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Phenolic and Flavonoid contents, DPPH antiradical scavenging and antidiabetic activities of the hydromethanolic extract of Nyambaka propolis

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ABSTRACT

This study proposes an alternative treatment based on Nyambaka propolis which can reduce the oxidative stress and hyperglycaemia caused by streptozotocin. Standard protocols were used to perform phytochemical screening. In vitro experiment was assessed to quantify the total of phenolic and flavonoid contents. The antioxidant activity of the extract was evaluated by the DPPH (2,2–diphenyl-1-picrylhydrazil) antiradical scavenging assay. Diabetes mellitus was induced using single intraperitoneal injection of streptozotocin (100mg/Kg). The phytochemical screening revealed the presence of compound such as: saponins, phenolics, flavonoids, terpenoids and steroids. The total of phenolic and flavonoid contents was respectively (893.1±0.5mg G.A.E/100g of extract) and (37.5±0.2 mg Q.E/100g of extract). The hydromethanolic (95/5) of propolis extract inhibited DPPH free radicals at (78.48±0.06%) with an IC_{50} of (0.096 mg/ml). Concerning antidiabetic activity, the dose of 500mg/Kg of propolis extract had the highest percentage reduction in glucose levels that means (78.13%) compared to the dose of 250 mg/Kg (65%) and the dose of 20mg/Kg of gibenclamid (77.6%). The hydromethanolic extract of Nyambaka propolis can be recommended to reduce the oxidative stress and hyperglycaemia caused by streptozotocin.

Keywords: Diabetes, Antioxidant, Propolis, Streptozotocin, DPPH.

INTRODUCTION

Diabetes is defined as a blood glucose level of more than 1.26 g/L after 8 hours fast, or more than 2g/L regardless of the time of collection and in the presence of clinical symptoms such as: polyuria, polydipsia and weight loss [1]. Type I diabetes, also known as insulino-dependent diabetes is a consequence of chronic hyperglycaemia due to the destruction of islets cells of langerhans [2]. It is a form of diabetes mellitus that appears abruptly in children and adolescents, but can also occur in young adults <40 years and concerns 10% of diabetics [3]. There are factors such as ROS (reactive oxygenated species) which cause metabolic changes inducing the appearance or development of diabetic disorders [2]. The low content of antioxidant enzymes in beta cells makes them sensitive to reactive oxygenated species. In fact, Streptozotocin (STZ) releases in the body toxic agents such: as methyl cations, methyl radicals, reactive oxygenated species (ROS) and nitric oxide (NO) [5]. After taking antidiabetic drugs some patients are victim of allergies, overweight and hypoglycaemia [6]. In order to alleviate these problems, the pharmacopoeia offers an alternative like the use of medicinal plants and propolis which is a natural substance made from a complex mixture of resins, wax, pollen and bees salivary secretions [11]. Previous works on phytochemical compounds of propolis revealed the presence of flavonoids, terpenoids, phenolics, tannins, alkaloids, glycosids, saponins, anthraquinones, fatty acids, coumarins, lignans, and sterols [8]. The chemical composition of propolis has been reported to be responsible of different biological properties such as: anti-ulcer [9], anti-inflammatory [10], antioxidant [11], antimicrobial [12], antinociceptive [17] and diabetes [13]. In this work, the phenolic and flavonoid compound of hydromethanolic extract were quantified using standard methods. The efficacity of the extract was evaluated on oxidative stress and hyperglycaemia caused by streptozotocin.

MATERIAL AND METHODS

Simple collection and extraction

The raw material for extraction was collected in April 2018 at Nyambaka (Adamawa-Cameroon) with the help of a beekeeper. 200g of raw propolis were extracted with the Methanol/H_2O (95:5) at room temperature during (48hx3) then, filtered with Whattman paper and concentrated with the rotavapor to dryness. The extract was recovered in labelled jars and stored for chemical tests.

Phytochemical screening

The phytochemical content of extract was evaluated according to the methods of Talla *et al*. [11] whith few modifications.

Anthocyanins

1ml of extract was prepared at 3mg/ml and added to 2ml of hydrochloric acid and 2ml of ammonia. The appearance of red or purple colour indicated the presence of anthocyanin.

Saponins

1ml of extract was added in distilled water and shaked for 5min. Persistent foam indicated the presence of Saponin.

Phenolic compounds

3 drops of a solution of $FeCl_3$ at 5% were adding to 3ml of dissolved extract, after shaking, the appearance of bluish-black colour indicated the presence of phenolic compounds.

Flavonoids

0.5g of extract was dissolved in 1ml of methanol and 5magnesium shavings and 1ml of concentrated HCl were added. The appearance of a brick red colour indicated the presence of flavonoids.

Terpenoids and steroids

An equal volume of concentrated sulphuric acid and acetic anhydride was added to the extract. 1minute later, the appearance of golden yellow colour indicated the presence of terpenoids and the presence of blue or green colour indicated the presence of steroid compounds.

Determination of total phenolic content

A volume of 100 µL of extract (0.2 mg/mL) was added to 200 µL of Folin-Ciocalteu and 1380 µL of distilled water. Then, 400 µL of Na_2CO_3 (20%) was added to the mixture, incubated at room temperature and protected from light for 1 hour. The spectrophotometer was used to measure the absorbance at 760nm. The calibration of gallic acid (0.2 mg/mL) was carried out by different concentrations (50, 75, 100, 125, 150,175,200 µg/mL) under the same conditions. The results obtained were expressed in grams of gallic acid equivalent per 100g of raw material (g E.A.G/100g RM). The formula (1) allowed to determine the content of phenolic compounds [11].

$$x = \frac{(y-b)V_1}{V_2.a.m} \times 100 \quad (1)$$

x: content of phenolic compounds;
y: calibration curve equation.

Determination of total flavonoid content

In order to quantify total flavonoid content, 400 µL of distilled water and 30 µL of Na_2NO_2 (5%) were added to 100 µL of each extract diluted in MeOH. Five minute later, 20 µL of a solution of $AlCl_3$ (10%) and 200 µL of Na_2CO_3 along with 25 µL of distilled water were also added and allowed to stand for 5 minutes. The mixture obtained was shaked in vortex and 1 mL was taken to read the absorbance [14]. The flavonoid content was calculated by formula (2):

$$F = \frac{0.05 \times A_{ext}}{A_q C_{ext}} \times 100 \quad (2)$$

F: content of total flavonoids; A_{ext}: absorbance of the extract; A_q: absorbance of quercetin; C_{ext}: concentration of propolis extract.

Antiradical activity

The antiradical test was carried out according to the method of Talla *et al*. [11] with slight modifications. The solutions of extract and vitamin C were prepared respectively at the following concentrations: 100, 200, 400, 600, 800, and 1000 µg/mL. Then, 1ml of DPPH was prepared at a concentration of 0.5 mM and added to the previous solution. The mixture was incubated in the dark at the temperature room for 30 minutes. The absorbance of each solution was read at 517 nm. The inhibition percentage (%IP) was calculated according to the formula (3):

$$\%\ IP = \frac{\text{Absorbance control} - \text{Absorbance}_{\text{extract}}}{\text{Absorbance control}} \times 100 \quad (3)$$

IP*:* inhibition percentage:

Antidiabetic activity

Animal preparation

Twenty-five male rats of age of ten weeks 190–205 g was divided into 5 groups. The animals were obtained from the Laboratory of Medicinal plants, Health and Galenic Formulation of the University of Ngaoundere. The rats were placed in transparent polypropylene cages with a cycle of 10/14 h (light/dark). To acclimating rats in the laboratory, they were fed with standard food for one week. Before the experiments, the rats were fasted during twelve hours with free access only to water.

Induction of diabetes

The protocol used for the induction of diabetes was according to those of Ueta *et al*. [15]. To evaluate the antidiabetic activity of the extract, type I diabetes was induced the first day of experiment by intraperitoneal injection of a single dose of streptozotocin (STZ; Sigma-Aldrich, St Louis, USA) at dose of 100 mg/kg body weight to all the rats except those of normal control. STZ was reconstituted just before administration in physiological NaCl. A blood glucose evaluation was performed 72 hours after injection at the main tail vein using a blood glucose meter.

Experimental design

Twenty-five rats were classified into five groups (5animals/group) and subjected to treatments. Group I received 1ml distilled water per 100g per day by oral gavage for three weeks and served as a normal control (N.C). The first day of the experiment, all the rats except those of normal control received a dose of 100mg/Kg of streptozotocin (STZ). Three days after induction, the rats of group II (STZ) received only distilled water during the three weeks and the others rats received the following treatments: group III received the dose of propolis extract at 250mg/Kg and serve as (STZ + D250); group IV received the dose of propolis extract at 500mg/Kg and serve as (STZ+D500) and group V received the dose of gibenclamid at 20mg/kg and serve as Positive Control (P.C).

Parameters monitored in the experiment

Blood glucose

Blood glucose levels were measured every three days using a blood glucose meter and test strips (one touch ultra) for each rat. After fasting for 12 hours, blood was taken by incision in the tail on unanesthetized rats.

Body weight evolution

The body weight was one of the parameters that determine the state of a diabetic due to the very considerable weight loss that accompanies the onset of the disease. The weight of the rats in each batch was measured every day using an electronic scale.

Statistical analysis

The results were expressed as means ± SD (Standard Derivation). Data were analysed using Graph Pad Prism Software, version 5.0. Two way analysis of variance was used for the treatment of the double-variable data. However One-way ANOVA followed by Turkey's post-test was used to analyse the single variable data.

Statistical significance was achieved at: 0.05 >p> 0.01 the difference was significant*, 0.05 > p > 0.001 the difference was very significant** and p < 0.001 the difference was highly significant***.

RESULTS

Phytochemical screening

The phytochemical screening revealed the presence of family compounds such as: saponins, phenolics, flavonoids, triterpens and steroids except anthocyanines.

Total of phenolic contents of the extract

The calibration curve of gallic acid (y = 0.0005 x+0.0345, r=0.981), allowed to determine the total of phenolic compounds it was found to be (893.1±0.1) mg G.A.E/100g of extract.

Total of flavonoid contents of the extract

The calibration curve of Quercetin (y=0.0015+0.0277, r=0.972), allowed to determine the total of flavonoid compound it was (37.5±0.2) mg Q.E/100g of extract.

DPPH antiradical scavenging activity

The DPPH antiradical activity was performed and we found that, the hydromethanolic extract inhibited free radicals with a percentage of (78.48±0.06%) and with an inhibitory concentration 50 (IC_{50}= 0,096) mg/ml. However, the activity of the extract was lower than the vitamin C which served as standard and inhibited free radicals with a high percentage of (94.50±0.30%) and IC_{50} of 0.089 mg/mL.

Antidiabetic activity

Blood glucose

The blood glucose level in rats depending on time was evaluated before induction that was the first day (D_0), after induction, the third day (D_3) and after treatment, the twenty-one days (D_{21}). The effect of propolis extract on blood parameter in STZ-induced diabetic rats day dependant was represented in (Fig. 1). A highly significant increase of glycaemia (P<0.001) on D_3 compare to D_0 was observed for animals which received the dose of streptozotocin (STZ). Then, we noticed, a significant increase of glycaemia day-dependent during all the experiment for the animals of negative control (STZ). From D_6 to D_{21} the blood sugar levels of the animals decreased particularly in positive control group with a percentage of (77.66%) and also for (STZ+D250) and (STZ+D500) groups with a percentage of (65.54 and 78.13 %) respectively. The dose of 500 mg/Kg of hydromethanolic extract of propolis reduced better the blood glucose of diabetic rats compared to the dose of 250 mg/Kg and the gibenclamid (20mg/Kg).

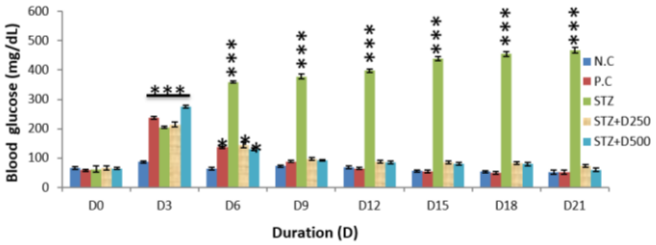

Figure 1: The effect of propolis extract on blood parameter in STZ-induced diabetic rats.

Data are expressed as the means: *p < 0.05, **p < 0.01, ***p < 0.001 versus the first day of experiment D_0.

Body Weight

The effect of propolis extract on body weight parameter in STZ-induced diabetic rats was illustrated in (Fig. 2). At D_3, the weight of animals in all groups except the normal control decreased significantly (P<0.05). The weight of animals of negative control (STZ) decreased by a percentage of (53.72%). However, at the end of treatment, the body mass of the rats of the others groups increased by a percentage of (34.30%), (32.05%) and (32.69%) respectively for positive control (P.C), (STZ+ D250) and (STZ+D500) groups.

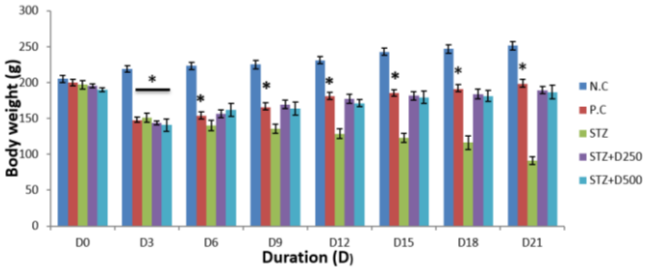

Figure 2: The effect of propolis extract on body weight parameter in STZ-induced diabetic rats. Data are expressed as the means *p < 0.05 versus the first day of experiment D_0.

DISCUSSION

This work was carrying out with the purpose of evaluate the antioxidant and antidiabetic activities of Nyambaka propolis. First of all, we made phytochemical screening of hydromethanolic extract of propolis and we found the presence of compounds such as: saponins, phenolics, flavonoids, triterpens and steroids. After that, we found that the total of phenolic content in the extract was 893.1±0.1 mg G.A.E/100g and the total of flavonoid content was 37.5±0.2 mg.Q.E/100g of extract. These results could be due to the high

polarity of the mixture solvent used to extraction which gave better yield of active compounds.

Subsequently, it was essential to evaluate the antioxidant activity of the extract. The results were expressed by the values of (IC_{50}=0,096 mg/ml) and the percentage of inhibition was (78.48±0.06 %). In fact, the hydroxyl groups of phenolic compounds present in the propolis extract inhibited the action of free radicals. So, the DPPH antiradical activity corroborated with the presence of phenolic compounds in the extract [17].

After evaluating the antioxidant activity of the extract, we determined its effects at doses of 250 and 500 mg/Kg on some parameters: blood glucose and weight. At D_3, the increase in blood glucose levels showed that STZ destroyed beta cells of islets of langerhans and made rats diabetic. The decrease of glycaemia levels from D_6 until D_{21} for animals treated with gibenclamid may be due to the mechanism of action of this drug which allowed the influx of ca2+ ions into beta cells and stimulated the production of insulin [18]. More than, we observed from D_6 to D_{21} a strong decrease in blood glucose levels for rats which received the doses of 250mg/Kg and 500mg/kg of extract. That may be explain by the capacity of propolis to regenerate the formation of beta cells and therefore stimulate insulin production [19]. At D_{21}, the percentage of blood sugar of rats which received extract of propolis at the dose of 500 mg/kg was lower than those which received gibenclamid (20mg/Kg). We could explain it by a synergistic action of the mixture of compounds such as flavonoids and polyphenols present inside the extract [19]. Previous work carried out on LC-MS of hydromethanolic extract of Nyambaka propolis revealed the presence of flavonoids such as: dimethylkuradin, pinobanksin-3-O-propionate and chrysin-5,7-dimethylether [16]. This compound could play a really important role in lowering blood sugar levels.

The decrease in body weight of diabetic rats after induction (D_3) was a consequence of dysfunction of mitochondrial which prevented the muscles from using glucose properly and therefore caused an atrophy [20]. From D_6 to D_{21}, the increase of weight of animals for positive control and (STZ+D.250)mg/Kg and (STZ+D.500) mg/Kg groups was the consequence of the capacity of propolis extract to restore the metabolic balance of the muscle cells and also allowed the regulation of the glucose level properly and decreased the muscle atrophy [19].

CONCLUSION

The hydromethanolic extract of Nyambaka propolis was rich in phenolic and flavonoid contents. These compounds were responsible of antioxidant and antidiabetic activities. The dose of 500mg/kg of propolis extract reduced more type 1 diabetes induced by streptozotocin than dose of 250mg/Kg and giblenclamid. This study showed that the propolis can be used in traditional medecine as a natural substance antioxidant and antidiabetic.

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Author contributions

Cecile Fabiola Balingui: investigation, data curation, writing-original craft. Talla Ernest Rodrigue: investigation, Talla Emmanuel: investigation and validation, Ngassoum Martin Benoit: supervision, Awalou Oumarou: investigation.

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Data Availability

All data obtained in this research are included whithin the manuscript data and the authors will make available any other information.

Declarations

Conflicts of interest statement

The authors declare that they have no conflict of interest.

Ethics Approval: Not applicable.

Consent to Participate: Not applicable.

Consent for Publication: Not applicable.

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