© 2021 Journal of Pharmacy & Pharmacognosy Research, 9 (2), 113-125, 2021
ISSN 0719-4250
http://jppres.com/jppres

Original Article

In utero exposure to *Hypoxis hemerocallidea* Fisch., C.A.Mey. & Avé-Lall. improves metabolic syndrome parameters in pregnant rats and offspring

[Exposición *in utero* a *Hypoxis hemerocallidea* Fisch., C.A.Mey. & Avé-Lall. mejora los parámetros del síndrome metabólico en ratas preñadas y crías]

Constance R. Sewani-Rusike[1*], Andiswa Buso[1], Godwill A. Engwa[2], Benedicta N. Nkeh-Chungag[2]

[1]Department of Human Biology, Faculty of Health Sciences, Walter Sisulu University PBX1, 5117, Mthatha, South Africa.
[2]Department of Biological and Environmental Sciences, Faculty of Natural Sciences, Walter Sisulu University PBX1, 5117, Mthatha, South Africa.
*E-mail: crusike@wsu.ac.za

Abstract

Context: Intrauterine and early life environments contribute to adult metabolic phenotype. Use of medicinal plants like *Hypoxis hemerocallidea* during pregnancy raises the question of whether this species may have epigenetic benefits or detriments due to intrauterine-programming, which is phenotypically expressed in the offspring.

Aims: To evaluate the effect of *H. hemerocallidea* on selected markers of metabolic syndrome on dams and their pups (offspring).

Methods: Pregnant female Wistar rats (n=18) were divided into three treatment groups (n=6/group): Ethanol extract of *H. hemerocallidea* at 150 and 300 mg/kg b.w and the control (distilled water) were administered for 21 days. Body weights were monitored and oral glucose tolerance was determined for dams on day 20 of gestation and for pups 28 days postpartum. Serum total antioxidant capacity (TAC), LDL and HDL were determined 28 days postpartum.

Results: *H. hemerocallidea* had no effect on body and organ weights of the treated dams. Pups born to *H. hemerocallidea* dams had reduced visceral fat compared to the untreated controls. *H. hemerocallidea* increased the glucose tolerance of treated dams and their pups compared to untreated controls. *H. hemerocallidea* extract increased serum HDL in treated dams while it decreased LDL in pups born to treated dams. *H. hemerocallidea* increased TAC in pups born to treated dams.

Conclusions: *H. hemerocallidea* protected pregnant dams and their pups from insulin resistance, improved lipid profiles, reduced visceral fat accumulation and boosted total antioxidant capacity in pups. These protective effects of *H. hemerocallidea* in pups may have resulted from intrauterine programming during pregnancy.

Keywords: *Hypoxis hemerocallidea*; intrauterine programming; insulin resistance; metabolic syndrome.

Resumen

Contexto: Los entornos intrauterinos y de la vida temprana contribuyen al fenotipo metabólico del adulto. El uso de plantas medicinales como *Hypoxis hemerocallidea* durante el embarazo plantea la cuestión de si esta especie puede tener beneficios o perjuicios epigenéticos debido a la programación intrauterina que se expresa fenotípicamente en la descendencia.

Objetivos: Evaluar el efecto de *H. hemerocallidea* sobre marcadores seleccionados del síndrome metabólico en madres y sus crías (descendencia).

Métodos: Se dividieron ratas Wistar hembras preñadas (n = 18) en tres grupos de tratamiento (n=6/grupo): Extracto etanólico de *H. hemerocallidea* fue administrado a 150 y 300 mg/kg p.c. y el control (agua destilada) durante 21 días. Se controlaron los pesos corporales y se determinó la tolerancia a la glucosa oral para las madres el día 20 de gestación y para las crías 28 días después del parto. La capacidad antioxidante total en suero (TAC), LDL y HDL se determinaron 28 días después del parto.

Resultados: *H. hemerocallidea* no tuvo ningún efecto sobre el peso corporal y de órganos de las madres tratadas. Las crías nacidas de madres de *H. hemerocallidea* habían reducido la grasa visceral en comparación con los controles no tratados. *H. hemerocallidea* aumentó la tolerancia a la glucosa de las hembras tratadas y sus crías en comparación con los controles no tratados. El extracto de *H. hemerocallidea* aumentó el HDL en suero en las hembras tratadas mientras que disminuyó el LDL en las crías nacidas de hembras tratadas. *H. hemerocallidea* aumentó el TAC en cachorros nacidos de madres tratadas.

Conclusiones: *H. hemerocallidea* protegió a las hembras preñadas y sus crías de la resistencia a la insulina, mejoró los perfiles de lípidos, redujo la acumulación de grasa visceral y aumentó la capacidad antioxidante total en las crías. Estos efectos protectores de *H. hemerocallidea* en las crías pueden ser el resultado de la programación intrauterina durante el embarazo.

Palabras Clave: *Hypoxis hemerocallidea*; programación intrauterina; resistencia a la insulina; síndrome metabólico.

ARTICLE INFO
Received: July 13, 2020.
Received in revised form: October 9, 2020.
Accepted: October 10, 2020.
Available Online: November 3, 2020.

INTRODUCTION

Obesity and metabolic syndrome are health conditions that commonly lead to diabetes and cardiovascular diseases (CVDs) (Saklayen, 2018). The consequences of metabolic syndrome, which include type 2 diabetes (T2D) and CVDs account for about 60% of all deaths worldwide (WHO, 2008). Metabolic syndrome is a complex condition characterized by a cluster of risk factors including obesity, hypertension, dyslipidemia, hyperglycemia and insulin resistance (Eckel et al., 2005). Obesity is known to be a primary contributing factor to the development of metabolic syndrome. When associated with increased visceral fat, obesity is associated with altered metabolism of lipids in the body leading to dyslipidemia, a condition associated with high levels of triglyceride, total cholesterol, free fatty acids (FFAs) and low density lipoprotein cholesterol (LDL) as well as reduced levels of high density lipoprotein cholesterol (HDL) (Ebbert and Jensen, 2013). More so, evidence indicates that obesity contributes directly to the development of insulin resistance (Torres-Leal et al., 2010). Insulin normally suppresses lipolysis in adipocytes and other peripheral tissues. The loss of antilipolytic effect of insulin in the adipocytes, results to increased FFAs, which compete with glucose for insulin action (Ormazabal et al., 2018). As such, insulin response to glucose uptake in the muscle and adipose tissues is impaired thereby limiting glucose clearance and metabolism (Samuel and Shulman, 2016). Insulin resistance is therefore a metabolic state in which cells fail to sufficiently respond to insulin and is characterized by hyperglycemia and glucose intolerance leading to the development of T2D and associated complications (Gutiérrez-Rodelo et al., 2017).

Known contributory factors to the development of metabolic syndrome include genetic factors, age, and lifestyle choices such high energy diet and lack of physical activity (Han and Lean, 2015). Emerging evidence has suggested that altered intrauterine environment may induce metabolic abnormalities in fetus predisposing the offspring after birth to develop metabolic disorders (Smith and Ozanne, 2006). As a result, the concept of the developmental origins of health and disease (DOHaD) was coined, which suggests that adverse influences early in development, particularly during intrauterine life, may result to permanent changes in the physiology and metabolism of the infant, which may in turn lead to an increased risk of metabolic diseases in adulthood (Inadera, 2013). According to Barker's "Early Origins" hypothesis, these diseases are mainly due to maternal disrupted metabolism that in turn disrupts intrauterine programming, which occurs during the critical stages of fetal development (Godfrey et al., 2010). Studies have shown undernutrition during pregnancy and rapid postnatal weight gain to be associated with obesity and T2D in the adulthood of offspring (Phillips, 1996). Also, intrauterine stress during pregnancy has been associated with increased stress responses (Phillips, 2007) and insulin resistance in offspring (Buhl et al., 2007). More so, there are increasing evidences that suggest intrauterine exposure to chemicals and some drugs have an impact on the development of metabolic disorders in offspring (Dalziel et al., 2005). Offspring of rat dams administered glucocorticoid as well as dexamethasone during pregnancy presented reduced birth weight, glucose intolerance (Nyirenda et al., 1998) and increased blood pressure in adulthood (Levitt et al., 1996). As a corollary, it is possible that there may be mechanisms by which *in utero* programming may prevent metabolic syndrome in later life. Indeed, experimentally, prenatal exposure to flavonoids, genistein, quercetin (Vanhees et al., 2011) and resveratrol (Vega et al., 2016) resulted in improved antioxidant status in the offspring and neonatal exposure to oleanolic acid reduced risk for the development of fructose-induced metabolic derangements (Nyakudya et al., 2018). These and other studies give insight into the possibility of targeting the fetus for potential prophylactic therapeutic interventions that can, through epigenetic mechanisms, reduce the genetic contribution towards the development of obesity, metabolic syndrome and their associated adverse health effects (Roche et al., 2005; Phillips, 2013).

In Africa, medicinal plants are commonly used during pregnancy as purported treatments to promote mother and fetal health (Nordeng et al., 2013). The use of medicinal herbs during pregnancy (*isihlambezo*) by South African women is well documented (Kaido et al., 1997; Abdillahi and Van Staden, 2013). One of the most commonly used plants is *Hypoxis hemerocallidea* Fisch., C.A.Mey. & Avé-Lall (family *Hypoxidaceae*), commonly known as African potato, inongwe (Xhosa) and Africa-patat (Afrikaans) is widely distributed in South Africa (Kooi and Theobald, 2006). *Hypoxis hemerocallidea*'s pharmacological anti-cancer, anti-inflammatory, anti-diabetic, anti-hypertensive and antioxidant properties have been demonstrated experimentally *in vitro* and in animal models (Ojewole, 2006; Ojewole et al., 2006). Phytochemicals associated with *H. hemerocallidea*'s medicinal properties include hypoxoside, glycosides, tannins, terpenes and phytosterols that include sitosterol, stigmasterol, stanol and rich in polyphenols (Drewes and Khan, 2004; Laporta et al., 2006). Pregnant women ingest the plant to prevent miscarriage and to allow for easy delivery of the baby at term (Nyinawumuntu et al., 2008). Additionally, diabetic and HIV positive pregnant mothers continue taking *H. hemerocallidea*'s as part of their self-medication (Peltzer et al., 2008). The use of this plant raises the question of whether this medicament may contribute to intrauterine-programming effects during pregnancy, which are phenotypically expressed in the offspring. Just like chemicals or drugs, we hypothesized that plant used during pregnancy may have epigenetic benefits or detriments in relation to later development of stress and metabolic syndrome in offspring. Therefore, the aim of this study was to investigate the effects of *H. hemerocallidea* on selected markers of metabolic syndrome in pregnant rats (dams) and offspring (pups) in the absence of an obesogenic diet.

MATERIAL AND METHODS

Preparation of extract

Hypoxis hemerocallidea corm was collected in March 2017 from a local traditional healer in Mkambati, Eastern Cape, South Africa (coordinates 31.28°S, 30.04°E). The plant was identified and authenticated by Dr Immelman, Department of Biological Sciences, Walter Sisulu University, Mthatha, Eastern Cape Province, South Africa. An herbarium voucher specimen was prepared and deposited in the Kei Herbarium with a voucher number: Buso1 (KEI). The corm was washed, chopped into small pieces, and pulverized into a pulp at high speed using a household blender. The pulp was dried at 40°C in a fan oven and extracted in 70% ethanol overnight at room temperature with continuous agitation in a platform shaker (Labcon, USA). The mixture was then filtered through a coarse cheesecloth followed by vacuum filtration through Whatman No.1 filter paper to obtain the filtrate. Ethanol was removed from the filtrate in a rotary evaporator (Buchi 461, USA). Water was then removed from the filtrate in a fan oven (Labcon 2085K, USA) at 50°C for 72 hours. The resulting solid extract was weighed and put in a screw cap tube and stored at -70°C and later used for animal treatments.

Animals

Eighteen adult female and nine male Wistar rats of reproductive age (150 g) were purchased from an authorized breeder and housed in the animal holding facility in the Department of Human Biology, Walter Sisulu University. Animals were allowed to acclimatize to the new environment for 2 weeks before initiation of the study. Rats were kept under light controlled conditions (12 h light: 12 h dark) and controlled temperature between 22°C and 26°C. During this and the entire experimental period, rats were fed standard rat pellets (Epol SA: protein 180 g/kg, moisture 120 g/kg, fat 25 g/kg, fiber 60 g/kg, calcium 18 g/kg and phosphorus 7 g/kg) and water *ad libitum*. Animals were treated in a humane manner adhering to national standards (SABS, 2008). Ethical clearance for this study was obtained from the Faculty of Health Sciences Research Ethics Committee (clearance number: 0023/009).

Study design

The rats were randomly cohabited 2 females:1 male per cage and allowed to mate for 5 days. The

presence of copulatory plug or sperm in vagina, checked every morning by vaginal smear, was denoted day 0 of pregnancy. Pregnant females were assigned to one of two groups and housed individually. The females were treated orally once daily throughout pregnancy (21 days) as per their body weight (bw).

The pregnant rats (dams) were divided randomly into three groups (n = 6 rats/group) and orally administered *H. hemerocallidea* extract daily for 21 days as follows: 1) Control group received 1 mL of distilled water. 2) Low dose treatment group received 150 mg/kg bw *H. hemerocallidea* in 1 mL volume. 3) High dose treatment group received 300 mg/kg bw *H. hemerocallidea* in 1 mL volume.

Doses used were based on those prescribed by traditional healers using the body surface area as a conversion factor of daily therapeutic dose from human to rat by a multiplication factor of 6.2 (Reagan-Shaw et al., 2008). The dams were weighed once before pregnancy and weekly for the duration of pregnancy (21 days) in order to adjust extract dose in relation to new body weights.

Delivery and pup care

After delivery, pups were left with their mothers for the 28-day duration of the study. Pups were weighed one day after delivery and weekly thereafter until day 28. There was no treatment intervention on the pups.

Oral glucose tolerance test

Oral glucose tolerance test (OGTT) was performed on each of the dams at day 18 of gestation and on pups at day 28 postpartum. Fasting glucose was measured using PalmLab glucometer (Covington, LA, USA) in mg/dL. The oral glucose tolerance test (OGTT) was performed to determine glucose clearance from the blood after a glucose load to reflect insulin response (Stumvoll et al., 2000). A glucose load of 3 g/kg in 1 mL volume was given orally to each fasted rat (dams and pups) after determination of fasting blood glucose at time 0. Blood glucose was measured after 30, 60, and 120 minutes.

Termination procedures

All animals were fasted for 12 hours before being euthanized by CO_2 inhalation in a closed chamber. The dams were terminated at day 28 after delivery and pups at 28 days postpartum. Blood was collected by cardiac puncture, allowed to clot at room temperature for 30 minutes and centrifuged (Eppendorf 5810 R, Hamburg, Germany) for 10 minutes at 10 000 rpm at room temperature to obtain serum. Vital organs (heart, spleen, kidneys and liver) and visceral fat were dissected out, excess connective tissue removed and weighed. Weights were expressed as percent of body weight. Serum was used to determine total antioxidant capacity and lipid profiles.

Lipid profiles

The HDL-c and LDL-c concentrations (µg/mL) were determined using a commercial quantification kit (Sigma-Aldrich, Catalogue MAK045) as per manufacturer's protocol. Serum HDL-c and LDL-c were first separated and then the cholesterol concentration of each was determined by a coupled enzyme assay, which resulted in a colorimetric product read at 570 nm in spectrophotometer (Phoenix-2000V UV-VIS, Biotech Engineering Management Co. Ltd. (UK).

Total antioxidant capacity

Total antioxidant capacity (TAC) was determined according to the ferric reducing antioxidant power (FRAP) assay described by Benzie and Strain (1996). In the presence of an antioxidant, which acts as a reducing agent, ferric-tripyridyltriazine is reduced to its ferrous form, a blue color at low pH. The TAC was determined by measuring absorbance at 593 nm (UV-spectrophotometer, Phoenix, USA). The TAC is directly proportional to the color change of the reaction mixture. Results were expressed as µg ascorbic acid (AA) equivalent/mL of serum.

Data presentation and statistics

Data was analyzed using GraphPad Prism version 8 (Graph-pad Software Inc., San Diego, CA, USA). The OGTT data was plotted and area under the curve (AUC) calculated using GraphPad prism employing the trapezoid method. All data was presented as mean ± standard error of means (SEM) in tables and figures. Analysis of variance (ANOVA) was used to compare mean differences of continuous variables between groups followed by Tukey *posthoc* test. P≤0.05 was considered statistically significant.

RESULTS

Body and organ weights of dams and pups

The body weight of dams treated with 150 mg/kg and 300 mg/kg *H. hemerocallidea* were similar to untreated controls throughout the 20 days of pregnancy (Table 1). Also, there was no difference (p>0.05) in visceral fat and organ weights of dams treated with 150 mg/kg and 300 mg/kg *H. hemerocallidea* compared to that of the untreated controls 28 days after delivery (Table 2). After delivery, the body weight of pups born by dams treated with 150 mg/kg and 300 mg/kg *H. hemerocallidea* increased (p<0.001) compared to the untreated controls (Fig. 1). Although organ weights were similar across the *H. hemerocallidea* exposed pups compared to untreated controls, visceral fat of pups born by dams treated with 300 mg/kg *H. hemerocallidea* was reduced compared to the untreated controls (Table 3).

Effect of *H. hemerocallidea* on glucose tolerance of dams and pups

At 28 days postpartum, *H. hemerocallidea* improved glucose tolerance in dams as the blood glucose level was higher (p<0.001) in untreated dams compared to dams treated with 150 mg/kg and 300 mg/kg *H. hemerocallidea* 30 minutes after oral glucose load (Fig. 2). Similarly, *H. hemerocallidea* also improved glucose tolerance in pups as pups born of untreated dams had higher (p<0.05) blood glucose level after 30 and 60 minutes of oral glucose load than those born of dams treated with 150 mg/kg and 300 mg/kg *H. hemerocallidea* (Fig. 3).

Total antioxidant capacity

The total antioxidant capacity (TAC) at 28 days postpartum of *H. hemerocallidea* treated dams was similar (p>0.05) to that of untreated controls. However, the TAC was increased (*p*<0.05) in pups born of dams treated with 150 mg/kg and 300 mg/kg *H. hemerocallidea* compared to the untreated controls (Fig. 4).

Table 1. Effect of *H. hemerocallidea* treatment on dams' body weights during pregnancy.

Pregnancy	Mean body weight (g)		
	Con	150 mg/kg HH	300 mg/kg HH
Day 0	194 ± 2.1	190 ± 3.0	193 ± 1.8
Day 14	217 ± 3.8	218 ± 4.3	218 ± 4.0
Day 20	213 ± 3.1	197 ± 3.6	205 ± 3.7

Data are presented as mean ± SEM (n = 6). SEM: Standard error of the mean; Con: untreated controls; HH: *Hypoxis hemerocallidea* ethanolic extract; p>0.05 indicates no significant difference with respect to the control.

Table 2. Effect of *H. hemerocallidea* treatment on dams' vital organs 28 days after delivery.

Organ	Organ Weight Index (% body weight)		
	Con	**150 mg/kg HH**	**300 mg/kg HH**
Visceral fat	1.43 ± 0.20	1.19 ± 0.10	1.20 ± 0.17
Heart	1.81 ± 0.17	1.57 ±0.18	1.86 ± 0.06
Liver	3.47 ± 0.32	3.34 ± 0.27	2.851 ± 0.07
Kidneys	0.70 ± 0.03	0.70 ± 0.03	0.68 ± 0.02
Spleen	0.24 ± 0.01	0.24 ± 0.01	0.25 ± 0.01

Values are mean ± SEM (n = 6). SEM: Standard error of the mean; Con: untreated controls; HH: *H. hemerocallidea* ethanolic extract; p>0.05 indicates no significant difference with respect to the control.

Table 3. Effect of *H. hemerocallidea* on pups' vital organs 28 days after delivery.

Organ	Organ weight index (% body weight)		
	Con	**150 mg/kg HH**	**300 mg/kg HH**
Visceral fat	1.60 ± 0.09	1.32 ± 0.15	1.19 ± 0.17*
Heart	0.36 ± 0.02	0.38 ± 0.01	0.34 ± 0.01
Liver	3.04 ± 0.02	3.18 ± 0.09	3.19 ± 0.12
Kidneys	0.66 ± 0.01	0.71 ± 0.03	0.65 ± 0.04
Spleen	0.30 ± 0.01	0.31 ± 0.01	0.32 ± 0.01

Values are mean ± SEM (n = 6). SEM: Standard error of the mean; Con: untreated controls; HH: *H. hemerocallidea* ethanolic extract. *p<0.05 indicates significant difference with respect to the control.

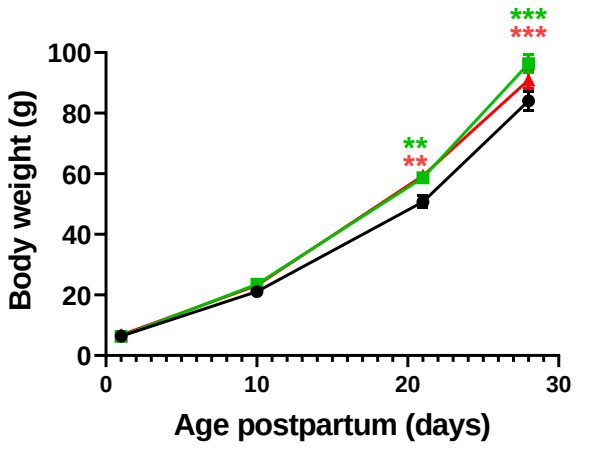

Figure 1. Effect of *in utero* exposure to *H. hemerocallidea* on pup's body weights.

Data is presented as mean ± SEM (n = 6). SEM: Standard error of the mean; Con: untreated controls; 150 mg/kg: 150 mg/kg bw of *H. hemerocallidea* ethanolic extract; 300 mg/kg: 300 mg/kg bw *H. hemerocallidea* ethanolic extract; bw: body weight; **p<0.01; ***p<0.001 compared to control.

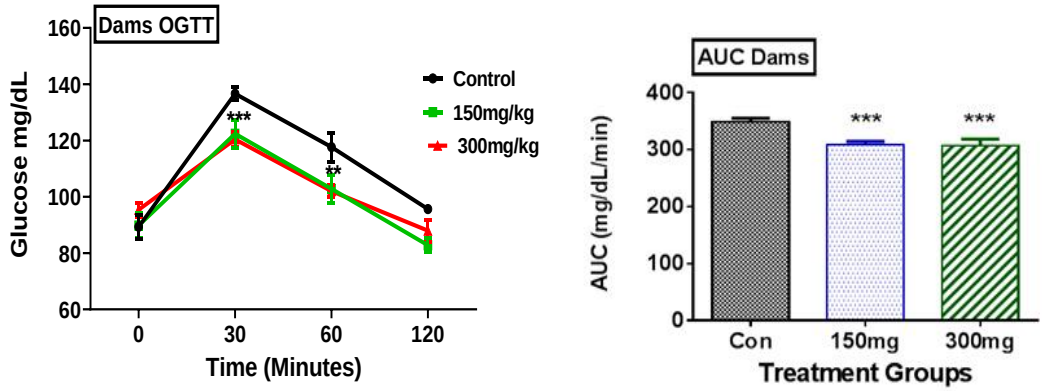

Figure 2. Effect of *H. hemerocallidea* on glucose tolerance in dams 28 days after delivery.

Data are presented as mean ± SEM (n = 6). SEM: Standard error of the mean; bw: body weight; Con: untreated controls; 150mg: 150 mg/kg bw of *H. hemerocallidea* ethanolic extract; 300mg: 300 mg/kg bw *H. hemerocallidea* ethanolic extract. ***p<0.001 indicates significant difference with respect to the control.

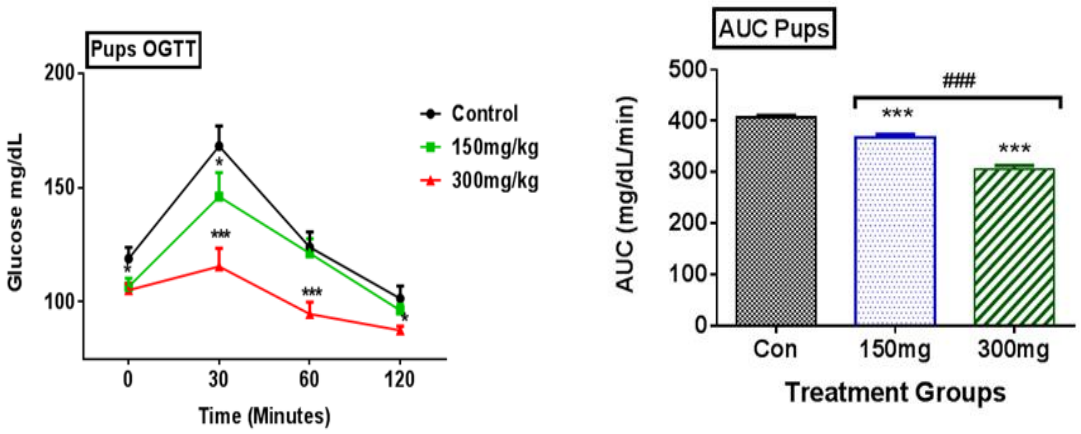

Figure 3. Effect of *H. hemerocallidea* on glucose tolerance in pups 28 days postpartum.

Data are presented as mean ± SEM (n = 6). SEM: Standard error of the mean; bw: body weight; Con: untreated controls; 150mg: 150 mg/kg bw of *H. hemerocallidea* ethanolic extract; 300mg: 300 mg/kg bw *H. hemerocallidea* ethanolic extract. *p<0.05; ***p<0.001 indicates significant difference with respect to the control; ###p<0.001 150 mg/kg indicates significant difference with respect to 300 mg/kg group.

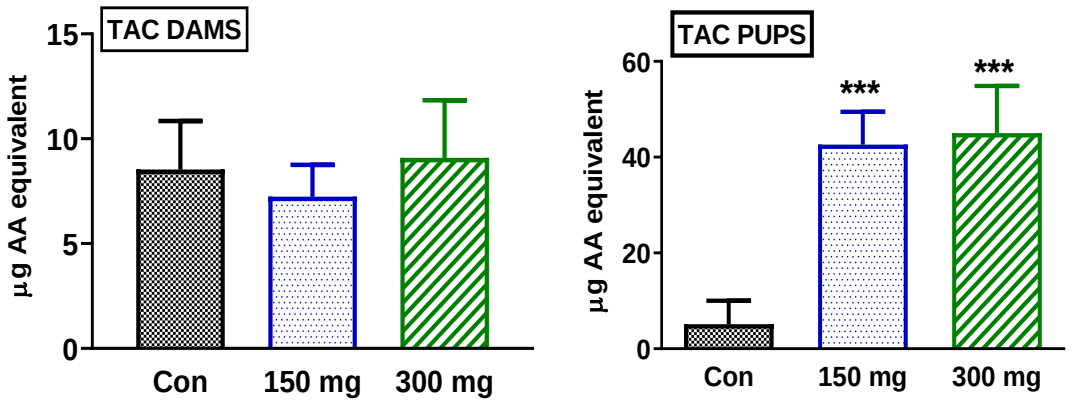

Figure 4. Effect of *H. hemerocallidea* on total antioxidant capacity in dams and pups 28 days after delivery.

Data are presented as mean ± SEM (n = 6). SEM: Standard error of the mean Con: untreated controls; 150mg: 150 mg/kg body weight of *H. hemerocallidea* ethanolic extract; 300mg: 300 mg/kg body weight *H. hemerocallidea* ethanolic extract. ***p<0.001 indicates significant difference with respect to the control.

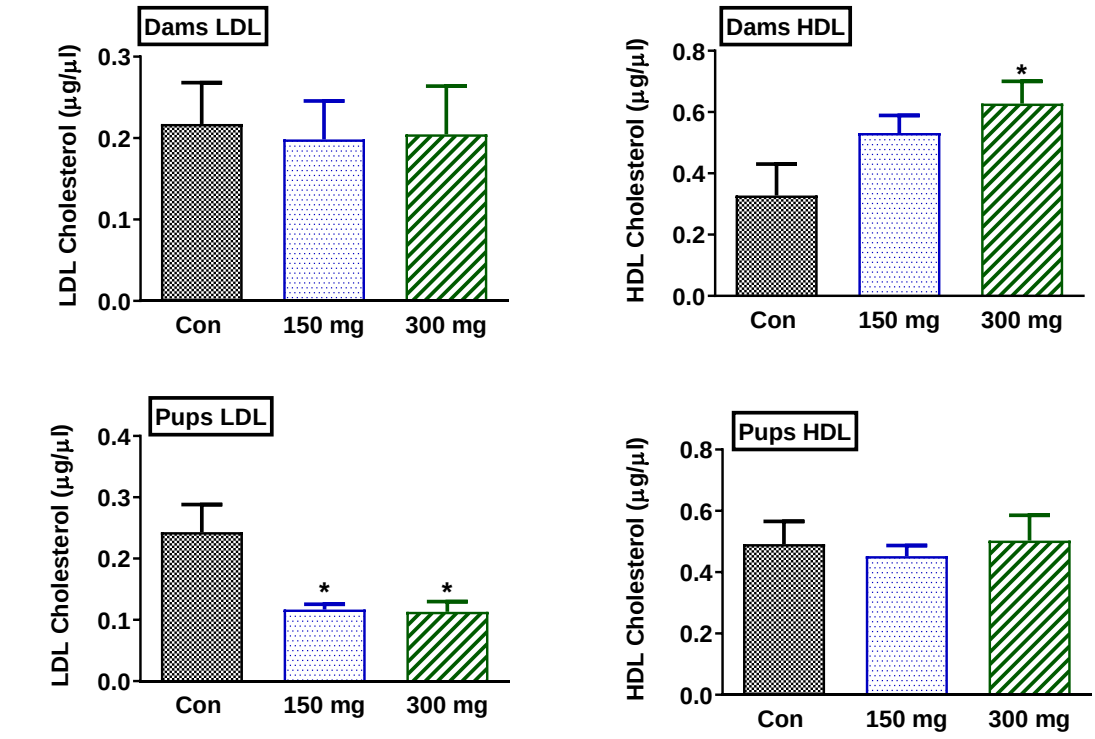

Figure 5. LDL and HDL of dams and pups. Effect of *H. hemerocallidea* treatment on LDL and HDL levels in dams and pups 28 days after delivery.

Data are presented as mean ± SEM (n = 6). SEM: Standard error of the mean Con: untreated controls; 150mg: 150 mg/kg body weight of *H. hemerocallidea* ethanolic extract; 300mg: 300 mg/kg body weight *H. hemerocallidea* ethanolic extract. *p<0.05 indicates significant difference with respect to the control.

LDL and HDL cholesterol of dams and pups

LDL level of dams treated with 150 mg/kg and 300 mg/kg *H. hemerocallidea* was similar ($p>0.05$) to that of the untreated controls. However, HDL level was increased ($p<0.05$) in 300 mg/kg *H. hemerocallidea* treated dams compared to the untreated controls. Pups born of dams treated with 150 mg/kg and 300 mg/kg *H. hemerocallidea* had lower LDL ($p<0.05$) compared to the untreated controls. Moreover, the HDL level of pups born to *H. hemerocallidea* treated dams was similar ($p>0.05$) to that of the untreated controls. Results are summarized in Fig. 5.

DISCUSSION

Metabolic syndrome associated diseases such as T2D and CVDs are known to originate from genetics, physical inactivity and increased intake of calorie-rich foods (Mottillo et al., 2010). Of late, there is evidence that supports the role of intrauterine programming that predisposes the offspring to develop metabolic syndrome later in life, associated with under- and over-nutrition, phytochemicals, drugs and stress during pregnancy (Ozanne, 2001; Boney et al., 2005). Medicinal plants collectively known as "*isihlambezo*" of which *Hypoxis hemerocallidea* is a part, are commonly used in South Africa for the duration of pregnancy to prevent premature labor, boost maternal immunity, encourage lactation, protect the fetus from evil spirits and to encourage growth and maturity of the fetus (Varga and Veale, 1997; Ngomane and Mulaudzi, 2012). It remains unclear whether *in utero* exposure to these medicaments may result to beneficial or detrimental metabolic-programming outcomes on the offspring later in life. This study, therefore, investigated the effects of *H. hemerocallidea* on metabolic syndrome parameters during and after pregnancy for the mothers and on their offspring. Findings of this study showed that treatment with *H. hemerocallidea* did not have an effect on body weight of dams throughout pregnancy. In line with a study by Laporta et al. (2006), treatment of mice with *H. hemerocallidea* had no effect on body weight. Since body weight during pregnancy is determined by fetal growth, food intake and metabolism of the dams, we conclude that *H. hemerocallidea* did not affect any of these parameters during pregnancy. Monitoring of organ weights gives information on the general wellbeing of animals (Tsai et al., 2003). *H. hemerocallidea* had no effect on vital organ (heart, liver, spleen and kidneys) weights of the treated dams and pups exposed in utero when compared to the control groups, thus, *H. hemerocallidea* had no gross toxic effects on these organs, also supported by our previous study in male rats using similar *H. hemerocallidea* doses (Tiya et al., 2017).

Uterine environmental perturbation during pregnancy may have epigenetic effects on offspring affecting metabolic function (Waterland and Jirtle, 2004). Studies have reported that birth weight may determine future risk of development of non-communicable diseases (Vehaskari et al., 2001). In the current study, although all offspring had similar birth weights, offspring exposed to *H. hemerocallidea* showed increased growth compared to controls by day 21 postpartum to termination of the study, 28 days postpartum. The observed growth may be attributed to muscle and bone growth since visceral fat was lower in *H. hemerocallidea* exposed offspring rats than in controls. It is therefore reasonable to surmise that *H. hemerocallidea* exposure may have stimulated growth hormone release at the hypothalamo-pituitary axis or increased peripheral tissue response through increased insulin-like growth factor (IGF) secretion. Indeed, a study in human children demonstrated that growth hormone response via IGF production was associated with genetic and epigenetic parameters (Ouni et al., 2015).

Insulin resistance is a condition in which cells fail to respond to the normal effects of insulin resulting in failure to metabolize glucose, which may result to T2D. Insulin resistance is commonly characterized by hyperglycemia and glucose intolerance as the action of insulin on glucose metabolism is impaired. Although pregnancy is naturally a state of insulin resistance adapted to deliver nutrients to the growing fetus (Sonagra et al., 2014), a shift to gestational diabetes is associated with epi-

genetic consequences promoting obesity, insulin resistance and diabetes in the offspring later in life (Li et al., 2017) *H. hemerocallidea* improved glucose tolerance of dams treated with both doses. This finding is hardly surprising since antidiabetic and hypoglycemic effects of *H. hemerocallidea* have been reported before (Mahomed and Ojewole, 2003; Oguntibeju et al., 2016). The unique finding in this study was the observed improved glucose tolerance of *H. hemerocallidea* exposed pups compared to the untreated controls. Studies in humans and animals have demonstrated that maternal gestational diabetes and hyperinsulinemia play a role in programming obesity and insulin resistance in offspring (Srinivasan et al., 2006; Wang et al., 2018). This suggests that intrauterine programming for improved glucose tolerance in the offspring might have occurred due to the anti-insulin resistance effects of *H. hemerocallidea* observed after maternal treatment. Indeed, fetal exposure to the antidiabetic drug metformin also resulted in improved glucose tolerance in offspring (Gregg et al., 2018).

Dyslipidemia is another factor of the metabolic syndrome characterized by increased triglyceride, total cholesterol and LDL, as well as reduced HDL level. LDL is known to be atherogenic "bad" cholesterol because it contains high amount of cholesterol while HDL rids the body of cholesterol, and thus considered as non-atherogenic "good" cholesterol (Adiels et al., 2008). In both dams and pups, *H. hemerocallidea* had protective effects by increasing HDL in dams and decreasing LDL as well as decrease visceral fat in pups. *H. hemerocallidea* might have had its effects by affecting enzymes involved in HDL or LDL metabolism in the liver or peripheral tissues. This finding also showed that *H. hemerocallidea* treatment in dams might have influenced epigenetic effects on the pups, which helped to reduce dyslipidemia and improved lipids.

Oxidative stress is associated with the development of chronic diseases including insulin resistance and T2D (Henriksen et al., 2011). Pregnancy and embryonic development are associated with increased production of reactive oxygen species (Sen and Simmons, 2010). Moreover, intrauterine stress has been shown to be associated with insulin resistance and may have influence on offspring (Buhl et al., 2007). Polyphenol that include flavonols found in *H. hemerocallidea*, are phytochemicals known to have antioxidant activity (Laporta et al., 2006). The total antioxidant capacity (TAC) in dams 28 days after treatment was similar in the *H. hemerocallidea* treated animals to that of the controls. This might have been so because of the period of assessment. Ideally, TAC should have been measured on day 20 of pregnancy or as close to delivery time as possible, but due to circumstances beyond our control, serum could only be collected 28 days after stopping treatment. Moreover, the pups born to *H. hemerocallidea* treated dams had higher TAC compared to the controls implying that *H. hemerocallidea* improved the antioxidant status of offspring. Previous studies have demonstrated that supplementation of polyphenols in pregnant rats resulted in increased protection against ROS in adulthood (Vanhees et al., 2011). The researchers associated this to possible genomic imprinting by the process of DNA methylation (Vanhees et al., 2011). Findings from the present study seem to suggest a similar effect of polyphenols on pups exposed during pregnancy.

CONCLUSIONS

H. hemerocallidea protected pregnant dams and their pups from insulin resistance, improved lipid profiles to be less atherogenic, reduced visceral fat accumulation and boosted total antioxidant capacity in pups born of *H. hemerocallidea* treated dams. These protective effects of *H. hemerocallidea* against metabolic syndrome in offspring may have resulted from intrauterine programming during pregnancy. An increase in HDL and a decrease in LDL coupled with improved glucose tolerance reduce the risk for development of T2D, atherosclerosis and CVDs. Therefore, *in utero* exposure to *H. hemerocallidea* may act as a prophylaxis to prevent future risk of metabolic syndrome in offspring.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

Authors acknowledge the South African Medical Research Council (SAMRC) for funding through the Self-Initiated Research (SIR) grant awarded to C.R. Sewani-Rusike (MRC-Rusike).

REFERENCES

Abdillahi HS, Van Staden J (2013) Application of medicinal plants in maternal healthcare and infertility: A South African perspective. Planta Med 79(07): 591–599.

Adiels M, Olofsson SO, Taskinen MR (2008) Overproduction of very low-density lipoproteins Is the hallmark of the dyslipidemia in the metabolic syndrome. Arteriosclerosis Thromb Vasc Biol 28 (7): 1225–1236.

Benzie IF, Strain JJ (1996) The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power. Analytical Biochem 239: 70–76.

Boney CM, Verma A, Tucker R, Vohr BR (2005) Metabolic syndrome in childhood: association with birth weight, maternal obesity, and gestational diabetes mellitus. Pediatrics 115: e290–e296.

Buhl ES, Neschen S, Yonemitsu S, Rossbacher J, Zhang D, Morino K, Flyvbjerg A, Perret P, Samuel V, Kim J, Cline GW, Petersen KF (2007) Increased hypothalamic–pituitary–adrenal axis activity and hepatic insulin resistance in low-birth weight rats. Am J Physiol Endocrinol Metab 293: E1451–E1458.

Dalziel SR, Walker NK, Parag V, Mantell C, Rea HH, Rodgers A, Harding JE (2005) Cardiovascular risk factors after antenatal exposure to betamethasone: 30-year follow-up of a randomised controlled trial. Lancet 365: 1856–1862.

Drewes SE, Khan F (2004) The African potato (*Hypoxis hemerocallidea*): a chemical historical perspective. S Afr J Sci 100: 425–430

Ebbert JO, Jensen MD (2013) Fat depots, free fatty acids, and dyslipidemia. Nutrients 5: 498–508.

Eckel RH, Grundy SM, Zimmet PZ (2005) The metabolic syndrome. Lancet 365(9468): 1415–1428.

Godfrey KM, Gluckman PD, Hanson MA (2010) Developmental origins of metabolic disease: life course and intergenerational perspectives. Trends Endocrinol Metab 21 (4): 199–205.

Gregg BE, Botezatu N, Brill JD, Hafner H, Vadrevu S, Satin LS, Alejandro EU, Bernal-Mizrachi E (2018) Gestational exposure to metformin programs improved glucose tolerance and insulin secretion in adult male mouse offspring. Sci Rep 8: 5745.

Gutiérrez-Rodelo C, Roura-Guiberna A, Olivares-Reyes JA (2017) Molecular mechanisms of insulin resistance: An update. Gac Med Mex 153: 197–209.

Han TS, Lean ME (2015) Metabolic syndrome. Medicine 43(2): 80–87.

Henriksen EJ, Diamond-Stanic MK and Marchionne EM (2011) Oxidative stress and etiology of insulin resistance and type 2 diabetes. Free Radical Biol Med 51(5): 993–999.

Inadera H (2013) Developmental origins of obesity and type 2 diabetes: molecular aspects and role of chemicals. Environ Health Prev Med 18:185–197.

Kaido TL, Veale DJH, Havlik I, Rama DBK (1997) Preliminary screening of plants used in South Africa as traditional herbal remedies during pregnancy and labour. J Ethnopharmacol 55(3): 185–191

Kooi R, Theobald S (2006) Traditional medicine in late pregnancy and labour: perceptions of Kgaba remedies amongst the Tswana in South Africa. Afr J Trad Compl Altern Med 3(1): 11–22.

Laporta O, Pérez-Fons L, Mallavia R, Caturla N, Micol V (2006) Isolation, characterization and antioxidant capacity assessment of the bioactive compounds derived from *Hypoxis rooperi* corm extract (African potato). Food Chem 101: 1425–1437.

Levitt NS, Lindsay RS, Holmes MC, Seckl JR (1996) Dexamethasone in the last week of pregnancy attenuates hippocampal glucocorticoid receptor gene expression and elevates blood pressure in the adult offspring in the rat. Neuroendocrinology 64: 412–418.

Li S, Zhu Y, Yeung E, Chavarro JE, Yuan C, Field AE, Missmer SA, Mills JL, Hu FB, Zhang C (2017) Offspring risk of obesity in childhood, adolescence and adulthood in relation to gestational diabetes mellitus: a sex-specific association. Int J Epidemiol 46(5): 1533–1541.

Mahomed IM, Ojewole JAO (2003) Hypoglycemic effect of *Hypoxis hemerocallidea* corm (African potato) aqueous extract in rats. Methods Find Exp Clin Pharmacol 25(8): 617–624.

Mottillo S, Filion KB, Genest J, Joseph L, Pilote L, Poirier P, Rinfret S, Schiffrin EL, Eisenberg MJ (2010) The metabolic syndrome and cardiovascular risk a systematic review and meta-analysis. J Am Coll Cardiol 56(14):1113–1132.

Ngomane S, Mulaudzi FM (2012) Indigenous beliefs and practices that influence the delayed attendance of antenatal clinics by women in the Bohlabelo district in Limpopo, South Africa. Midwifery 28(1): 30–38.

Nordeng H, Al-Zayadi W, Diallo D, Ballo N, Paulsen BS (2013) Traditional medicine practitioner's knowledge and views on treatment of pregnant women in three regions of Mali. J Ethnobiol Ethnomed 9: 67.

Nyakudya TT, Mukwevho E, Erlwanger KH (2018) The protective effect of neonatal oral administration of oleanolic acid against the subsequent development of fructose-induced metabolic dysfunction in male and female rats. Nutr Met 15(1): 82.

Nyinawumuntu A, Awe EO, Ojewole JAO (2008) Uterolytic effect of *Hypoxis hemerocallidea* Fisch. & C.A. Mey. (Hyxidaceae) corm ['African Potato'] aqueous extract. J Smooth Muscle Res 22(1): 91–96.

Nyirenda MJ, Lindsay RS, Kenyon CJ, Burchell A, Seckl JR (1998) Glucocorticoid exposure in late gestation permanently programs rat hepatic phosphoenolpyruvate carboxykinase and glucocorticoid receptor expression and causes glucose intolerance in adult offspring. J Clin Invest 101: 2174–2181.

Oguntibeju OO, Meyer S, Aboua YG, Goboza M (2016) *Hypoxis hemerocallidea* significantly reduced hyperglycaemia and hyperglycaemic-induced oxidative stress in the liver and kidney tissues of streptozotocin-induced diabetic male Wistar rats. Evidence-Based Compl Altern Med 2016: ID 8934362.

Ojewole JA (2006) Antinociceptive, anti-inflammatory and antidiabetic properties of *Hypoxis hemerocallidea* Fisch. & CA Mey.(Hypoxidaceae) corm ['African Potato'] aqueous extract in mice and rats. J Ethnopharmacol 103(1): 126–134.

Ojewole JAO, Kamadyaapa DR, Musabayane CT (2006) Some *in vitro* and *in vivo* cardiovascular effects of *Hypoxis hemerocallidea* Fisch & CA Mey Hypoxidaceae) corm (African potato) aqueous extract in experimental animal models: cardiovascular topics. Cardiovasc J S Afr 17(4): 166–171.

Ormazabal V, Nair S, Elfeky O, Aguayo C, Salomon C, Zuñiga FA (2018) Association between insulin resistance and the development of cardiovascular disease. Cardiovasc Diabetol 17(1): 122.

Ouni M, Castell AL, Linglart A, Bougnères P (2015) Genetic and epigenetic modulation of growth hormone sensitivity studied with the IGF-1 generation test. J Clin Endocrinol Met 100(6): E919–E925.

Ozanne SE (2001) Metabolic programming in animals. Br Med Bull 60: 143–52.

Peltzer K, Friend-du Preez N, Ramlagan S, Fomundam H (2008) Use of traditional complementary and alternative medicine for HIV patients in KwaZulu-Natal, South Africa. BMC Public Health 8(1): 255.

Phillips CM (2013) Nutrigenetics and metabolic disease: current status and implications for personalised nutrition. Nutrients 5(1): 32–57.

Phillips DI (1996) Insulin resistance as a programmed response to fetal undernutrition. Diabetologia 39: 1119–1122.

Phillips DI (2007) Programming of the stress response: a fundamental mechanism underlying the long-term effects of the fetal environment? J Intern Med 261: 453–460.

Reagan-Shaw S, Nihal M, Ahmad N (2008) Dose translation from animal to human studies revisited. FASEB J 22(3), 659–661.

Roche HM, Phillips C, Gibney MJ (2005) The metabolic syndrome: the crossroads of diet and genetics. Proc Nutrition Soc 64(3): 371–377.

SABS (2008) South African National Standard: The Care and Use of Animals for Scientific Purposes, 1st edn. (SANS 10386:2008), 232 pp. Pretoria, South Africa: South African Bureau of Standards, Standards Division.

Saklayen MG (2018) The global epidemic of the metabolic syndrome. Curr Hyperten Rep 20: 12.

Samuel VT, Shulman GI (2016) The pathogenesis of insulin resistance: integrating signaling pathways and substrate flux. Nm J Clin Invest 126(1): 12–22.

Sen S, Simmons R (2010) Maternal antioxidant supplementation prevents adiposity in the offspring of Western diet-fed rats. Diabetes 59: 3058–3065.

Smith NH, Ozanne SE (2006) Intrauterine origins of metabolic disease. Rev Gynaecol Perinatal Pract 6: 211–217.

Sonagra AD, Biradar SM, Dattatreya K, Jayaprakash MDS (2014) Normal pregnancy-a state of insulin resistance. J Clin Diagnostic Res 8(11): CC01–CC03.

Srinivasan M, Aalinkeel R, Song F, Mitrani P, Pandya JD, Strutt B, Hill DJ, Patel MS (2006) Maternal hyperinsulinemia predisposes rat fetuses for hyperinsulinemia, and adult-onset obesity and maternal mild food restriction reverses this phenotype. Am J Physiol Endocrinol Met 290(1): E129–E134.

Stumvoll M, Mitrakou A, Pimenta W, Jenssen T, Yki-jarvinen H, VanHaeften T, Renn WG (2000) Use of the oral glucose tolerance test to assess insulin release and insulin sensitivity. Diabetes Care 23: 295–301

Tiya S, Sewani-Rusike CR, Shauli M (2017) Effects of treatment with *Hypoxis hemerocallidea* extract on sexual behaviour and reproductive parameters in male rats. Andrologia 49(8): e12742.

Torres-Leal FL, Fonseca-Alaniz MH, Rogero MM, Tirapegui J (2010) The role of inflamed adipose tissue in the insulin resistance. Cell Biochem Funct 28(8): 623–631.

Tsai PP, Stelzer HP, Hedrich HJ, Hackbarth H (2003) Are the different enrichment designs on the physiology and behaviour of DBA/ 2 mice consistent? Lab Anim 37: 314–327.

Vanhees K, Godschalk RW, Sanders A, van Waalwijk van Doorn-Khosrovani SB, van Schooten FJ (2011) Maternal quercetin intake during pregnancy results in an adapted iron homeostasis at adulthood. Toxicology 290(2-3): 350–358.

Varga CA, Veale DJH (1997) Isihlambezo: utilization patterns and potential health effects of pregnancy-related traditional herbal medicine. Social Sci Med 44(7): 911–924.

Vega CC, Reyes-Castro LA, Rodríguez-González GL, Bautista CJ, Vázquez-Martínez M, Larrea F, Chamorro-Cevallos GA, Nathanielsz PW, Zambrano E (2016) Resveratrol partially prevents oxidative stress and metabolic dysfunction in pregnant rats fed a low protein diet and their offspring. J Physiol 594(5): 1483–1499.

Vehaskari VM, Aviles DH, Manning J (2001) Prenatal programming of adult hypertension in the rat. Kidney Int 59(1): 238–245.

Wang X, Yang T, Miao J, Liu H, Wu K, Guo J, Chen J, Li T (2018) Correlation between maternal and fetal insulin resistance in pregnant women with gestational diabetes mellitus. Clin Lab 64(6): 945–953.

Waterland RA, Jirtle RL (2004) Early nutrition, epigenetic changes at transposons and imprinted genes, and enhanced susceptibility to adult chronic diseases. Nutrition 20: 63–68.

WHO - World Health Organization (2008) 2008–2013 Action Plan for the Global Strategy for the Prevention and Control of Noncommunicable Diseases, Geneva Switzerland: World Health Organization.

AUTHOR CONTRIBUTION:

Contribution	Sewani-Rusike CR	Buso A	Engwa GA	Nkeh-Chungag BN
Concepts or ideas	x			x
Design	x			x
Definition of intellectual content	x			
Literature search		x	x	
Experimental studies		x		x
Data acquisition		x		
Data analysis	x	x	x	
Statistical analysis	x		x	
Manuscript preparation		x	x	
Manuscript editing	x		x	x
Manuscript review	x	x	x	x

Citation Format: Sewani-Rusike CR, Buso A, Engwa GA, Nkeh-Chungag BN (2021) *In utero* exposure to *Hypoxis hemerocallidea* Fisch., C.A.Mey. & Avé-Lall. improves metabolic syndrome parameters in pregnant rats and offspring. J Pharm Pharmacogn Res 9(2): 113–125.