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Evaluation of hexane extract of *Chlorophytum alismifolium* on hyperglycaemia and neuropathic pain in high-fat diet and streptozotocin-induced diabetic Wistar rats

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ABSTRACT

Background & Aim: Diabetes mellitus is an intricate metabolic disorder of the endocrine system associated with complications that result in morbidity and mortality. This study was carried out to establish the antihyperglycaemic profile and the effect of hexane extract of *Chlorophytum alismifolium* (HECA) on diabetic neuropathy in high-fat diet and streptozotocin-induced hyperglycaemic Wistar rats.

Experimental: *C. alismifolium* tubers were extracted with n-hexane using a soxhlet apparatus. The LD_{50} was determined using the OECD 425 guideline. The animals were served high fat diet for 6 weeks and then administered 40 mg/kg of streptozotocin to induce diabetes. Experimental groups were set up using normal rats in group I and hyperglycaemic rats in 5 groups of 5 rats each. Group I and II were the normal and hyperglycaemic controls given distilled water (1 mL/kg); groups III, IV and V were given categorized doses of HECA (150, 300 and 600 mg/kg) respectively. Group VI received pioglitazone (20 mg/kg). Fasting glycaemic level was evaluated and diabetic neuropathy was investigated using thermal and mechanical methods.

Results: Phytochemical screening showed the presence of flavonoids, alkaloids, steroids and triterpenes while the oral median toxic dose was estimated to be >5000 mg/kg. Administration of HECA at all doses evidently ($P<0.05$) reduced the glycaemic level relative to the hyperglycaemic group and over time. Treatment with HECA also remarkably ($P<0.05$) lowered the withdrawal times in both thermal and mechanical hyperalgesic methods of diabetic neuropathy compared to the hyperglycaemic control.

Recommended applications/industries: The findings revealed that the hexane extract of *Chlorophytum alismifolium* has beneficial effects and can be applied in the management of diabetes and some of its complications.

1. Introduction

Diabetes mellitus (DM) is a protracted metabolic disease that is depicted by a relative or total lack of insulin and thereby resulting in hyperglycaemia. (ADA, 2010). Type 2 diabetes DM is a major cause of complications and responsible for mortality (Cordero *et al.,* 2016). Globally, people living with DM have been approximated to be about 464 million; Middle East and North Africa (55 million), Sub-Saharan Africa (19 million), Europe (59 million), North America and Caribbean (48 million), South and Central America (32 million), South and East Asia (88 million) and Western Pacific (163 million) and this shocking figure is projected to rise to an estimated 578 million by the year 2030 (IDF 2019). Microvascular and macrovascular complications are linked with type 2 DM and often occur concurrently (Bramlage *et al.,* 2014; Kontopantelis *et al.,* 2015). The presence of microvascular disease increases the danger of morbidity and mortality in subjects with type 2 DM (Brownrigg *et al.,* 2016) because of an associated damage of target organs and tissues like the kidneys, eyes and nerves (Laha and Paul, 2019).

Protracted hyperglycaemia is attributed to metabolic disruptions and result in vascular destruction by initiating several biochemical mechanisms which are associated with abnormal cellular homeostasis and development of systemic complications (Vincent *et al.,* 2011; Mohammedi *et al.,* 2017). The commonest complication of DM is neuropathy and approximately 30-50% of all diabetic patients acquire diabetic peripheral neuropathy (Vincent and Feldman, 2004; Yang *et al.,* 2019). The management of diabetes and its complications with orthodox medications is associated with grave undesirable effects and poor prognosis (Alicic *et al.,* 2017). Therefore, there is need to explore natural products because of their supposed efficacy and reduced harmful consequences (Peng *et al.,* 2019).

Chlorophytum alismifolium Baker belongs to the family liliaceae and it is a stunted herbal stem with tubers (Burkill, 1995). Hitherto, *C. alismifolium* had demonstrated a significant degree of safety in Wistar rats (Abubakar *et al.,* 2019a) and it has been evaluated for its antihyperglycaemic (Abubakar *et al.,* 2018), anti-inflammatory and antinociceptive (Abubakar *et al.,* 2019b; 2020) activities. This research is aimed at exploring the hexane extract of *C. alismifolium* for hyperglycaemia and neuropathic pain in an animal model of type 2 DM.

2. Materials and Methods

2.1. Drugs and chemicals

The standard drug used was Pioglitazone (Micro Laboratories LTD., POHH0014, India). Chemicals used include streptozotocin (STZ) (MP Biomedicals M 3219k, France) and n-hexane (Sigma Chemical Co. St. Louis, USA).

2.2.Experimental animals

Wistar rats (150-200g) were gotten from the Animal Husbandry of the Department of Pharmacology and Therapeutics, Ahmadu Bello University Zaria, Nigeria. They were kept under normal laboratory specifications and given unrestricted access to feed and water *ad libitum*. The studies were performed with the authorization of the Ahmadu Bello University Committee on Animal Use and Care with the Ethical Approval Number: ABUCAUC/2020/31.

2.3. Processing of extract and phytochemical screening

The whole plant of *Chlorophytum alismifolium* was collected from Toro Local Government Area of Bauchi state in July, 2019. It was identified and authenticated at the Herbarium Section of the Department of Botany, Ahmadu Bello University, Zaria, Nigeria. The plant was issued a voucher specimen number (No. 6785) after comparing with an existing reference voucher specimen. The tubers were dried and processed into powdered material (1 kg) which was then subjected to extraction with 2.5 L of n-hexane using a soxhlet extractor for 48 h (Redfern *et al.,* 2014). The extractobtained was dried on a water bath set at 45^{o}C. The extract was coded hexane extract of *Chlorophytum alismifolium* (HECA) and stored in a desiccator until further use. The extractive value was established and it was subjected to phytochemical investigations using standard procedures as described by Evans (2009).

2.4. Acute toxicity study

The median lethal dose (LD_{50}) of HECA was established in accordance with the Organization for

Economic Co-operation and Development (OECD, 425) guideline (OECD, 2001) using five rats which were fasted overnight before dosing with HECA at 5000 mg/kg orally. One rat was initially dosed and food was withheld for 4 h followed by observation for the first 24 h. Four rats were additionally dosed and then observed for 14 days for symptoms and signs of toxicity such as; changes in mucous membranes, skin, fur and eyes, circulatory, respiratory, somato-motor activity and behaviour pattern and mortality. Thereafter, the LD_{50} was estimated.

2.5. Antihyperglycaemic study

Fifty (50) rats were fed with high fat diet (HFD) which was made of animal pelletized feed and margarine (10:1) and 20 % fructose solution as drinking water for 6 weeks as described by Okoduwa *et al.* (2017). Experimental hyperglycaemia was produced by the administration of 40 mg/kg of streptozotocin (STZ) through the intraperitoneal route (Parveen *et al.*, 2010). The rats were administered 10% dextrose solution for 24 h to avert death as a result of early hypoglycaemia associated with STZ. The rats were given access to water and food. The blood glucose levels were monitored 3 days post STZ injection and then validated after 7 days to confirm hyperglycaemia with the aid of a glucometer (Accu-check Active, Roche, Germany). The rats with fasting blood glucose level above 190 mg/dL were designated hyperglycaemic and selected for the study. Group I served as the normal control group (Non hyperglycaemic) administered distilled water (1 mL/kg), group II *served* as the hyperglycaemic control administered distilled water 1 mL/kg, groups III-V were hyperglycaemic rats administered HECA at 150, 300 and 600 mg/kg respectively while group VI was administered pioglitazone 20 mg/kg for 28 days.

2.6. Evaluation of diabetic neuropathy

2.6.1. Tail immersion test

Thermal hyperalgesia was evaluated in the hyperglycaemic rats using tail immersion method as described by Anjaneyulu *et al*. (2006). The tail of each animal was dipped in a beaker containing hot water maintained at 45.5 ± 0.5 ºC monitored with a thermometer. The tail flicking responses of rats were observed and the time taken to flick tail was recorded. Three tests separated by at least 10 min were performed for each rat and the mean for each rat was recorded.

2.6.2. Paw-pressure test

Mechanical hyperalgesia is a clinical feature of peripheral neuropathy (Callaghan *et al.*, 2012). Increase sensitivity to pain as a result of the probable destruction of nociceptors was determined using Ugo Basile Analgesy-Meter (Model 7200, Italy), as described by Randall and Selitto (1957). The pain reflex was quantified using the analgesy-meter which produces a force that is applied to the rat's hind paw. The force is applied to the animal's paw at a rate of 64 g/s. The cut off pressure of 450 g was maintained to avoid mechanical damage to the paw skin. The mean nociceptive threshold which is the force in grams at which a rat struggled to withdraw its hind-paw was recorded.

2.7. Statistical analysis

Data evaluation was done with the aid of SPSS software (Version 20) and was presented as mean ± standard error of the mean (S.E.M.). Variations between means were examined by one-way and repeated measures analysis of variance (ANOVA) followed by Bonferroni post hoc test where necessary. Values of $P \leq 0.05$ were considered statistically significant. The results were presented in charts and table where applicable.

3. Results and discussion

Oral administration of HECA neither elicited any clear symptom of toxicity nor death in rats over a period of 14 days, hence the LD_{50} was estimated to be greater 5000 mg/kg. Evaluation of the acute toxicological propensity of compounds is needed to establish unwanted adverse effects attributed single or brief deliberate or accidental contact (Colerangle, 2017). The LD_{50} of HECA was greater than 5000 mg/kg which indicates that it is practically non-toxic following brief oral administration (Erhirhie *et al.*, 2018).

In the antihyperglycaemic study, there was a substantial ($P<0.001$) elevation in blood glucose level in the hyperglycaemic group when compared to the

normal control. Administration of HECA at 150 mg/kg evidently (P<0.05) lowered the fasting blood glucose level on day 7 in comparison to the hyperglycaemic group. HECA at 150 and 300 mg/kg extensively (P<0.05) reduced the fasting blood glucose level on day 14 relative to the hyperglycaemic group. Equally, on days 21 and 28, there was a remarkable (P<0.05) reduction in blood glucose level at 150 and 600 mg/kg in comparison to the hyperglycaemic group. On comparison over time, HECA at 150 mg/kg substantially (P<0.05) reduced the fasting blood glucose level on day 7 in comparison with day 0. The extract also lowered blood glucose level at 150 and 300 mg/kg (P<0.001, P<0.01) respectively on day 14 in comparison with day 0. On days 21 and 28, there was an evident (P<0.001) reduction in the blood glucose level at 150 and 600 mg/kg when compared to day 0 (Figure 1). STZ is a diabetogenic agent which can destroy pancreatic β-cells and cause hyperglycaemia (Szkudelski, 2001). Feeding rats with HFD and induction of hyperglycaemia with STZ is a classical model simulating type 2 DM and used to evaluate complications (Ma *et al.,* 2020). In this study, administration of STZ and HFD caused hyperglycaemia in the rats which was significantly decrease by the administration of HECA relative to the hyperglycaemic control and over time. The chemical profiling of HECA through GC-MS showed the presence of some compounds with established antihyperglycaemic activity (Abubakar *et al.,* 2019b). Hyperglycaemia especially in type 2 diabetes mellitus is not only caused by compromised insulin emission from the pancreas but also by the diminished insulin sensitivity in the target tissues (Taylor *et al.,* 1994). Hence, an increase in insulin sensitivity is vital for obtaining normoglycaemia. Isoxazolidine, one of the compounds found in HECA elicits its antihyperglycaemic activity by decreasing insulin resistance or improving insulin sensitivity in the target tissues (Shinkai *et al.,* 1998). Isothiazole is also another compound present in HECA which has been reported to act through the selective inhibition of aldose reductase, an enzyme in the polyol pathway which catalyzes the formation of sorbitol and thereby reducing some diabetic complications (Clerici *et al.,* 2008).

The paw pressure test results established the occurrence of peripheral neuropathy with significant (P<0.05) higher paw withdrawal force in the hyperglycaemic group when compared to the normal control. Treatment with HECA at 150 and 600 mg/kg substantially (P<0.05) lowered the withdrawal force compared to the hyperglycaemic control (Figure 2). The tail immersion test results established the occurrence of peripheral neuropathy manifested by a significantly (P<0.05) longer tail flick withdrawal latency for the hyperglycaemic group in comparison to the normal control. However, administration of HECA at 150 and 600 mg/kg evidently (P<0.05) lowered the withdrawal time in comparison to the hyperglycaemic group (Figure 3). Peripheral neuropathy is a serious complication of DM linked with demyelination and deterioration of neuronal fibers, impairment of sensory fibers and altered microvasculature (Kosacka *et al.,* 2012). In the current investigation, HFD-STZ-induced diabetic rats displayed a sustained hypoalgesia with decreased sensitivity to mechanical and thermal stimuli. Treatment with HECA reduced the withdrawal force and time due to increased pain sensitivity and response as a result of improved sensory signaling underlying the impairment of pain feeling linked with diabetic neuropathy (Lennertz *et al.,* 2011). GC-MS analysis of HECA (Abubakar *et al.,* 2019b) revealed several compounds with established efficacy in neuropathic pain. Triazolo-triazine derivatives are agonists of adenosine receptor ligands and applied for the treatment of inflammation, neuropathic and perioperative pains (Müller and Jacobson, 2010). Thiazole derivatives have been reported to be effective in preventing a wide variety of disorders related to diabetes, obesity, neuropathic pain and neurodegenerative disorders (Leoni *et al.,* 2017). Isothiazole and benzothiazole derivatives elicit pain-alleviating effect in peripheral diabetic neuropathy (Hong *et al.,* 2017). 8-Nonynoic acid is an alkaloidal derivative which ameliorates allodynia, a feature of peripheral neuropathy (Ling *et al.,* 2014). On a similar note, propanamide derivatives improve allodynia associated with diabetic neuropathy in murine models (Schmiedebergs, 2017).

Extraction of 1 kg of the powdered plant material gave 24 g of HECA and the percentage yield was

calculated to be 2.4% w/w. Phyto-constituents found in HECA include alkaloids, flavonoids, triterpenes, and steroids (Table 1). Phytochemical screening of HECA revealed the presence of secondary metabolites similar to a previous report (Abubakar *et al.*, 2019b). Flavonoids, alkaloids and triterpenes have been shown to elicit antihyperglycaemic activity (Al-Ishaq *et al.*, 2019; Patil and Khatib, 2020). Flavonoids have also been reported to be effective in neuropathic pain (Basu and Basu, 2020). Alkaloids also possess anti-allodynic effect and ameliorate neuropathic pain (Ling *et al.*, 2014; Forouzanfar and Hosseinzadeh, 2018). On a similar note, steroidal and triterpenoidal compounds elicit a beneficial effect in diabetic neuropathy (Dutra *et al.*, 2012). Enhanced effect of the aforementioned compounds with other phytochemical constituents may be attributed to the observed antihyperglycaemic activity and the amelioration of neuropathic pain in the diabetic rats.

Table 1: Phytochemical constituents of hexane extract of *Chlorophytum alismifolium*

Constituents	Test	Observation	Inference
Glycosides	Keller-Killiani	A Purple ring at the interface	Absent
Saponins	Frothing	Honey comb froth	Absent
Flavonoid	Shinoda	Red coloration of the solution	Present
Alkaloids	Dragendorff	Red to brownish precipitate	Present
Tannins	Goldbeaters skin	The skin turns dark brown	Absent
Steroids	Salkowski	Cherry red coloration at interphase	Present
Triterpenes	Liebermann-Burchard	A reddish brown ring	Present
Anthraquinones	Bontrager	Cherry red/pink coloration	Absent

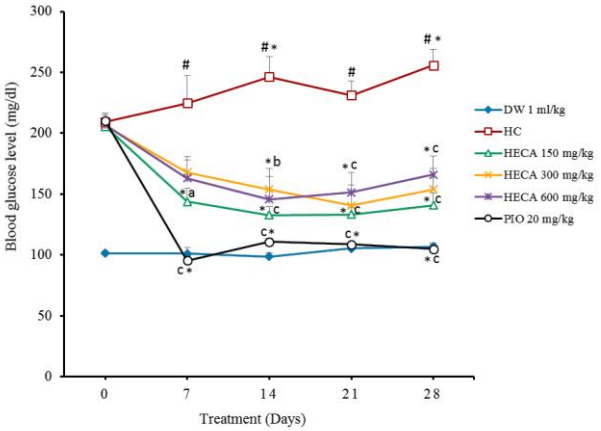

Figure 1: Effect of hexane extract of *Chlorophytum alismifolium* tubers on blood glucose levels of hyperglycaemic rats. Values are mean ± S.E.M., # = P<0.001 compared to DW group, a = P<0.05, b = P<0.01, c=P<0.001 as compared HC group, * = P<0.05 compared to day 0 – Repeated measure ANOVA followed by Bonferroni post hoc test, n = 5, DW = Distilled water, HC = Hyperglycaemic control, HECA = Hexane extract of *Chlorophytum alismifolium*, PIO = Pioglitazone

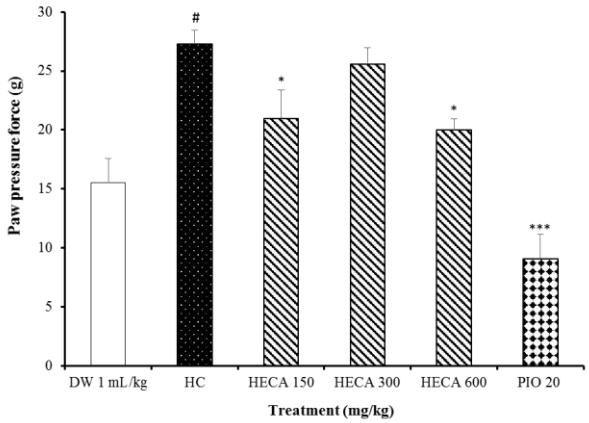

Figure 2: Effect of hexane extract of *Chlorophytum alismifolium* tubers on paw pressure force in rats. Values are mean ± S.E.M., # = P<0.05 compared to DW group, * = P<0.05, ** =P<0.01, ***=P<0.001 as compared HC group – One way ANOVA followed by Bonferroni post hoc test, n = 5, DW = Distilled water, HC = Hyperglycaemic control, HECA = Hexane extract of *Chlorophytum alismifolium*, PIO = Pioglitazone

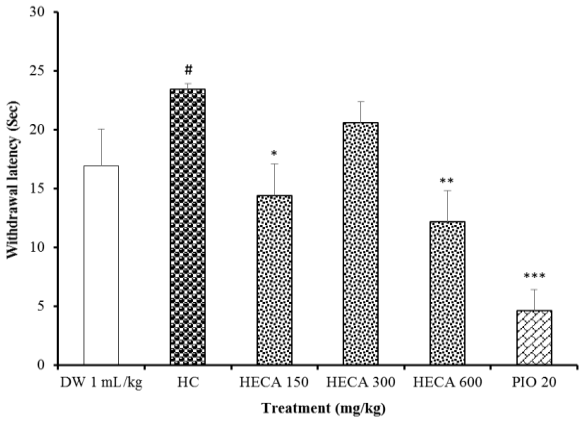

Figure 3: Effect of hexane extract of *Chlorophytum alismifolium* tubers on withdrawal latency in rats. Values are mean ± S.E.M., # =P<0.05 compared to DW group, * = P<0.05, ** = P<0.01, *** = P<0.001 as compared HC group – One way ANOVA followed by Bonferroni post hoc test, n = 5, DW = Distilled water, HC = Hyperglycaemic control, HECA = Hexane extract of *Chlorophytum alismifolium*, PIO = Pioglitazone

4. Conclusion

The hexane extract of *Chlorophytum alismifolium* has beneficial effects in alleviating hyperglycaemia and neuropathic pain in diabetic rats.

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