


ORIGINAL CONTRIBUTION

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The phytochemical and pharmacological screening of three crude extracts of *Desmodium canum* (strong back)

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Abstract

Introduction: *Desmodium canum* (Strong Back) is deemed a versatile traditional medicine, where it is used to treat diabetes, hypertension, asthma and erectile dysfunction.

Aim: To identify the various phytochemicals present within extracts of *D. canum*, their antioxidant capabilities and their effects on blood glucose levels, haemodynamic parameters and testosterone levels in healthy Sprague-Dawley (S-D) rats.

Method: Crude extracts were obtained using hexane, ethyl acetate and methanol. These were analysed for various phytochemicals and their antioxidant potential assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. The extracts were investigated for hypoglycaemic potential using the Oral Glucose Tolerance Test (OGTT), where extracts were administered intravenously (50 mg/kg BW) to fasted rats and their blood glucose readings monitored at 30 min intervals. The hypotensive effect of the extracts were also investigated where rats were administered intravenously at 50 mg/kg BW. These haemodynamic parameters were monitored using the CODA 6 machine at 5 min intervals for a total of 20 min. Additionally, the effect on testosterone level was investigated in male rats where extracts were administered daily by oral gavage. Serum testosterone levels were then determined using an ELISA kit.

Results: The different extracts showed varying phytochemical and pharmacological properties. The methanol extract showed antioxidant capabilities, while the ethyl acetate extract showed significant hypoglycaemic and hypotensive effects when compared with the control. The hexane extract showed significant activity in increasing the testosterone when compared with the control.

Conclusion: *D. canum* extracts showed significant pharmacological activities in normal Sprague- Dawley rats.

Keywords: *Desmonium canum*, Strong back, Hypoglycaemic, Hypotensive, Oral glucose tolerance test (OGTT), CODA 6

Introduction

Traditional medicine, commonly called folk-medicine, has a long history and existed as the sole source of medicine in the past. The practice is used in the maintenance of health, the prevention and treatment of physical and mental illnesses; where herbs, animal parts and minerals

are employed to be the source of medicine [1]. It has a rich influence within the Caribbean region, where folkloric claims have formed a part of the backbone of modern medicine [2]. With this knowledge, many researchers are turning to the traditional roots to investigate these folkloric claims. It is well documented that the presence of the various classes of phytochemicals are associated with many of the pharmacological properties observed [3, 4]. To date, many plants have been extensively studied and phytochemicals (secondary

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metabolites) have been isolated with various pharmacological activities including anti-diabetic properties, hypotensive, and aphrodisiac, among others.

Desmodium canum, commonly known as Strong Back, is one such plant that is used traditionally in several Caribbean islands for the treatment of various illnesses [5]. The plant exists as a perennial herb that grows up to about 70 cm and has red stems and petioles. It grows wild and is often used as a therapeutic agent against diabetes, asthma, hypertension, abdominal pains and the common cold [5]. Additionally, it is extensively used in aphrodisiac tonics, where it is believed to increase libido by increasing testosterone production and penile erection [6]. There is also the belief that the full potential of the plant remains unlocked [7] and therefore research into the bioactivity of the plant may lead to the development of pharmaceuticals for the treatment of diabetes, hypertension and low libido.

This research sets out to investigate the effect of extracts of *Desmodium canum* on blood glucose levels, blood pressure, as well as, testosterone levels in healthy Sprague-Dawley rats. It is imperative that novel methods of maintaining normal levels of the listed parameters are discovered because of the implications associated with their imbalance within the body. For example, a chronic increase in the blood glucose levels can lead to the metabolic condition known as Diabetes Mellitus (DM). DM is “a group of metabolic diseases characterized by hyperglycaemia resulting from defects in insulin secretion, insulin action or both” [8]. Majority of the cases of diabetes falls into two broad etiopathogenic conditions, namely Type 1 and Type 2 Diabetes Mellitus [1, 8]. Type 1 is characterized by a cellular mediated autoimmune destruction of the beta-cells of the pancreas. In this case, the pancreas is unable to secrete the hypoglycaemic hormone, insulin. Type 2 on the other hand, is characterized by relative insulin deficiency to predominantly insulin secretory defects with insulin resistance [8].

The pathophysiology of Type 2 Diabetes includes an elevation of reactive oxygen species (ROS) due to the glycation and hence deactivation of many of the endogenous antioxidants [9]. Consequently, tissues are more susceptible to oxidative attack which results in an increase in the complications associated with diabetes; such as hypertension, coronary heart disease, and erectile dysfunction [9, 10].

Hypertension is one of the main vascular complications associated with diabetes, where, as much as 50 to 80% of all type 2 diabetics are hypertensive [11]. Hypertension is defined as a chronic increase in the pressure of the blood exerted on the walls of the blood vessels, where measurements are expressed as systolic blood pressure (SBP) over diastolic blood pressure (DBP). Typically, hypertension related diabetes is a product of the

decrease in the flexibility of the veins and arteries, resulting in the resistivity of blood flow in the vascular network [11, 12]. In instances of diabetes related hypertension, oxidative stress decreases the bioactivity of nitric oxide that typically promotes the relaxation of the smooth muscles of the blood vessels. Additionally, as a result of insulin deficiency in type 2 DM, the cells resort to metabolizing lipids and proteins which can promote elevation of low density lipoproteins within the blood. This increase causes resistance to blood flow within the blood vessels, which causes an increase in blood pressure [13].

Patients that suffer from diabetes and/or hypertension typically are at greater risk of being plagued by erectile dysfunction (ED) [14]. The pathogenesis of diabetes related ED is similar to that of diabetes associated hypertension, where the oxidative species deactivated NO which plays a pivotal role in erectile responses. Chronic hyperglycaemia may damage nerves and blood vessels that play significant roles in the erectile response, thus introducing an imbalance in the mechanism. There may also be glycation of the smooth muscles of the penis which decreases the elasticity of these muscles, consequently leading to erectile dysfunction [15].

The male primary sex hormone, testosterone, plays a significant role in the erectile responses in men. Studies have shown that men with higher testosterone levels typically have a greater sex drive. Additionally, the hormone up-regulates the expression of the endothelium nitric oxide synthase, an enzyme responsible for the synthesis of NO. Theoretically, this suggests that an increase in testosterone levels may decrease the incidence of erectile dysfunction due to oxidative stress in diabetic men, however, not much work has been done.

This study focuses on identifying various phytochemicals within the extracts of *D. canum* as well as investigating several pharmacological properties of the plant, namely the effect of the crude extracts on blood glucose levels, blood pressure and testosterone levels in healthy Sprague-Dawley rats, as well as, its antioxidant capabilities.

Materials and methodology

Materials

Solvents (HPLC or ACS grade) purchased from Pharmco Products, IL, USA, dimethyl sulfoxide (DMSO) and DPPH were purchased from Sigma Aldrich Co., USA., glucose and corn oil from Hilo (local) supermarket, sodium hydroxide, ferric chloride (GR Grade), hydrochloric acid, ammonium molybdate, sulfuric acid from BDH chemicals. ACCU-Check Active Blood Glucose Monitoring System, Büchi Rotavapor R-124 and Büchi water bath B-481, Spectroline MiniMax UV Lamp (Model UV-4NFW), Kent CODA 6 Non-Invasive Blood

Pressure System (Monitor, animal restraints, electrical warming pad and O- and V-cuffs), Analytical Balance, Ohaus triple beam balance).

Plant collection and extraction

The stem and the leaves of *Desmodium canum* were collected in the hills of rural St. Andrew and was authenticated and given the voucher number: 36277 by Mr. Patrick Lewis, a botanist in the Herbarium Department of Life Sciences, The University of the West Indies (UWI), Mona. The plant was air dried and milled into a fine grounded powder using an electrical grinder. The milled plant was then weighed and packed into a glass column for sequential extraction using hexane, ethyl acetate and then methanol. Each solvent was allowed two separate 8 h periods followed by a 24 h period. The solvents were removed using a rotary evaporator and the remaining extracts were weighed and stored in a freezer for future bioassay.

Phytochemical screening

Phytochemical screening of the crude extracts was done using a modified method described by Iqbal, Salim & Lim in 2015; Hossain et al., in 2013 [16, 17]. Colour change comparisons were made to the various solvents used to dissolve the extracts (hexane, ethyl acetate and methanol) and also to known members of the phytochemical categories for positive comparisons.

Test for alkaloids

Separate solutions of the extracts (1 mg/mL, 2 mL) were made up in their respective solvents. To each solution, Dragendorff's reagent was added and the presence of turbidity or formation of an orange-red precipitate was used as an indication for the presence of alkaloids.

Test for flavonoids

To solutions of the extracts (1 mg/mL), dilute NaOH solution was added. The formation of an intense yellow/orange colour that became colourless with the addition of a few drops of dilute HCl was an indication of the presence of flavonoids.

Test for tannins

The extracts (2 mg) were dissolved in 2 mL of their respective solvents. To this, a few drops of 10% ferric chloride were added. The formation of blackish-blue colour was used for the indication of the presence of tannins.

Test for saponins

Each extract (0.5 g) was boiled in distilled water (10 mL) for 5 min. The tubes were stoppered and vigorously shaken for 5 min and then left to stand for 30 min. The

formation of frothing in the tubes was used as an indication for the presence of saponins.

Test for triterpenoids

The extracts (5 mg) were dissolved in 2 mL of chloroform after which, 1 mL of acetic anhydride was added. This was then followed by the addition of 1 mL of concentrated sulfuric acid. The formation a reddish violet colour indicated the presence of triterpenoids.

Test for steroids (the Liebermann-Burchard's test)

Extracts (0.5 g) were each dissolved in anhydrous chloroform (10 mL) and filtered. The solutions collected were mixed with 1 mL of acetic anhydride, to which, 1 mL of concentrated sulfuric acid was added to form a lower layer. The formation of a green colour was used as an indication of the presence of steroids.

Antioxidant assay

The antioxidant assay was carried out using a modification of the method described by Proestos et al., 2013 where 2,2-diphenyl-1-picrylhydrazine (DPPH) served as a source of stable free radicals [18]. Various concentrations (200, 100, 50, 25, 10, 1 µg/mL) of the plant extracts and ascorbic acid (standard) were made up in methanol to a volume of 2 mL. This was then incubated with DPPH (0.1 mM, 2 mL) in the dark for a period of 1 h. Subsequently, the absorbance values were measured using a UV/VIS spectrophotometer at 517 nm. The decrease in DPPH radicals were monitored using a UNICO® 1100RS Spectrophotometer at 517 nm. The decrease was expressed as:

$$\% \text{inhibition} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

Abs control- absorbance of a solution of methanol and DPPH

Abs sample- absorbance of solution of extract incubated with DPPH

Ethical consideration

Ethical Approval was granted by The University of the West Indies Ethics (Mona) Committee for the protocol described below.

Animal care

Healthy S-D rats (200–250 g) were used for the biological assays. The animals were housed at The University of the West Indies, Mona Animal House where they were fed standard rodent lab chow and tap water ad libitum. The room was maintained at standard room temperature and pressure ($23 \pm 2^\circ\text{C}$ and 1 atm) and the animals were exposed to 12/12 h light/dark cycle.

The effects of crude extracts on blood glucose levels

The oral glucose tolerance test (OGTT) was used to determine the effect of the crude extracts on blood glucose levels. The study was done similarly to the method described by Alexander-Lindo, Salmon & McGrowder [19]. Twenty-four (24) normal S-D rats were obtained from the Animal House and divided into 4 groups ($n = 6$). Group 1 was treated with the hexane extract intravenously at 50 mg/kg body weight (BW). Group 2 was treated with the ethyl acetate crude extract intravenously at 50 mg/kg BW. Group 3 was treated with the methanol crude extract intravenously at 50 mg/kg BW while group 4 served as the control, receiving 0.3 mL of vehicle, dimethyl sulfoxide (DMSO).

The animals were fasted overnight with water ad libitum for approximately 12 h, subsequently their fasting blood glucose levels were monitored using an Accu-chek glucometer. Group 1 was then administered 50 mg/kg BW hexane extract dissolved in 0.3 mL DMSO. Their blood glucose was then monitored at 30 min intervals for 1 h, after which a glucose load of 1.75 g/kg BW dissolved in 0.3 mL of water was administered orally. Blood glucose level was then monitored at 30 min intervals for a further 2½ h. This was then repeated for groups 2 to 4 and the results were noted and compared.

The effects of the extracts on blood pressure

In accordance to ethical considerations to minimize the number of rats used, the animals above were rested for 2 weeks, after which they were reused for the determination of the effect of the crude extracts on blood pressure. They were acclimatized to a restraint prior to the study by allowing them three separate 15 min periods. The Kent CODA 6 non-invasive machine was used to determine the basal blood pressure of the animals. This was done by placing the animals in a restraints, followed by attaching the occlusion (O) cuff and volume pressure recorder sensor cuff (V). The systolic, diastolic and mean arterial pressure were monitored. The animals were treated with the respective crude extracts by intravenous injections of 50 mg/kg BW. The O and the V cuffs were then placed on the tail of the animals and the blood pressure readings taken at 5 min intervals for 20 min.

The effects of the crude extracts on testosterone levels

The study was adapted from Bai & An 2015. Thirty-six male S-D rats were obtained from the UWI Animal House and divided into six groups with $n = 6$. By oral gavage, group 1 was administered 250 mg/kg body weight (BW) of the hexane extract dissolved in 0.3 mL corn oil. Group 2 was given the ethyl acetate extract (250 mg/kg BW), again dissolved in 0.3 mL corn oil. Group 3 received the methanol extract (250 mg/kg BW) dissolved in 0.3 mL of 10% dimethylsulfoxide (DMSO).

Groups 4 and 5 served as negative controls where the carrier solvent, corn oil and DMSO were respectively administered to the two groups. Groups 1–5 were treated daily for a period of 28 days by oral administration of the respective treatment regime described above. Group six served as a positive control in which the rats received a 5 mg/kg BW per week treatment of testosterone via subcutaneous injections. After 28 days of treatment, all the rats were weighed and then anaesthetized by intraperitoneal injections of sodium pentobarbital (60 mg/kg BW). Blood was then collected from the renal arteries, centrifuged at 3000 rpm for 10 min. Serum was removed and used for the quantification of testosterone using a testosterone mouse/rat ELISA kit purchased from Crystal Chem Inc [20].

Statistical analysis

Values were expressed as mean \pm standard error of the mean and were analysed using the student's *t* test, where $p < 0.05$ was considered statistically significantly different.

Results and discussion

Antioxidant capabilities of the crude extracts of *D. canum* (Fig. 1)

Antioxidant capabilities of the extracts were compared to ascorbic acid, where *indicates a significant difference when compared with the positive control. The methanol extract showed inhibition of %DPPH activity that was comparable with the ascorbic acid at 200 μ g/mL and 100 μ g/mL. This is of great importance as consumption of fruits and vegetables rich in antioxidants have shown positive results in decreasing the morbidity of diseases associated with oxidative stress, such as, coronary heart diseases and atherosclerosis [9, 10]. Isolation of these compounds and further investigation of their IC₅₀ values may show their efficacy in reducing oxidative stress.

The effects of the crude extracts on blood glucose levels (Fig. 2)

Intravenous administration of the extracts had varying effects on the blood glucose levels of the rats. The DMSO control indicated the usual increase in the blood glucose levels at 90 min due to the administration of oral glucose load at the 60 min interval. Throughout the remaining of the curve, there was a general decrease in the blood glucose level due to the action of insulin. There was no effect on the blood glucose concentration. The ethyl acetate extract showed the most significant hypoglycaemic activity throughout the OGTT curve. This was observed in the fasting state at 60 min (4.08 ± 0.21 mmol/L vs the DMSO control of 4.69 ± 0.26 mmol/L; $p < 0.01$) as well as the post prandial state at 120 min ($4.89 \pm$

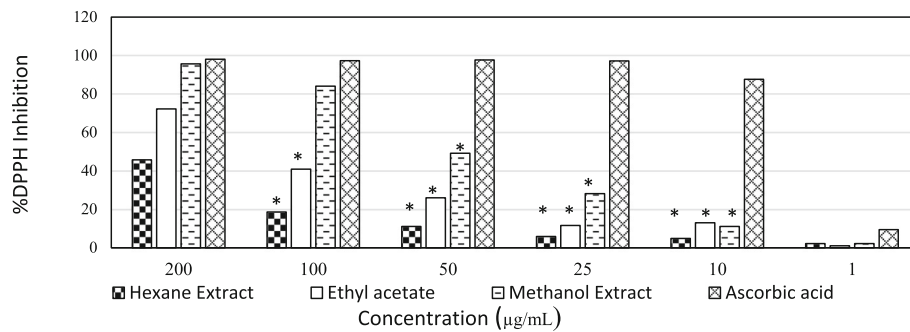


Fig. 1 The antioxidant capabilities of crude extracts from *Desmodium canum* (Strong Back)

0.0833 mmol/L vs 5.50 ± 0.17 mmol/L; $p < 2.4 \times 10^{-4}$) and 150 min (4.42 ± 0.22 mmol/L versus 4.64 ± 0.25 mmol/L; $p < 8.0 \times 10^{-3}$) compared with the control. The greatest activity was observed in the post-prandial region, where the values fell to those similar to fasting conditions. Post-prandial hyperglycaemia has been implicated with the development of several complications including obesity and Type 2 diabetes. Manipulation of this by α -amylase and α -glucosidase enzymes was suggested to improve the progression of diabetes by as much as 25% [21]. The modulation of the post-prandial glycaemia is therefore a necessary step in curbing impaired glucose tolerance and the ethyl acetate extract showed great potential in doing this. Table 1 shows that phytochemical screening of the ethyl acetate extract indicated the presence of steroids, alkaloids, terpenoids, saponins and reducing sugars. Agrawal and co-authors (2013) demonstrated that some alkaloids possess antihyperglycaemic properties, chiefly, by being insulinotropes or acting as inhibitors of α -amylase and α -glucosidase enzymes [22]. It may also be possible that the hypoglycaemic compounds present within the

ethyl acetate extract may be from another class of phytochemicals listed above. However, it is certain that the ethyl acetate extract works best in the fed state and may indeed act as inhibitors to the enzymes mentioned or may act as insulinotropes. Further work needs to be done to isolate these active natural products and determine their mechanisms of action.

The effects of the crude extracts on haemodynamic parameters

The crude ethyl acetate extract from the plant had the most significant hypotensive effect when compared with the DMSO control. This was seen at times: 10 min (124.24 ± 4.69 mmHg vs 150.92 ± 5.33 mmHg; $p < 0.003$), 15 min (124.82 ± 4.65 mmHg vs 158.10 ± 5.99 mmHg, $p < 0.001$) and 20 min (134.21 ± 6.2 vs 154.00 ± 3.34 ; $p < 0.01$). The methanol extract also showed significant activity compared with the DMSO control as seen at the 15 min interval (133.7 ± 4.61 mmHg vs. 158.10 ± 5.99 mmHg; $p < 0.01$) and the 20 min interval (135.0 ± 3.00 mmHg vs. 155.89 ± 6.89 mmHg; $p < 0.033$).

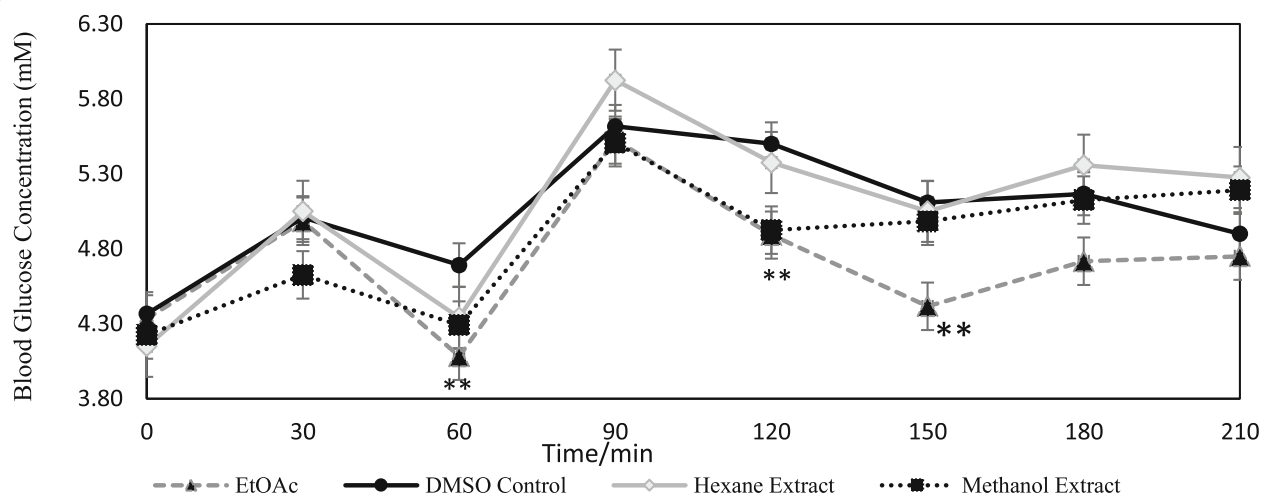


Fig. 2 The effects of the crude extracts of *Desmodium canum* (Strong Back) on blood glucose levels in normal Sprague-Dawley rats (IV @ 50 mg/kg BW). ** $p < 0.01$

Table 1 The various classes of phytochemicals present in the crude extracts of *Desmodium canum*

Phytochemicals	Hexane Extract	Ethyl acetate Extract	Methanol Extract
Phenols	–	–	+
Tannins	–	–	+
Flavonoids	–	–	+
Saponins	–	+	+
Terpenoids	+	+	+
Steroids	+	+	–
Alkaloids	+	+	–

Key

+ Indicating a positive response

– Indicating a negative response

The ethyl acetate extract showed the most significant activity when compared with the DMSO control. This was observed at: 10 (97 ± 8.49 mmHg vs 121.63 ± 5.95 mmHg, $p < 0.041$), 15 (99.94 ± 6.63 vs. 129.24 ± 3.59 mmHg, $p < 0.004$) and 20 min intervals (108.73 ± 4.55 vs 126.96 ± 3.64 mmHg, $p < 0.01$).

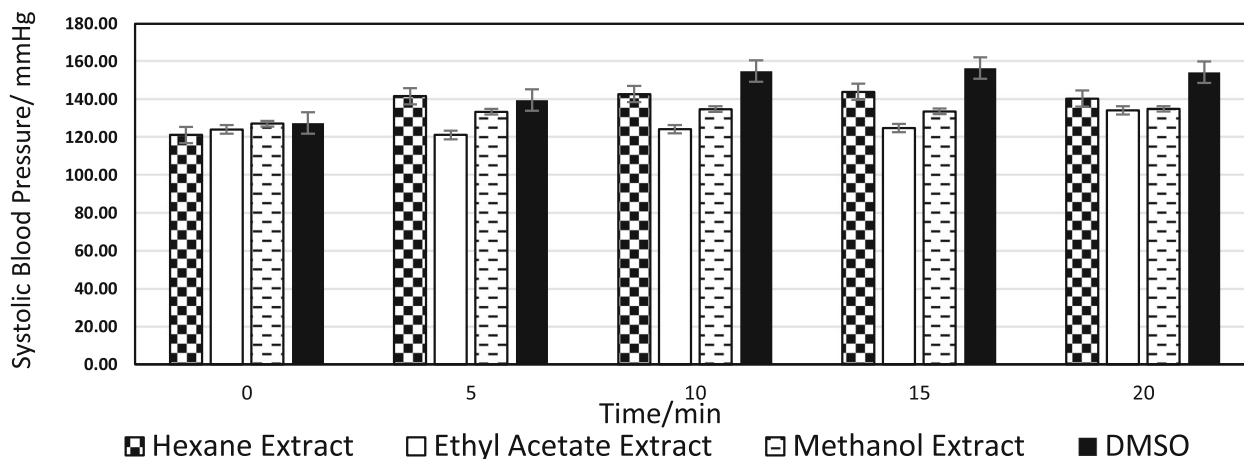
The ethyl acetate extract showed the most significant lowering of the MAP when compared with the DMSO. This was observed at the 10 min interval (105.72 ± 8.49 mmHg vs 131.13 ± 5.98 mmHg; $p < 0.017$), 15 min (107.94 ± 6.63 mmHg vs 138.53 ± 3.59 mmHg, $p < 0.0019$) and 20 min (114.60 ± 4.55 mmHg vs 135.91 ± 3.64 mmHg; $p < 0.01$). The methanol crude extract also showed hypotensive mean arterial pressure when compared with the control. The effects were seen at times of 15 min (117.70 ± 5.63 mmHg vs 138.53 ± 3.59 mmHg, $p < 0.015$) and 20 min (119.9 ± 3.0 mmHg vs. 135.91 ± 3.64 mmHg, $p < 0.017$).

It was noted that with administration of the DMSO, all three parameters being monitored increased above

the normal values and may be as a result of the animals becoming agitated or stressed due to the IV injection. However, all four animal groups were exposed to the same conditions and as such, comparisons can be made among the groups. On that basis, acute administration of both the ethyl acetate and the methanol extracts showed significant activity in lowering the SBP, DBP and MAP (Figs. 3, 4 and 5). As mentioned, Diabetes Mellitus and hypertension are interrelated, where as much as 50 to 80% of type 2 diabetics are also hypertensive [11]. It is therefore imperative that more effective methods be established in managing the diseases. The greatest activity was seen in the systolic blood pressure at 10 min post- administration of the ethyl acetate extract. SBP is defined by the pressure of the blood exerted on the walls of the arteries as the heart contracts and is usually the value that is targeted in anti-hypertensive therapy [23]. Both the ethyl acetate and the methanol extracts showed hypotensive effect. SBP was further decreased at the 15 and 20 min intervals. The results indicate that the ethyl acetate extract of *D. canum* possesses the potential to improve the therapeutic approach to diabetes and hypertension. Daily administration of the methanol extract may lead to further decline of the blood pressure due to its antioxidant capabilities. Antioxidants are scavengers for reactive species and thus prevent the oxidative attack on the muscles of the blood vessels.

The effects of the crude extracts on serum testosterone levels (Fig. 6)

The hexane extract (2.21 ± 0.44 ng/mL) and testosterone (2.99 ± 0.31 ng/mL) both showed significant increase in the serum testosterone levels when compared with the corn oil control (0.80 ± 0.13 ng/mL). There was no

**Fig. 3** The effects of the crude extracts of *D. canum* on systolic blood pressure in normal Sprague-Dawley rats (IV @ 50 mg/kg BW). * $p < 0.05$. ** $p < 0.01$

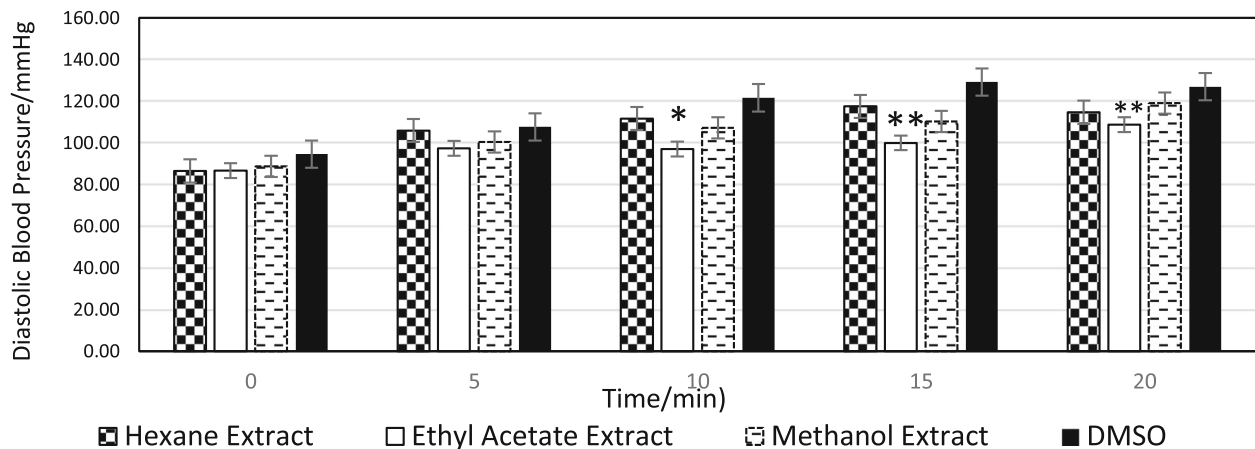


Fig. 4 The effects of the crude extracts of *D. canum* on the diastolic blood pressure of normal Sprague-Dawley rats (IV @ 50 mg/kg BW). * $p < 0.05$. ** $p < 0.01$

significant difference between the hexane extract and the testosterone treated groups. The ethyl acetate crude extract showed no significant difference to the control indicating no effect on the testosterone level.

There was no significant difference between the methanol extract and its control. Indicating no effect on the testosterone level. The testosterone treated group was significantly higher than these two groups.

Qualitative analysis of the hexane extract indicated the presence of steroids, alkaloids and terpenoids. This significantly increased the serum testosterone levels in the animals, where the values were comparable with the testosterone treated group. Several studies have established that many plants such as *Moringa oleifera* and *Tribulus alatus* are aphrodisiacs, where they typically increase testosterone level and/or inhibit the phosphodiesterase-5 enzyme [24, 25].

Low testosterone levels can be as a result of chronic illnesses, stress, trauma, lifestyle, congenital issues, among others. The use of testosterone as a therapeutic

agent to infertility, erectile dysfunction, athletic performance and low libido is well documented. Exogenous testosterone, however, has many side effects which include suppression of intratesticular testosterone development, which is required for spermatogenesis [26]. The effect of the crude hexane extract in increasing the production and secretion of testosterone is an ideal approach to treating hypogonadism in men, which is a common cause of ED. However, a prudent approach is necessary in establishing a method that utilizes this plant in the treatment of hypogonadism, erectile dysfunction and low sex drive.

Conclusion

Desmodium canum (Strong Back) has been used for many generations for its pharmacological properties in treating diabetes, hypertension, erectile dysfunction and the common cold. This paper investigated some of the folkloric claims, where extracts were obtained from the plant and bio-assayed. The different extracts showed

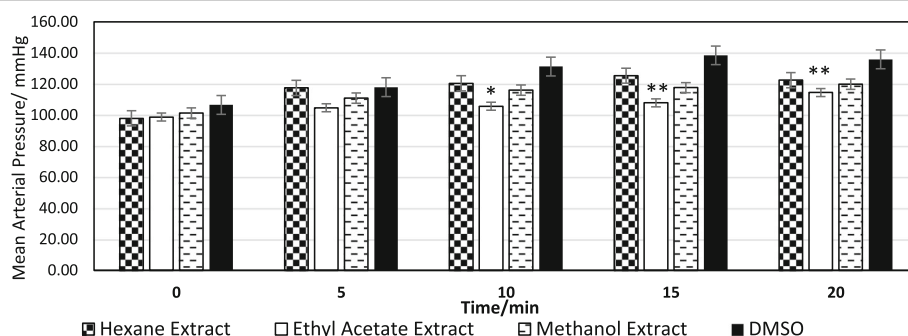


Fig. 5 The effects of the crude extracts of *D. canum* on the mean arterial pressure of normal Sprague-Dawley rats (IV @ 50 mg/kg BW). * $p < 0.05$. ** $p < 0.01$

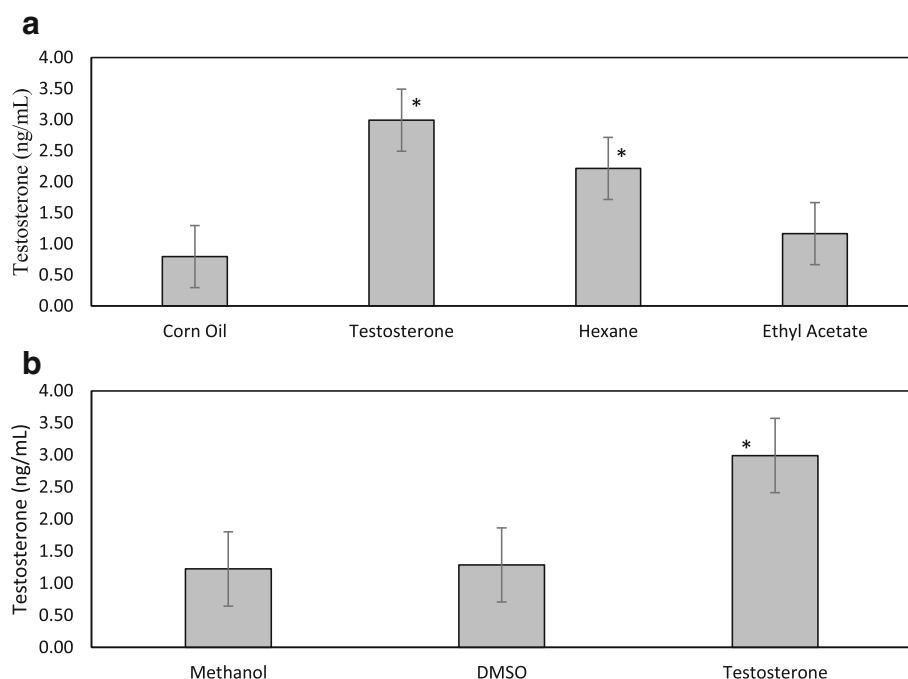


Fig. 6 a The effects of the hexane and the ethyl acetate crude extracts from *D. canum* on the serum testosterone levels in normal Sprague-Dawley rats. *Indicates significant difference when compared with the corn oil control, $p < 0.05$. **b** The effect of the methanol crude extract from *D. canum* on the serum testosterone level in normal Sprague-Dawley rats. *Indicates significant difference when compared with the control, $p < 0.05$

varying pharmacological activities, that is, the hexane extract significantly increased serum testosterone levels in normal male rats, while the ethyl acetate extract significantly lowered the blood glucose level and blood pressure and the methanol extract showed significant antioxidant capabilities. Further studies may lead to the isolation of the natural products responsible for the activities observed and may introduce new therapeutic approaches to these illnesses.

Abbreviations

μ L: Microlitre(s); BW: Body weight; *D. canum*: *Desmodium canum*; DBP: Diastolic blood pressure; DM: Diabetes Mellitus; DMSO: Dimethyl sulfoxide; DPPH: 2,2-Diphenyl-1-picrylhydrazyl; ED: Erectile dysfunction; i.v.: Intravenous; g: Grams; kg: Kilogram(s); L: Litre(s); mg: Milligram(s); min: Minute(s); mL: Millilitre(s); mmol: Millimole(s); MAP: Mean arterial pressure; NO: Nitric oxygen; O-Cuff: Occlusion cuff; OGTT: Oral glucose tolerance test; ROS: Reactive oxygen species; SBP: Systolic blood pressure; S-D: Sprague-Dawley; V-Cuff: Volume Pressure Recording cuff; WHO: World Health Organization

Acknowledgements

The authors wish to express gratitude to the Office of Graduate Studies, The University of the West Indies, Mona and The Ministry of Health Research Grant for funding this study.

Authors' contributions

A total of four individuals contributed towards the conception, experimental design, execution, data analysis and writing of this paper. RA played a significant role by designing the study and constructed the framework for the study to be undertaken. She also assisted with data analysis and edited

previous versions of this paper before submission. KL performed the experiments and wrote the bulk of the information provided in the paper. He also analysed the data obtained and along with RA sought research grants for funding. RP provided some of the reagents utilized in the study. He also provided advice as it relates to methodology to be utilized and edited previous versions of the paper. Similarly, CN provided advice and aided with the analysis and proof-reading of the paper. All authors read and approved the final manuscript.

Funding

Funding for the purchase materials and equipment for the current study was provided by The University of the West Indies' Office of Graduate Studies and Research along with the Ministry of Health. The funding was provided in the form of research grants and were utilized for the purchase of reagents, material and equipment.

Availability of data and materials

Datasets used or analysed in this paper are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate

Ethical Approval was granted by The University of the West Indies Ethics (Mona) Committee for the protocol described below.

Consent for publication

Not applicable.

Competing interests

The authors have no conflict of interest to declare.

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Received: 25 March 2020 Accepted: 3 November 2020

Published online: 09 November 2020

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