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A comparative study of the histopathological modifications of adrenal gland in STZ-induced diabetic Wistar rats administered with selected herbal plants versus Glimepiride

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This study investigated the comparative study of four herbal extract versus Glimepiride on the histomorphological modification of adrenal gland in STZ induced diabetic rats with a view to understanding their antidiabetic properties. Forty- two healthy adult Wistar rats (*Rattus novergicus*) with an average weight of 153.4 g were randomly divided into seven groups (n=6). STZ (65 mg/kg) dissolved in citrate buffer was administered intraperitoneally to animals in groups B to G while animals in group A received equivalent volume of citrate buffer. Plant extracts (100 mg/kg) were administered daily (orally) to animals in groups C to F and glimepiride (anti-diabetic drug) to animals in group G for 14 days. After the expiration of the study, the animals were sacrificed and the adrenal gland was excised, fixed in 10% formol saline for histology and morphometric analysis. Result showed that body weights of diabetic rats significantly decreased when compared with control and other groups. Also, adrenal weight, and thickness of the cortex were significantly increased (*P*<0.05) in diabetic rats compared with control and other groups. Also, thickness in medulla of adrenal gland of group B was decreased significantly (p < 0.05) when compared with control and other groups. The histology and morphometric analysis revealed that the adrenal gland in the group treated with *Citrullus lanatus* seed shaft showed a better histoarchitectural outline of all the four plant extracts used. This study suggested that *C. lanatus* seed shaft could be a better alternative therapy in ameliorating diabetic-associated disorders of the adrenal gland.

Key words: Diabetes, *Psidium guajava, Veronia amygdalina, Ficus mucuso, Citrullus colocynthis,* adrenal gland.

INTRODUCTION

The extracts of medicinal plants have been established to ameliorate and protect different diseases which have been used by the majority of the world population for thousands of years. Herbal drugs are prescribed and used

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widely because of their effectiveness in curative, less side effects and not expensive.

Citrullus colocynthis (CC) (family of Cucurbitaceae) commonly known as colocynth or bitter apple, is one of the plants which has been used for anti-diabetic in traditional medicine (Shafaei et al., 2014). The name of this extract was derived from its bitter flavor which is similar to colocynthine (Shafaei et al., 2014). The colocynth which originated from tropical Asia and Africa is now widely distributed in other parts of the world (Azzi et al., 2015). *C. colocynthis* contained lycopene, ascorbic acid and citruline which are valued source of natural antioxidants. These mentioned functional ingredients act as protection against chronic health disorders like cancer insurgence and cardiovascular disorders (Shafaei et al., 2014). Lycopene is a lipophilic carotenoid stored in adipose tissues that reduces the obesity and hyperglycemic conditions (Madhava et al., 2011).

V. amygdalina (VA) is from Asteraceae family. It is a perennial plant with height between 1 and 6 m. It is soft wooded and a multipurpose with rapid regenerating shrub (Nwosu et al., 2013). All parts of the plant are useful in pharmacologically research and both the roots and leaves are useful in phyto-medicine for management of various diseases in humans and animals (Tugume et al., 2016). The leaves could also used traditionally to induce fertility in women (Adedapo et al., 2014). It possesses antioxidant benefits (Oyeyemi et al., 2017), enhances the immune system, decreased blood sugar when compared to untreated diabetic animals in a study conducted using streptozotocin-induced diabetic laboratory animals (Oyeyemi et al., 2017) and *in vitro* antihelmintic and antiparasitic properties (Ademola and Eloff, 2011). A number of researches have shown the anti-diabetic properties of VA (Oyeyemi et al., 2017). The *in vivo* anti-diabetic activity has also been demonstrated (Tugume et al., 2016).

Ficus mucuso (FM) with a common name called fig is a semi-deciduous spreading savannahs tree with greenish flower and a very tiny numerous seeds (Ahoua et al., 2012). Apes and indeed humans (Kamanzi, 2002) depends so much on *Ficus* as part of their diet because of the high nutritive value. The antioxidant status and beneficial effects of *Ficus* have been documented (Ahoua et al., 2012) as well as ameliorative role of its extracts on the biochemical profiles (Ayoka et al., 2014).

Guava (*Psidium guajava* L.) (PG) possess some phytochemicals which had been documented, such as phenolic compounds, carotenoids and vitamins, mainly ascorbic acid (C) and tocopherol (E), which are effective free-radical scavengers (Jiménez-Escrig et al., 2001; Tesfahun and Habtamu, 2017). Some of these substances are effective in the treatment of the diseases (Tesfahun and Habtamu, 2017). The regular consumption of significant amounts of fruits and vegetables of *P. guajava* has been promoted by specialists to prevent degenerative and chronic diseases due to its antioxidants property (Chiari-Andreo et al., 2017).

Diabetes is a group of metabolic diseases characterized by hyperglycaemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycaemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the adrenal gland eyes, kidneys, nerves, heart, and blood vessels (Ismail et al., 2016). Diabetes mellitus (DM) is a chronic disease leading to impairment of the functions of many systems, such as the cardiovascular, immune, and central nervous systems through hyperglycemia, polyuria, polydipsia, and natriuresis (Ismail et al., 2016).

Diabetes mellitus (DM) is primarily caused when there is dysfunction of the hypothalamic-pituitary-adrenal (HPA) axis. Studies investigating the association between diabetes and adrenal gland morphology had documented that adrenal volume is increased in patients with diabetes (Carsin et al., 2016; Schneller et al., 2014).

This research is significant because it could help diabetics to know the comparative study of four herbal extract versus Glimepiride on the histomorphological modification of adrenal gland in STZ induced diabetic rats in the treatment of diabetes patients.

MATERIALS AND METHODS

Animal management

Forty- two healthy adult Wistar rats (*Rattus novergicus*) with an average weight of 153.4 g were procured from the animal house of College of Health Sciences, Obafemi Awolowo University, Ile - Ife, Osun State. The animals were kept under standard laboratory condition of good lighting, moderate temperature, and adequate ventilation in a hygienic environment. They were feed on standard rat chow of balance diet. The animals were placed under standard laboratory protocols as stipulated by the Institutional Animal Care and Use Committee.

Animal grouping and treatment

The animals were randomly divided into seven groups of 6 animals each: Group A, control normal rats administered with equivalent volume of citrate buffer by oral method; Group B, experimentally-induced diabetic rats with streptozotocin (65 mg/kg), administered intraperitoneally; Group C, induced diabetic rats with streptozotocin (65 mg/kg) treated with aqueous extract of VA leaves (100 mg/kg), dissolved in normal saline for 14 days administered orally; Group D, induced diabetic rats with streptozotocin (65 mg/kg) treated with aqueous extract of shaft of CC seeds (100 mg/kg), dissolved in normal saline for 14 days given orally; Group E, induced diabetic rats with streptozotocin (65 mg/kg) treated with aqueous extract of PG (100 mg/kg) orally, dissolved in normal saline for 14 days; Group F, induced diabetic rats (65 mg/ kg) treated with aqueous extract of FM (100 mg/kg) orally, dissolved in normal saline for 14 days; Group G, experimentally-induced diabetic rats with streptozotocin (65 mg/ kg) treated with a standard antidiabetic drug (2 mg/kg of glimepiride) dissolved in normal saline for 14 days administered orally.

Table 1. The effects of extracts on relative weight of adrenal gland.

Group	Absolute weight of adrenal glands (g)	Relative weight of adrenal glands (%)
A (Control)	0.06 ± 0.01[a]	0.06 ± 0.01[ab]
B (Diabetic)	0.04 ± 0.00[a]	0.03 ± 0.00[a]
C (Diabetic +*Veronia amygdalina*)	0.06 ± 0.01[a]	0.03 ± 0.00[a]
D (Diabetic + *Citrullus lanatus* seed shaft)	0.08 ± 0.01[a]	0.03 ± 0.00[a]
E (Diabetic + *Psidium guajava*)	0.05 ± 0.00[a]	0.03 ± 0.00[a]
F [Diabetic + *Fiscus mucuso* (SPP)]	0.20 ± 0.13[b]	0.09 ± 0.06[b]
G (Diabetic + Glimepiride)	0.05 ± 0.01[a]	0.02 ± 0.00[a]

Values are given as Mean ± SEM in each group. In the table ab differs significantly at p<0.05 while a and b does not differ significantly at p < 0.05 (using one way ANOVA with SNK).

Preparation of extracts

The plant leaves were procured from a local market in Ile-Ife metropolis in Osun state, Nigeria. The leaves were taken to the herbarium in the Department of Botany, Obafemi Awolowo University, Nigeria for identification. The leaves and shaft of the plants were air dried and powdered in a warring blender. The extraction process of the plant leaves of *V. amygdalina* (425 g), *P. guajava* (970 g), *F. mucuso* (370 g) and shaft of *C. colocynthis* (615 g) were prepared by dissolving it in 2.9 L, 3.19 L, 3.5 L and 2.2 L of distilled water respectively for 72 h with intermittent shaking. Thereafter, the solution was filtered using a filter paper. The filtrate was then concentrated *in vacuo* at 35°C using a rotator vacuum evaporator (Buchi Rotavapor, R110 Schweiz). The extracts were oven dried at 37°C, and the respective percentage yield (3.00, 2.65, 5.34 and 1.76 g) were stored until ready to use. The aliquot portion of each of the extracts were weighed and dissolved in normal saline for use on each day of the experiment.

Induction of diabetes

Diabetes mellitus was induced in groups B, C, D, E, F, and G by a single intraperitoneal injection of 65 mg/kg body weight of streptozotocin (Tocris Bioscience, UK) dissolved in 0.1 M sodium citrate buffer (pH 6.3) (Topal et al., 2013). Diabetes was confirmed in animals 48 h after induction, by determining fasting blood glucose level using a digital glucometer (Accu-chek® Advantage, Roche Diagnostic, Germany) consisting of a digital meter and the test strips using blood samples obtained from the tail vein of the rats. The animals were stabilized for 28 days before the commencement of extract and glimepiride administration. The fasting blood glucose was subsequently monitored throughout the experimental period. Animals in group A were given equal volume of citrate buffer used in dissolving streptozotocin intraperitoneally.

Method of administration of extracts

The animals were fed orally using orogastric tube. The animals were held with a glove with the left hand such that the neck region was held by the fingers to still the neck while being fed. Treatment was done at 07.00 h every day before the animals were fed over a period of two weeks (14 days).

Sacrifice and specimen collection

The animals were sacrificed by cervical dislocation 24 h after the expiration of research. The Adrenal glands were excised and weighed, following midline-abdominal incision.

Histological evaluation

The harvested adrenal glands were fixed in 10% formal saline for a minimum of 48 h and processed routinely for paraffin embedding. Serial sections were obtained at 5 μm from a rotary microtome (Bright B5040, Huntington England) and stained using routine haematoxylin and eosin method.

Photomicrography

Stained sections were viewed under a Leica DM750 microscope (Leica Microsystems, Heerbrugg, Switzerland) with digital camera attached (Leica ICC50) and digital photomicrographs were taken which were also imported onto the ImageJ version 1.48 (NIH, Bethesda, MD, USA) software for histomorphometric analysis of thickness of cortex and medulla in adrenal gland.

Statistical analysis

Data were expressed as mean ± SEM and analysed using One-way ANOVA, followed by Student Newman-Keuls (SNK) test for multiple comparisons. Significant difference was taken as p<0.05.

RESULTS

Effects of extracts on relative weight of adrenal gland

Body weights of diabetic rats significantly decreased (*P*<0.05) compared with other groups. Adrenal weight was significantly increased (*P*<0.05) in diabetic rats compared with other groups. Herbal plants extract-treated diabetic rats showed a significant increase in body weight (*P*<0.05) and a significant decrease in adrenal weight (*P*<0.05) in comparison with diabetic rats (Table 1).

Effects of extracts on histomorphometric thickness of cortex and medulla

There was significant decrease (*P*<0.05) in the thickness

Table 2. The effects of extracts on histomorphometric thickness of cortex and medulla.

Group	Adrenal cortex			
	Zona Glomerulosa (µm)	Zona Fasciculata (µm)	Zona Reticulosa (µm)	Adrenal Medulla (µm)
A (Control)	30.75±1.15	120.5 ± 2.34	43.61 ± 2.97	257.2 ± 31.69
B (Diabetic)	38.74 ± 0.63	131.5 ± 2.97	56.64 ± 1.93	91.97 ± 0.85
C (Diabetic + *Veronia amygdalina*)	32.39 ± 1.60	125.1 ± 2.90	48.02 ± 3.18	182.3 ± 10.07
D (Diabetic + *Citrullus lanatus* seed shaft)	31.13 ± 1.47	125.1 ± 2.36	44.92 ± 4.50	229.7 ± 42.28
E (Diabetic + *Psidium guajava*)	32.69 ± 2.27	127.1 ± 5.12	48.41 ± 2.85	138.9 ± 20.07
F [Diabetic + *Fiscus mucuso* (SPP)]	35.89 ± 1.50	127.4 ± 4.76	50.65 ± 1.57	151.7 ± 13.15
G (Diabetic + Glimepiride)	32.99 ± 2.52	128.2 ± 5.22	48.75 ± 2.58	155.7 ± 1.96

Values are given as Mean ± SEM (using one way ANOVA with SNK).

of the cortex of group B (Diabetic) compared with groups C, D, E, F and G and decreased significantly (P<0.05) in diameter of medulla in adrenal gland of diabetic rats when compared with the control and other groups. The herbal plants extract-treated diabetic rats showed a significant (P<0.05) increase in cortex and medulla total thickness when compared with diabetic (group B) (Table 2).

Histological findings

The adrenal glands of control rats revealed normal appearance and were seen surrounded by thin connective tissue capsule (Figure 1A). Sections of STZ diabetic rat's adrenal glands (Figures 1B) showed distortion in zona glomerulosa, zona fasciculate, dilated and congested sinusoids in the cortex. Normal zona glomerulosa and zona fasciculate were revealed in the extract administration groups (Figures 1C-1F) as compared to standard drug administration group (Figure 1G).

DISCUSSION

Experimental diabetes was induced by streptozotocin (STZ), led to the alteration of adrenal gland in wistar rats. This has been reported earlier that diabetes can be induced by means of chemical destruction or surgical removal of a part of the β-cell mass, feeding high-sugar diets, and drugs such as streptozotocin (STZ) and alloxan (Carsin-vu et al., 2016). In the present study, the effect of four different herbal plants on the adrenal gland in STZ-induced diabetes was carried out.

The histological result revealed that groups B treated with STZ had distortion of the histology of the adrenal gland tissues while group C to F improved hyperglycemia by four herbal plants which could be attributed to the fact that some components of herbal plants enhanced the insulin-stimulated glucose uptake of rat adipocytes (Obike et al., 2014).

In this new research, STZ-induced diabetes resulted in a significantly decreased in the rat's body weight and a significantly increased in adrenal gland weight mostly in group B. This is in accord with the findings of Mustata et al. (2005) and Ghada et al. (2015), who reported that diabetic rats were significantly reduced in body weight and significantly increased in adrenal gland weight. Reduction in body weight in group B might be due to the increased in muscle wasting and loss of tissue proteins (Shirwaikar et al., 2006). It could also be lack or deficiency of carbohydrate needed for the energy metabolism, which resulted in degradation of structural proteins (Pepato et al., 1996). Significant increased in the body weight of diabetic rats treated with the four different herbal plants compared with the diabetes group might be due to the blood glucose stabilization effect which is more effective for each other and prevents the loss of body weight differently.

The findings of this study shows that STZ-induced diabetes had significant increase in the total thickness of adrenal cortex and increased in the diameter of medullar, which may be explained on the basis of the increased in the thickness of zona fasciculate, zona glomerulosa and zona reticulosa in the cortex as well as increase in diameter of medullar in adrenal gland. Ghada et al. (2015) reported that STZ-induced diabetes causes a notable hypertrophy of the cells of the zona fasciculata, which may explain the increase in total thickness of the adrenal cortex and medullar in the present study.

Administration of the extracts improves the histoarchitecture of the Adrenal gland and by extension restores its functionality. The groups administered with *C.colocynthis* extract demonstrated a distinct regenerative capacity over the other three extract.

Previous studies have reported some similar histopathological findings (Wu et al., 2004). The plant extracts used for the study, are common herbal plant used traditionally in the management of diabetes, amongst the South Wast, Nigeria. Three of these plants (*C. colocynthis, V. amygdalina* and *P. guajava*) have been

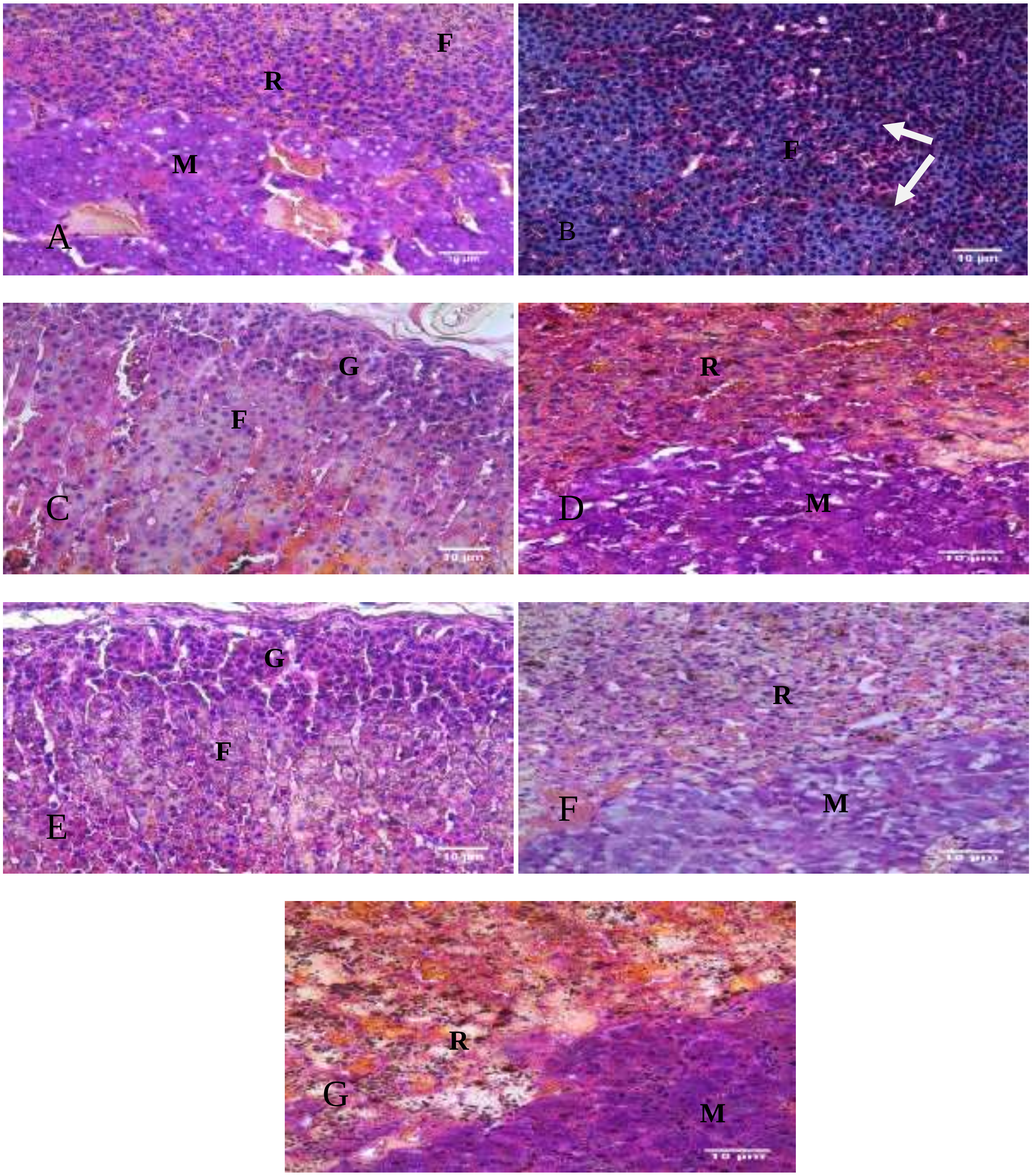

Figure 1. Photomicrographs of Adrenal Gland of Groups A, B, C, D, E, F, and G. (H&E x400). G, zona glomerulosa; F, zona fasciculate, R: zona reticulosa; M, medulla. The white arrow pointed to distorted in zona fasciculate. The extract treated groups (C-F) reveals Adrenal tissue regenerations which shows a remarkable reversible cellular injury as compared with group B.

reported to possess anti-diabetic properties (Akpaso et al., 2011). The four medicinal plants used in this study are well known for their antioxidant properties which are due to their high level content of flavonoids (komolafe et al., 2013).

Conclusion

Treatment of diabetic rats with four herbal plants markedly improves ultrastructure of the adrenal gland with their antioxidant properties which are due to their high level content of flavonoids. Therefore this study suggests that *C. colocynthis* could be better in ameliorating diabetic-associated disorders of the adrenal gland.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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