein/cardiac puncture was used to obtain blood, which was then allowed to clot. Clotted blood was then centrifuged at 3000 rpm for 15 minutes {41, 42}. The serum was decanted and properly preserved in a refrigerator for later biochemical analyses.

Biochemical analysis

Serum biochemical analysis kits used were made by Randox Laboratories (UK) or by commercial biochemical kits manufactured by Olympus in Hamburg (Germany) used on an automated analyzer (Olympus AU800). To examine serum biochemical markers, standard experimental protocol were followed thus: total protein {43}, albumin {44} creatinine {45}, and urea {46}, Serum lipid profile: Total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) {47}, and triglycerides (TGs) {48} were measured using colourimetric techniques, while low-density lipoprotein cholesterol (LDL-C) was calculated as [TC (HDL + very LDL (VLDL) {49}. the serum electrolyte content was measured using procedures outlined by {50} Hydroxylmethylglutaryl (HMG)-CoA reductase activity {51}.

Statistical Analysis

GraphPad Prism version 9.0 (GraphPad Software, Incorporated, San Diego, CA, USA) was used to analyze the data collected. The analyzed results were expressed as the mean ± standard error of mean (SEM), and the differences between treated and control groups were statistically assessed using one-way ANOVA, followed by post hoc Turkey multiple range test and LSD. The differences were considered significant at $p < 0.05$.

Results

Body weight gain after Bisphenol-A induction

At the beginning of the experiment, the result reflected no significant ($p<0.05$) difference among the body weights of all the rats (**Figure 1a and 1b)**. Exposure of the Wistar rats to BPA after four weeks showed a significant ($p<0.05$) increase in body weight (134.09g ±4.50, 136.58g ±3.77, 137.10g ±6.91, 155.58g ±10.03) when compared to the normal control group (103.07g ±2.78).

Effect of *A.cordifolia* treatment on body weight of BPA-induced obese male Wistar

The effects of *A.cordifolia* treatment on the body weight of Bisphenol A-induced Wistar rat treated with *A.cordifolia* is shown in **Figure 2a and 2b**. At the end of the experiment at (week 8), it was observed that there was a significant ($p<0.05$) increase in the obese control (207.67g ±18.60) when compared with the normal control (165.59g ±8.36). The treatment with 30 mg/kg of orlistat significantly ($p<0.05$) lowered the body weight (146.00±11.38) of BPA-induced Wistar rat when compared with negative control (165.59g ±8.36). However, 1000 mg/kg of *A.cordifolia* significantly lowered the body weight of BPA-induced obese Wistar rats compared to obese control. Moreover, compared with normal control, the percentage weight difference between normal control and obese control was (20.03), for orlistat (13.42), while 500 mg/kg & 1000 mg/kg of *A.cordifolia* had 10.12 and 7.25, respectively. Overall, amongst treatments given to Wistar rats, treatment with orlistat had the most effective impact on body weight.

Anthropometric parameters

Effect of BPA-induced Obesity on waist Circumference of male Wistar rats

The effect of Bisphenol A (BPA) administration on the waist circumference of rats for a duration of 4 weeks is presented in **Figure 3a**. At week one, there was no significant difference in the waist circumference of all the rats, however after induction at week four, groups 2 to 5 had significantly higher waist circumferences (13.13cm ±0.26, 13.14 cm ±0.35, 13.67 cm ±0.46) when compared with normal control (11.83 cm ±0.17).

Effect of *A.cordifolia* treatment on waist circumference of BPA-induced obese male Wistar rats

The effect of *A.cordifolia* treatment on the waist circumference of Bisphenol A-induced obese Wistar rats is shown in **Figure 3b**. After treatment at week 8, the waist circumference of the obese control or negative control was significantly higher (14.67 cm ±0.33) than normal control (12.50 cm ±0.29) and every other treatment group (12.75 cm ±0.22, 13.20 cm ±0.33, 13.20 cm ±0.21). Furthermore, treatment with orlistat significantly lowered (12.75 cm ±0.22) the waist circumference of Bisphenol A-induced obese Wistar rats compared with negative control (14.67 cm ±0.33). Treatment with 500 mg/kg of *A.cordifolia* significantly reduced waist circumference (13.20 cm ±0.33), Similarly, treatment with 1000 mg/kg significantly also reduced waist circumference (13.20 cm ±0.21).

Effect of BPA-induced Obesity on Lees Index of Wistar rats

Figure 4a shows the Lees index of Wistar rats before and after Bisphenol A administration for four weeks. At week one, there was no significant difference among the Lees index of all the rats, however after induction at week four, when compared with normal control (273.69±2.40), all other groups had significantly higher values of Lees index (310.19±3.03, 304.21±3.78, 305.91±2.82, 309.97±2.31).

Effect of *A.cordifolia* treatment on lees index of BPA-induced obese male Wistar rats

The effect of *A.cordifolia* treatment on Lee's index of Bisphenol A-induced obese Wistar rats is shown in Figure 4b. After treatment at week 8, the Lees index of groups 2-5 (257.91±4.95, 260.48±2.12, 259.53±1.15) had significantly lower values (P<0.05) compared with obese control (296.79±5.22).

Effect of *A.cordifolia* treatment on fasting blood glucose (FBG) of BPA-induced obese male Wistar

The Effects of *A.cordifolia* treatment on FBG of Bisphenol A-induced obese Wistar rats is shown in Figure 5. Rats in group 2 exposed to BPA showed a significant increase in FBG at week 8 (108.50mg/dl ± 4.29) compared to normal control (79.50mg/dl ± 0.01). After treatment, by the end of the 8th week, compared with obese control rats treated with low (500mg/kg) and high dose (1000mg/kg) of *A.cordifolia* as well as 30mg of Orlistat had a marked decrease in FBG levels (79.00mg/dl ± 4.49; 70.50mg/dl ± 1.02; 78.00mg/dl ± 2.86) respectively compared with the obese control (108.50mg/dl ± 4.29).

Effect of *A.cordifolia* treatment on serum glucose, total protein and albumin levels of BPA-induced obese male Wistar rats

Table 1 shows the effects of *A.cordifolia* treatment on serum glucose, total protein and albumin levels of Wistar rats after BPA administration. Rats exposed to BPA showed a significant increase in serum glucose and a significant decrease in total protein, but there was no significant difference in serum albumin when compared with the normal control. After treatment at week 8, all treatment groups showed a significant decrease in serum glucose (66.56mg/dl ±7.46; 96.13mg/dl ±17.73; 64.12mg/dl ± 11.28) and a significant increase in total protein (3.63g/dl ± 0.30; 2.76g/dl ± 0.12; 2.32g/dl ± 0.89) which was consistent with the serum glucose (69.61mg/dl ± 7.25) and total protein (3.31g/dl ± 0.26) of normal control. There was also no significant difference in albumin levels after treatment compared with the obese control.

Effect of *A.cordifolia* on serum creatinine, urea, and uric acid levels of BPA-induced obese Wistar rats

Table 2 illustrates the effects of *A. cordifoila* treatment on the creatinine, urea, and uric acid levels of Wistar rats following BPA administration. Rats given 50mg/mg of BPA had considerably greater levels of creatinine (1.39±0.01 mg/dl), urea (68.66±2.96 mg/dl), and uric acid (16.18±1.07 mg/dl) than normal controls (0.23±0.01 mg/dl, 42.0±71.49 mg/dl, and 9.60±0.88 mg/dl, respectively). When compared to BPA-untreated controls, treatment with 30 mg/kg of orlistat and 1000 mg/kg of *A. cordifolia* had the most potent effect on the serum levels of creatinine (0.20 mg/dl, 0.20 mg/dl), urea (32.18 mg/dl, 31.52 mg/dl), and uric acid (8.77 mg/dl, 12.16 mg/dl), respectively.

TABLE 1:

Effects of *A.cordifolia* treatment on serum glucose, total protein and albumin levels of BPA-induced obese Wistar rats

Groups	Treatments	Serum Glucose (mg/dl)	Total protein (g/dl)	Albumin (g/dl)
1	Normal control	69.61±7.25^{a}	3.31±0.26^{ab}	1.94±0.07^{a}
2	BPA untreated obese control (50mg/kg)	119.20±9.77^{b}	2.07±0.21^{a}	1.52±0.17^{a}
3	BPA + Orlistat (30mg/kg)	66.56±7.46^{a}	3.63±0.30^{b}	1.70±0.18^{a}
4	BPA + *A,.cordifolia* (500mg/kg)	96.13±17.73^{ab}	2.76±0.12^{ab}	1.56±0.24^{a}
5	BPA + *A,.cordifolia* (1000mg/kg)	64.12±11.28^{a}	2.32±0.89^{ab}	1.51±0.23^{a}

Results are presented as Mean±SEM. Means with the same alphabets (such as a, a and b,b) are not significantly different, however, means with different alphabets (such as a,b and b,c) are significantly different P<0.05.

TABLE 2:

Effect of *A.cordifolia* on serum creatinine, urea, and uric acid levels of BPA-induced obese Wistar rats

Groups	Treatments	Creatinine (mg/dl)	Urea (mg/dl)	Uric acid (mg/dl)
1	Normal control	0.23±0.01^{a}	42.07±1.49^{b}	9.60±0.88^{b}
2	BPA untreated obese control (50mg/kg)	1.39±0.01^{b}	68.66±2.96^{c}	16.18±1.07^{a}
3	BPA + Orlistat (30mg/kg)	0.20±0.05^{a}	32.18±0.27^{a}	8.77±0.81^{b}
4	BPA + *A,.cordifolia* (500mg/kg)	0.23±0.03^{a}	43.83±2.25^{b}	11.05±0.21^{b}
5	BPA + *A,.cordifolia* (1000mg/kg)	0.20±0.01^{a}	31.52±2.27^{a}	12.16±0.18^{b}

Results are presented as Mean±SEM. Means with the same alphabets (such as a,a and b,b) are not significantly different, however, means with different alphabets (such as a,b and b,c) are significantly different P<0.05. *n = 6*

Kidney weight and Serum electrolytes

The effect of an ethanol extract of *A. cordifolia* on kidney weight and serum electrolyte levels in Wistar rats following BPA treatment is shown in Table 3. The administration of BPA caused a significant increase (p<0.05) in the kidney weight of rats in BPA untreated obese control (group 2) (2.27±0.13 g) when compared with normal control (group 1) (0.94±0.03 g). Treatment with 500mg/kg and 1000mg/kg of *A.cordifolia* significantly lowered the kidney weight of BPA-induced rats (1.19±0.05 g; 1.07±0.08 g), respectively when compared with BPA untreated rats (2.27±0.13 g). In addition, the serum sodium (Na^{+}) and chloride (Cl^{-}) ion levels in the BPA-untreated control group significantly decreased (p<0.05) as a result of the rats receiving 50mg/kg of BPA. The potassium (K^{+}) ion levels in the BPA untreated control, however, were non-significantly higher (3.17±0.47 g) than in the normal control (2.94±0.15 g). Treatment with both doses of *A.cordifolia* significantly decreased K^{+} levels and increased Na^{+} and Cl^{-} levels in a dose-dependent manner.

TABLE 3:

The effect of an ethanol extract of *A. cordifolia* on kidney weight and serum electrolyte levels in BPA-induced obese Wistar rats

Groups	Treatments	Kidney (g)	Na^+ (mEq/L)	K^+ (mmol/l)	Cl^- (mmol/L)
1	Normal control	0.94 ± 0.03^a	280.00 ± 45.08^{ab}	2.94 ± 0.15^a	128.57 ± 13.91^a
2	BPA untreated obese control (50mg/kg)	2.27 ± 0.13^c	166.40 ± 32.29^a	3.17 ± 0.47^a	101.48 ± 19.86^a
3	BPA + Orlistat (30mg/kg)	1.11 ± 0.08^b	236.00 ± 34.11^{ab}	2.60 ± 0.46^a	111.77 ± 12.00^a
4	BPA + *A,.cordifolia* (500mg/kg)	1.19 ± 0.05^b	253.60 ± 71.20^{ab}	3.04 ± 0.11^a	121.55 ± 10.91^a
5	BPA + *A,.cordifolia* (1000mg/kg)	1.07 ± 0.08^{ab}	352.80 ± 73.28^b	2.75 ± 0.15^a	135.28 ± 5.08^a

Results are presented as Mean±SEM. Means with the same alphabets (such as a,a and b,b) are not significantly different, however, means with different alphabets (such as a,b and b,c) are significantly different $P<0.05$. *n = 6*

Effect of *A.cordifoila* treatment on Lipid profile parameters of Wistar rats after BPA administration

Table 4 shows the effect of *A.cordifoila* treatment on Lipid profile parameters of BPA-induced obese Wistar rats. The result shows that the obese control group had significantly higher levels of TC (263.89 mg/dl ±8.05), TG (197.00 mg/dl ±31.15), LDL-c (75.32 mg/dl ±8.70) and VLDL-c (75.32 mg/dl ±8.70), and lower levels of HDL-c (57.22 mg/dl ±5.8) than the normal control group. However, treatment with *A.cordifolia* at both doses (500 mg/kg and 1000 mg/kg) resulted in significant reductions in TC (210.67 mg/dl ±2.64, 173.33 mg/dl ±39.91) and TG (170.70 mg/dl ±21.05, 119.61 mg/dl ±6.67) levels compared to the obese control group (263.89 mg/dl ±8.05, 197.00 mg/dl ±31.15). In addition, treatment with 1000 mg/kg of *A.cordifolia* resulted in a significant increase in HDL-c (145.74 mg/dl ±11.59) levels compared to the obese control group (57.22 mg/dl ±5.81). With respect to LDL-c and VLDL-c, treatment with 500mg/kg and 1000mg/kg of *A.cordifolia* significantly lowered LDL-c (49.35 mg/dl ±0.30, 10.50 mg/dl ±1.24) and VLDL-c (25.60 mg/dl ±5.53, 15.78 mg/dl ±3.73) values in a dose-dependent manner when compared with obese control (75.32 mg/dl ±8.70, 45.09 mg/dl ±9.52).

TABLE 4:

The effect of *A.cordifoila* treatment on Lipid profile parameters of BPA-induced obese Wistar rats.

Groups	Treatments	TC (mg/dl)	TG (mg/dl)	HDL-c (mg/dl)	LDL-c (mg/dl)	VLDL-c (mg/dl)
1	Normal control	119.44±4.03^{a}	140.71±17.80^{ab}	100.23±17.88^{b}	10.04±2.56^{a}	11.91±1.78^{a}
2	BPA untreated obese control (50mg/kg)	263.89±8.05^{d}	197.00±31.15^{b}	57.22±5.81^{a}	75.32±8.70^{c}	45.09±9.52^{b}
3	Orlistat (30mg/kg)	202.08±15.67^{c}	138.37±17.28^{ab}	140.69±14.97^{c}	34.53±3.08^{b}	10.76±1.81^{a}
4	*A.cordifolia* (500mg/kg)	210.67±2.64^{c}	170.70±21.05^{ab}	130.67±19.14^{c}	49.35±0.30^{b}	25.60±5.53^{a}
5	*A.cordifolia* (1000mg/kg)	173.33±39.91^{b}	119.61±6.67^{a}	145.74±11.59^{c}	10.50±1.24^{a}	15.78±3.73^{a}

Results are presented as Mean±SEM. Means with the same alphabets (such as a, a and b,b) are not significantly different, however, means with different alphabets (such as a,b and b,c) are significantly different P<0.05. *n = 6*

TC – Total cholesterol; TG – Triglycerides; HDL-c – High-Density Lipoprotein; LDL-c – Low-Density Lipoprotein; VLDL-c – Very Low-Density Lipoprotein

Effect of *A.cordifoila* treatment on HMG-CoA reductase and lipase activity in BPA-induced obese Wistar rats

Table 5 shows the effect of ethanol extract of *A.cordifoila* leaves treatment on HMG-CoA reductase and lipase activity in BPA-induced obese Wistar rats, The Tissue Mevalonic Acid Ratio (TMAR) was significantly (p<0.05) lower in the obese control (0.93±0.05) compared to the normal control (1.06±0.22). However, treatment with 1000 mg/kg of *A. cordifolia* showed the highest effect by significantly increasing the TMAR (1.41±0.18) compared to other treatment groups (15±0.29, 10±0.17). With regards to the Magnitude of Cholesterol synthesis (MCS), the obese control showed a slight difference compared to the normal control (50.31±8.36). Treatment with (1000 mg/kg) of *A. cordifolia* significantly reduced the MCS (42.52±4.57) in comparison to other treatment groups (56.05±5.55, 53.14±9.28). Furthermore, the percentage reduction of HMG-CoA reductase activity was significantly higher (p<0.05) in the obese control (50.99±2.23) when compared with the normal control (40.31±10.36). Moreover, the group treated with (1000 mg/kg) *A. cordifolia* showed the most significant effect by greatly lowering the (32.52±4.57) percentage reduction of HMG-CoA reductase activity thereby increasing HMG-CoA reductase activity. The result for the lipase activity showed a significant increase in the obese control (254.10±6.37) compared to the normal control (175.45±8.15). The treatment group that received 30mg/kg of orlistat (197.63±5.84) and 1000 mg/kg of *A. cordifolia* (201.67±5.84) showed a significant reduction in lipase activity when compared with the obese group (254.10±6.37).

Table 5:

The effect of ethanol extract of *A.cordifoila* leaves treatment on HMG-CoA reductase and lipase activity in BPA-induced obese Wistar rats

Groups	Treatments	TMAR	MCS (%/g liver weight)	% reduction of HMG CoA activity	Lipase (U/L)
1	Normal control	1.06±0.22^{a}	50.31±8.36^{a}	40.31±10.36^{a}	175.45±8.15^{a}
2	BPA untreated obese control (50 mg/kg)	0.93±0.05^{a}	60.99±2.23^{b}	50.99±2.23^{b}	254.10±6.37^{c}
3	Orlistat (30 mg/kg)	1.15±0.29^{ab}	56.05±5.55^{b}	49.38±11.36^{b}	197.63±5.84^{a}
4	*A.cordifolia* (500 mg/kg)	1.10±0.17^{ab}	53.14±9.28^{ab}	43.14±9.28^{ab}	242.00±9.88^{c}
5	*A.cordifolia* (1000 mg/kg)	1.41±0.18^{b}	42.52±4.57^{a}	32.52±4.57^{a}	201.67±5.84^{b}

Results are presented as Mean±SEM. Means with the same alphabets (such as a, a and b,b) are not significantly different, however, means with different alphabets (such as a,b and b,c) are significantly different P<0.05. *n = 6*

TMAR – Tissue Mevalonic Acid Ratio; MCS – Magnitude of Cholesterol synthesis; HMG CoA – Hydroxylmethylglutaryl CoA

Effect of *A.cordifoila* treatment on weights of adipose tissue and liver of Wistar rats after BPA administration

Table 6 shows the effect of *A.cordifoila* treatment on the adipose tissue and liver weight of Wistar rats after BPA administration. The result obtained showed that the administration of BPA caused a significant (p<0.05) increase in the weight of the adipose tissue indicated in the obese control (2.49 g ±0.15) compared to the normal control group (1.16 g ±0.30)**.** Treatment with orlistat significantly (p<0.05) reduced the weight of adipose tissues (1.27 g ±0.17), while treatment with (500 mg/kg) of *A. cordifolia* (2.07 g ±0.54) showed no significant difference. However, treatment with *A. cordifolia* (1000 mg/kg) showed a more significant reduction in adipose tissues (1.52 g ±0.07) weight compared to the obese control (2.49 g ±0.15) (figure 1). Concerning the liver, BPA administration resulted in a significant increase in the liver weight of the obese control (7.62 g ±0.17) compared with normal control (5.07g ±0.17). Moreover, treatment with orlistat, 500 mg/kg and 1000 mg/kg of *A.cordifolia* significantly (p<0.05) reduced liver weight (5.29 g ±0.05, 5.11g ±0.25) compared with obese control (7.62g ±0.17). Additionally, 1000 mg/kg treatment had the most significant (p<0.05) effect on the liver weight of all *A. cordifolia* treated rats.

TABLE 6:

Effect of *A.cordifoila* treatment on the adipose tissue and liver weight of Wistar rats after BPA administration.

Groups	Treatments	Adipose tissue (g)	Liver (g)
1	Normal control	1.16±0.30^{a}	5.07±0.17^{a}
2	BPA untreated obese control (50 mg/kg)	2.49±0.15^{b}	7.62±0.17^{b}
3	Orlistat (30 mg/kg)	1.27±0.17^{a}	5.79±0.33^{a}
4	*A.cordifolia* (500 mg/kg)	2.07±0.54^{ab}	5.29±0.05^{a}
5	*A.cordifolia* (1000 mg/kg)	1.52±0.07^{a}	5.11±0.25^{a}

Results are presented as Mean±SEM. Means with the same alphabets (such as a, a and b,b) are not significantly different, however, means with different alphabets (such as a,b and b,c) are significantly different P<0.05.

Discussion

BPA altered the normal weight gain of experimental rats in this study leading to a significant increase in body weight. This result is consistent with Sherma {52} and Bujalance-Reyes *et al* {53}. They observed a significant increase in body weight in rats and mice respectively after BPA exposure. This could be attributed to the toxic effects of BPA on several homeostatic pathways, including control in appetite and satiety in the brain when it crosses the blood-brain barrier. However, treatment with 500 mg/kg and 1000 mg/kg of *A.cordifolia* significantly reduced the body weight of BPA-induced obese rats. Furthermore, results from this study also showed increased waist circumference in the rats exposed to BPA. Waist circumference (WC) is an important indicator for assessing the risk of hypertension and CVD {54}. A recent study showed the association of BPA levels with BMI and WC in children {8}. Another study also reported that higher BPA exposure was significantly associated with elevated WC and obesity in adults {55}. However, the WC of the experimental rats in the study was reduced significantly following treatment with 500mg/kg and 1000 mg/kg of *A.cordifolia*. Additionally, results showed an increase in Lee's index after BPA exposure. Lee's index is a measure of body fat in rodents {56}. In this study, treatment with 500 mg/kg and 1000 mg/kg of *A.cordifolia* resulted in a significant decrease in lees index compared with obese control. The exact mechanism through which A. cordifolia carries out its anti-obesity function is not yet understood, however, Since many natural products can regulate appetite, enhance insulin sensitivity, inhibit pancreatic lipase and amylase, metabolically and thermogenically stimulate the body, inhibit adipogenesis, induce apoptosis in adipocytes, and alter host metabolic processes and maintain glucose homeostasis {57}future research should look at which amongst these mechanism is employed by this plant in effecting its anti-obesity property

There was a significant rise in fasting blood glucose (FBG) and serum glucose (SG) levels in the experimental rats following BPA administration. This finding aligns with findings from previous studies conducted by Menale *et al.* {58} and Tunduri *et al* {59}. A separate study reported that BPA exposure in adult mice produced a state of hyperglycemia, glucose tolerance and impaired lipid metabolism {60}. Treatment with 500 mg/kg and 1000 mg/kg of *A.cordifolia* significantly reduced the elevated levels of FBG and SG. This demonstrates the hypoglycemic effects of *A.cordifolia*.

Bisphenol A (BPA) was found to exert detrimental effects on the liver, as evidenced by a notable increase in serum total protein levels within the obtained results. The concentration of serum protein maintains equilibrium through the interplay between protein synthesis and degradation rates {61}. The induction of obesity in rats by BPA might have disrupted the inherent integrity and functioning of the liver, given its role in the biosynthesis of plasma proteins. These findings align with the observations of Abdel-Rahman *et al.* {62}. Fortunately, the administration of *A. cordifolia* at doses of 500 mg/kg and 1000mg/kg led to a significant reduction in total protein levels. This implies the potential of *A. cordifolia* to fortify the cellular membrane of the liver and preserve its capacity for protein biosynthesis.

Scientific evidences have linked BPA exposure to proteinuria related to increased urinary albumin excretion {63}. In contrast, some studies have reported a decrease in serum albumin of BPA-treated rats following acute and sub chronic exposure {64}. However, in this study, there was no significant difference in serum albumin concentration after BPA administration when compared with the normal control. Although the reason for this is not completely understood, it is certain that serum albumin balance can be affected following exposure to BPA within the experimental dose.

The results of this study are congruent with those of Bindhumol *et al.* {65}, who found that oral exposure of young male rats to BPA was linked to a substantial increase in kidney weight in the BPA-untreated animals compared to the control group. Since BPA is a xenoestrogen and the kidneys have several estrogen receptors, BPA might stimulate

those receptors, resulting in the proliferation of epithelial cells and an increase in the weight of the kidneys {66}. Additionally, the elevated serum concentrations of urea, creatinine, and uric acid indicate a potential adverse influence of BPA administration on the renal function of the rats. This result is consistent with the altered levels of the aforementioned parameters reported in rats experiencing BPA-induced renal damage within the dosage range of 50 mg/kg to 150 mg/kg {67}. Treatment with *A.cordifolia* improved the alterations of the urea, creatinine, and uric acid levels in the kidneys.

Significantly increased concentrations of sodium, chloride, and potassium were observed following administration of BPA, suggesting that bisphenol A upsets electrolyte balance and causes perturbation of kidney functions, thus leading to alterations in cellular processes in which these electrolytes are involved. This is in line with findings by Ezeonu *et al.* {68} which showed that BPA exposure at both acute and sub-chronic caused dysfunction in electrolyte balance in rats. However, *A.cordifolia* treatment significantly lowered sodium, chloride, and potassium levels, restoring electrolyte balance and renal function.

Furthermore, this study revealed that BPA administration increased serum TC, TG and VLDL-c of Wistar rats compared with the normal control group. This finding is consistent with a study by Xu *et al*. {69} and Huang *et al.* {70} which investigated the effects of BPA exposure on lipid metabolism in rats. The study found that BPA exposure (50 µg/kg/day) resulted in significant increases in total cholesterol and LDL cholesterol levels and a decrease in HDL cholesterol levels. The mechanisms by which BPA induces obesity and alters lipid metabolism are not fully understood. However, studies suggest that BPA may interfere with the normal functioning of hormones involved in energy balance, such as leptin and insulin, as well as with the peroxisome proliferator-activated receptors (PPARs), which play a crucial role in lipid metabolism {71}. However, the treatment with both doses (500 mg/kg and 1000 mg/kg) resulted in a significant reduction in the TC and TG compared to the obese control. In addition, treatment with *A.cordifolia* at a dose of 1000 mg/kg resulted in a significant increase in HDL-c levels compared to the obese control group. With respect to LDL-c and VLDL-c, treatment with 500 mg/kg and 1000 mg/kg of *A.cordifolia* significantly lowered LDL-c and VLDL-c values in a dose-dependent manner when compared with obese control. These results are consistent with the findings of Thomford *et al.* {72}. which showed the hypolipidemic potentials of ethanolic leaf extracts of *A. cordifola*.

With respect to the activity of HMG CoA reductase, BPA administration led to a significant increase in HMG CoA reductase activity compared to the normal control group. In line with this, Akash *et al.* {73} found that the administration of BPA at a dose of 70 mg/kg caused a significant increase in HMG CoA reductase activity in the liver of rats. However, treatment with (1000 mg/kg) *A. cordifolia* significantly lowered HMG-CoA reductase activity. The anti-HMG-CoA reductase activity of the *A. cordifolia* leaf extract could be linked to the presence of some polyphenols and flavonoids.

It was observed during the present study that Wistar rats after administration of BPA showed that the weight of adipose tissues was significantly increased. Similar studies have been found to support this. Huang *et al.* {74} investigated the effects of BPA exposure on adipose tissue in mice. The study found that exposure to BPA at a dose of 50 µg/kg caused an increase in adipose tissue mass and changes in the expression of genes involved in adipogenesis. Another study by Hao *et al.* {75}found that exposure to BPA at a dose of 50 µg/kg caused significant changes in the morphology of adipose tissue, including a reduction in adipocyte size and an increase in the number of small adipocytes. However, treatment with *A. cordifolia* (1000 mg/kg) showed a more significant reduction in adipose tissue weight.

With regards to the liver, BPA administration resulted in a significant increase in the liver weight of the Wistar rats and treatment with *A. cordifolia* (1000 mg/kg) showed the most significant effect on liver weight. According to Weng *et*

al. {76}, the increase in liver weight could be attributed to various factors, such as liver inflammation, increased cholesterol production, and accumulation of hepatic lipids. However, the liver weight reduction observed after administering *A.cordifolia* treatment might be attributed to the actions of chlorogenic acid and quercetin found in the plant extract {23}.

Conclusion

The ethanol leaf extract of *Alchornea cordifolia* demonstrated potential in the management of BPA-induced obesity, according to the study's results, with extracts exhibiting anti-hyperlipidemic action similar to that of Orlistat.

Abbreviations

ACEE:

Alchornea cordifolia Ethanolic extract

BMI

Body Mass Index

WC

Waist Circumference

HDL-C

High Density Lipoprotein Cholesterol

LDL-C

Low Density Lipoprotein Cholesterol

VLDL-C:

Very Low Density Lipoprotein Cholesterol

TC

Total Cholesterol

TG:

Triglycerides

BPA

Bisphenol A

TMAR

Tissue mevalonic Acid Ratio

MCS

Magnitude of cholesterol synthesis

HMG-CO A

Hydroxymethylglutaryl-CoA

Declarations

Availability of data and materials

All the necessary data and materials are included in this publication

Contributions

This work, from the experiment to the manuscript was a collaborative effort of all listed authors. Authors; Abdulhakeem Rotimi Agboola, Chinedum Martins Ekeleme and Esien David-Oku conceptualized the work, All authors participated in the laboratory works, Abdulhakeem Rotimi Agboola Chinedum Martins Ekeleme, , Gideon Bassey Agbor and Esien David-Oku curated the data. Chinedum Martins Ekeleme carried out the statistical analysis, Abdulhakeem Rotimi Agboola wrote the original draft while the final draft was proofread, edited and approved by all authors

Corresponding Author

Abdulhakeem Rotimi Agboola, Email: agboolar06@gmail.com.

Ethics approval and consent to participate

All experimental and surgical procedures were carried out in line with University and International animal care guidelines after getting necessary clearance from the Faculty Animal Research Ethics Committee, Faculty of Basic Medical Sciences (FAREC-FBMS) University of Calabar (Approval No: 245BCM3823)

Consent for publication

The authors have given approval for this work to be published work in Clinical Phytoscience.

Competing interests

The authors declare that there is no conflict of interests regarding the publication of this article.

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Figures

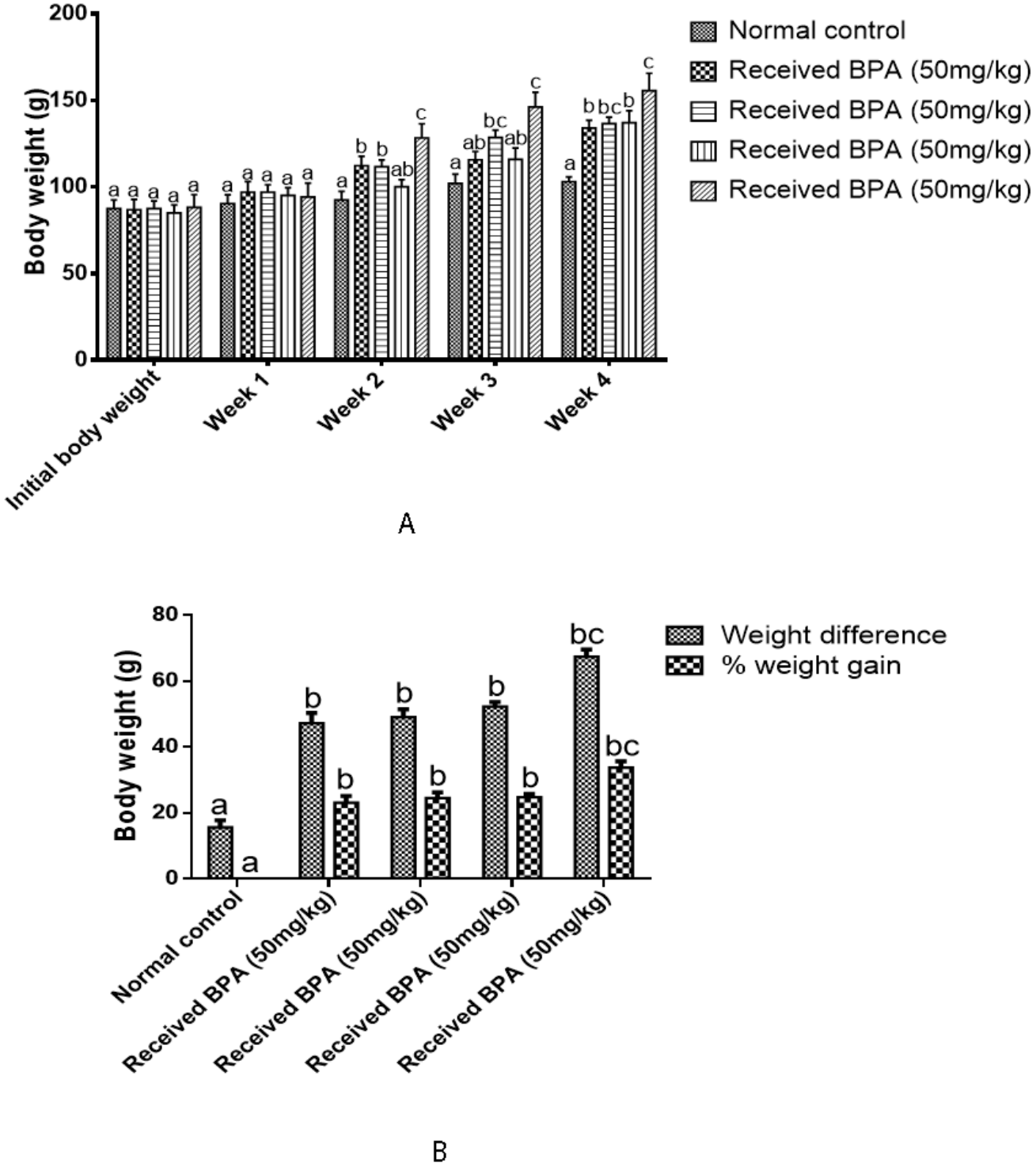


Figure 1

a: Bodyweight of BPA-induced obese Wistar rats (Induction phase)

b: Bodyweight difference and % weight gain compared with normal control of BPA-induced obese Wistar rats (Induction phase)

Results are presented as Mean±SEM. Bars with the same alphabets (such as a,a and b,b) are not significantly different, however, means with different alphabets (such as a,b and b,c) are significantly different P<0.05

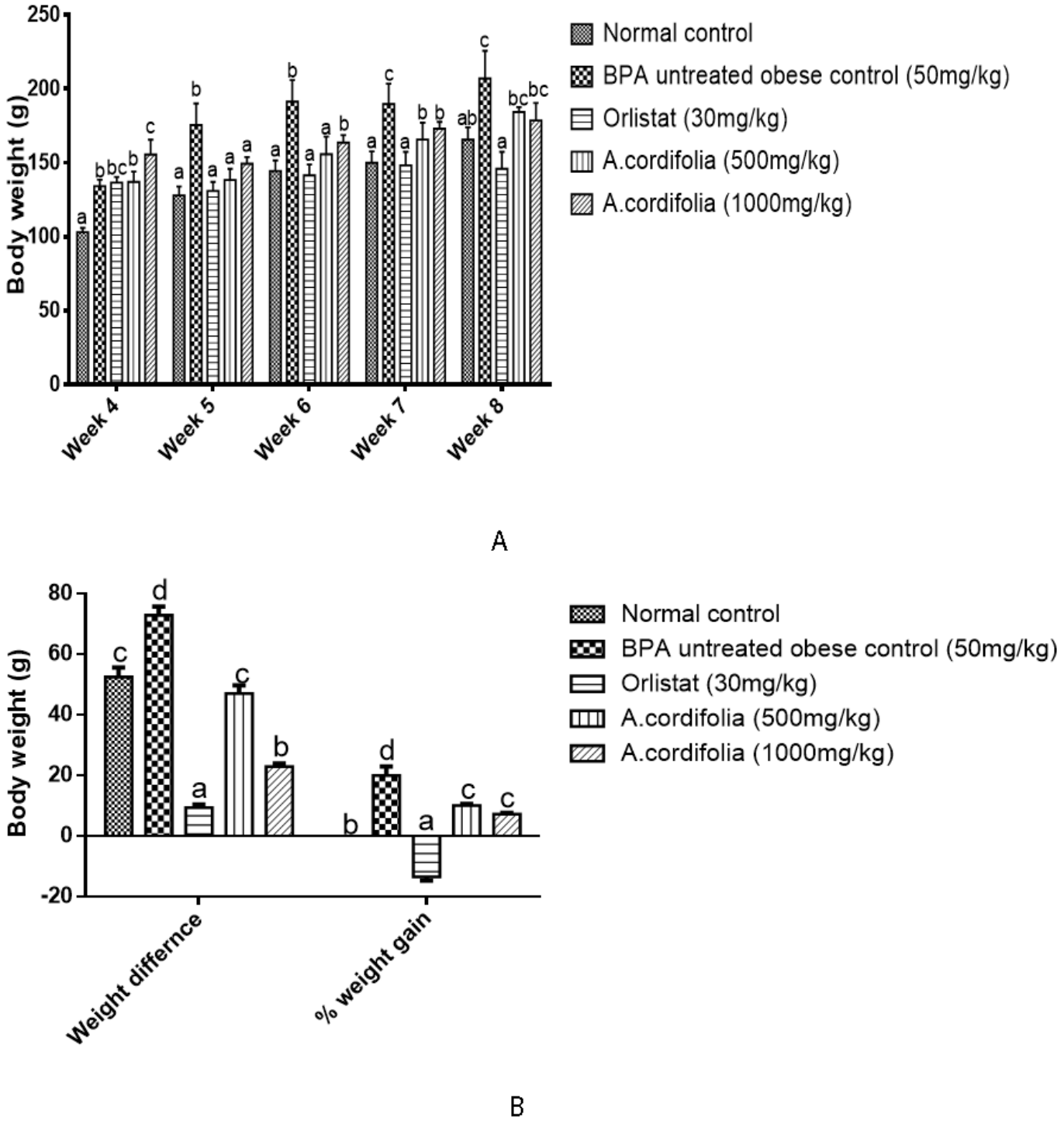
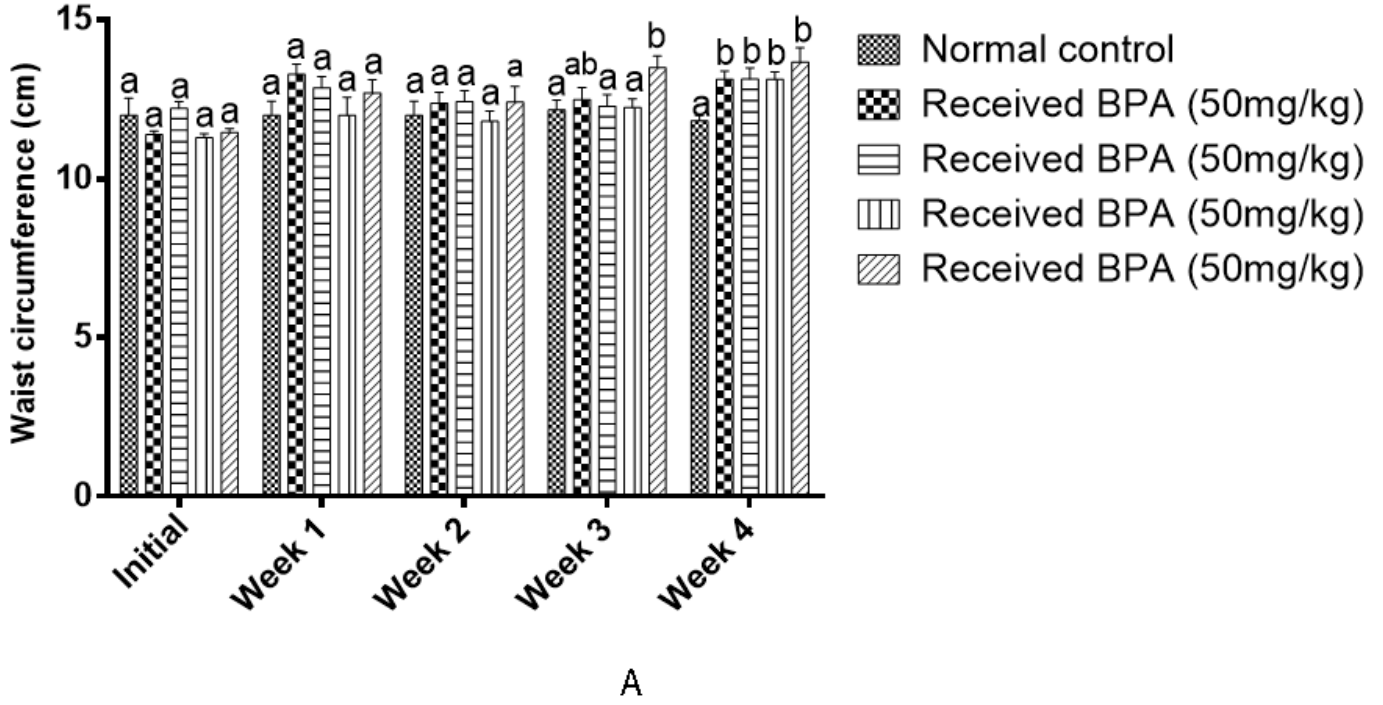


Figure 2

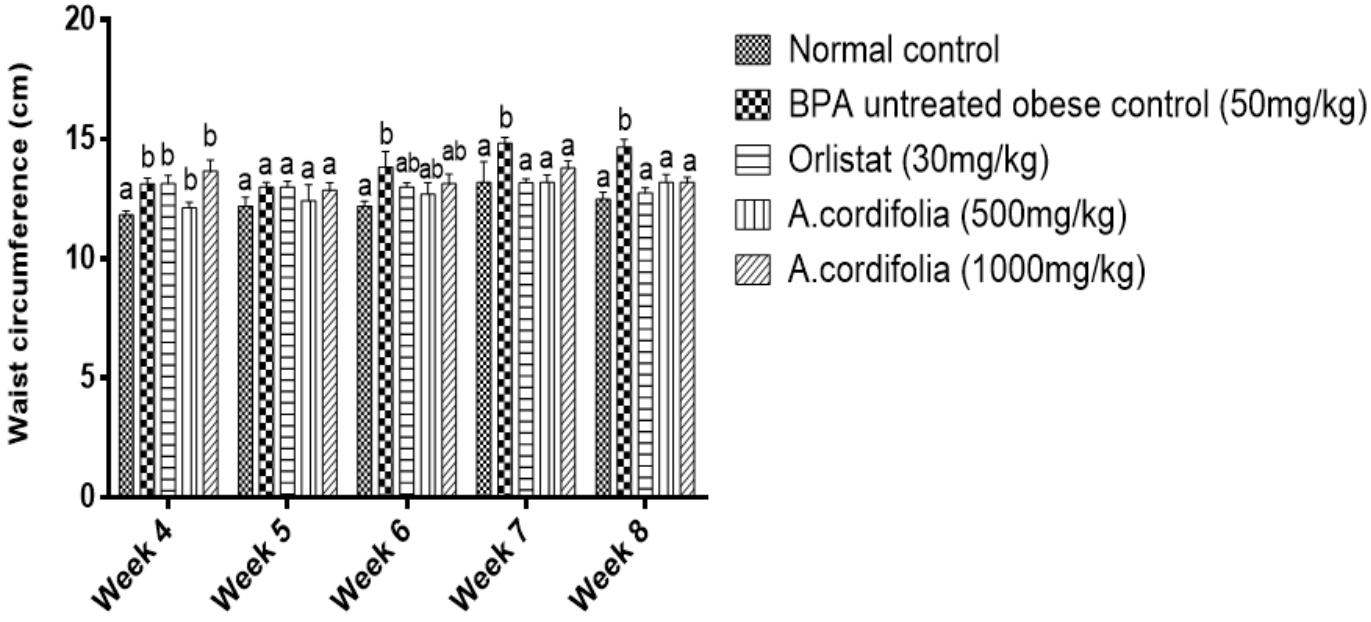
a: Bodyweight of BPA-induced obese Wistar rats treated with ethanol extract of A.cordifolia leaves (Treatment phase –Week 5 – 8)

b: Bodyweight difference and % weight gain compared with normal control of BPA-induced obese Wistar rats (Treatment phase – Week 5 - 8)

Results are presented as Mean±SEM. Bars with the same alphabets (such as a,a and b,b) are not significantly different, however, means with different alphabets (such as a,b and b,c) are significantly different P<0.05.



A



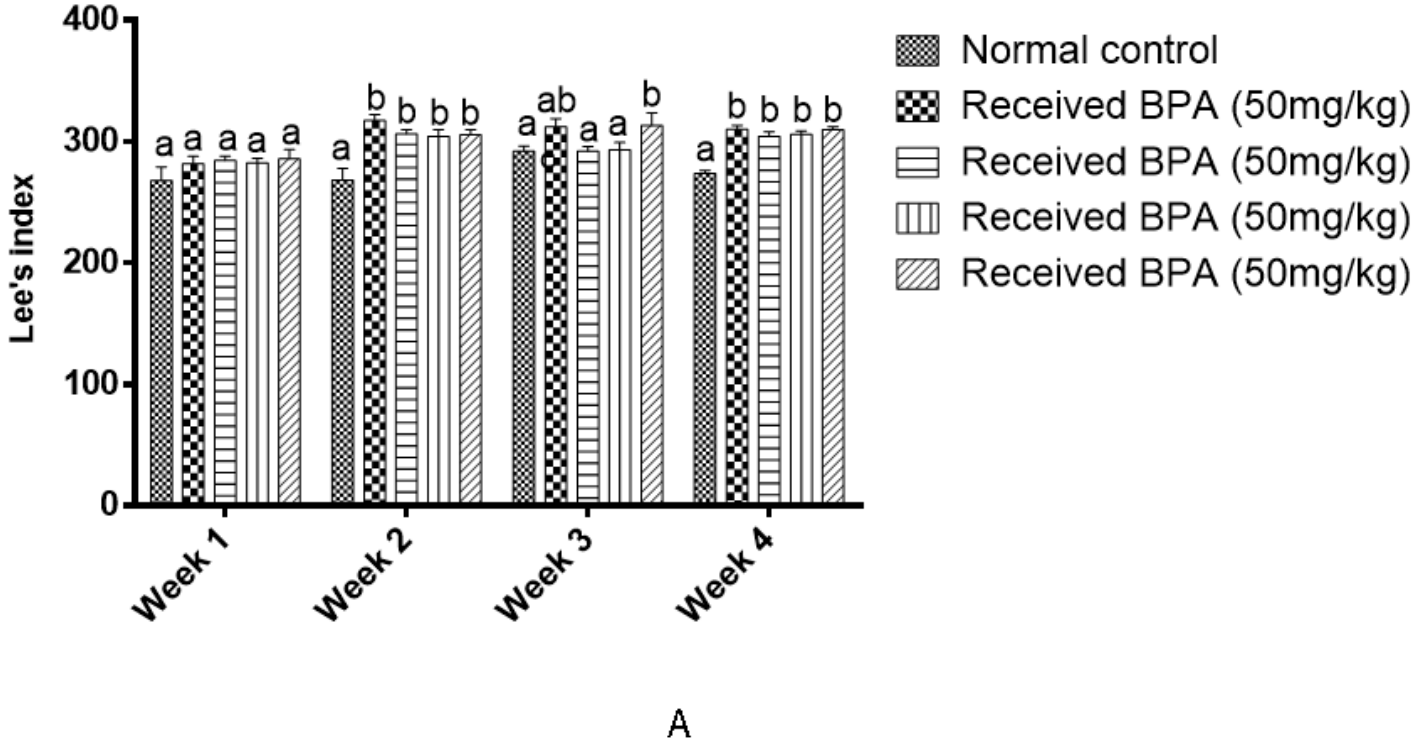
B

Figure 3

a: The effect of Bisphenol A (BPA) administration on the waist circumference (induction phase)

b: Waist circumference of BPA-induced obese Wistar rats treated with ethanol extract of A.cordifolia leaves (Treatment phase)

Results are presented as Mean±SEM. Bars with the same alphabets (such as a,a and b,b) are not significantly different, however, means with different alphabets (such as a,b and b,c) are significantly different P<0.05.



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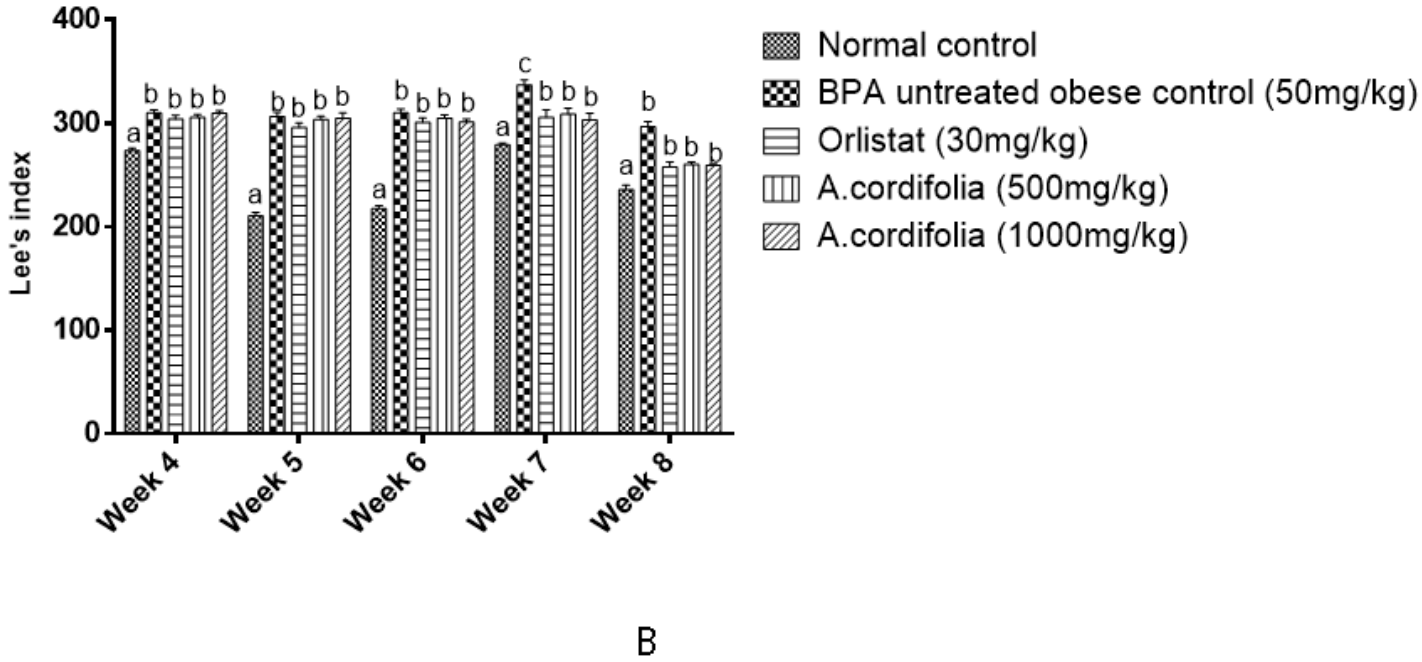

B

Figure 4

a: The effect of Bisphenol A (BPA) administration on the Lees index (induction phase)

b: Lees index of BPA-induced obese Wistar rats treated with ethanol extract of A.cordifolia leaves (Treatment phase)

Results are presented as Mean±SEM. Bars with the same alphabets (such as a, a and b,b) are not significantly different, however, means with different alphabets (such as a,b and b,c) are significantly different $P<0.05$.