Effect of aqueous extract of *Stachytarpheta indica* leaves on glycaemia of rabbits

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Abstract
Methods: For this study, the aqueous maceration of the leaves was subjected to a tri-phytochemical scanning by the conventional method proposed by the OAU (1985) followed by the evaluation of the antihyperglycemic activity on rabbits, some of which have been rendered hyperglycemic by gavage at a dose of 4 g / l glucose solution. The blood sugar of each rabbit was noted before and after gavage. These rabbits were organized into 8 homogeneous lots of 3, with 3 normoglycemic lots. These latter lots were treated with distilled water and the extract at 0.25 and 40 mg / ml, respectively. The hyperglycemic lots received 40, 10 and 2.5 mg / ml of extracts and 0.25 mg / ml of glibenclamide, respectively. The volume of solution per gavage is 0.6 ml per 20 grams of body weight of rabbit. Blood glucose levels of each rabbit were noted every 30 minutes for 180 minutes.
Results: The results obtained show that the macerated contains total alkaloids, quinonics and saponosides. For blood glucose, the basal mean was 1.02 g / L ± 0.08. This mean, in hyperglycemic rabbits, reached 1.40 g / l ± 0.06. Gavage of glibenclamide lowered hyperglycemia to approximately 0.80 g / L after 180 min. The 40 mg / ml extract reduced hyperglycemia by 40% and that of glibenclamide stabilized at 58%. Glibenclamide 0.25 mg / ml and extract 40 mg / ml had a significant hypoglycaemic effect in rabbits.
Conclusion: Herbal medicine has produced hypoglycemic effect, certainly this activity could be linked to the presence of alkaloids in the extract, constituents which may be active as an antidiabetic.

Keywords: Côte-d'Ivoire, ethnopharmacology, medicinal plants, traditional healers

Introduction
Diabetes, a chronic disease caused by absolute or relative lack of insulin, causes disability and death and appears like a major public health problem (1). This metabolic disorder exists all over the world. The global prevalence of diabetes increases daily and now the situation is very alarming for developing countries [1, 2]. Available statistics has shown that the global estimated prevalence for the disease stands at 285 million adults (6.4%) of the world population in 2010. This figure is projected to rise to 439 million adults (7.7%) by 2030 [3].

Diabetes prevalence is on the rise worldwide due to accumulating risk factors well pronounced in developing countries. An estimated 69% rise is observed for the prevalence of the disease in adults in developing countries versus 20% for adults in developed countries [3-5]

The percentage of deaths attributable to high blood glucose or diabetes that occurs before age 70 is higher in low and middle-income countries than in high-income countries [6]. Most deaths are caused by diabetes complications such as heart disease and stroke, neuropathy, nephropathy, cataracts, micro angiopathy, atherosclerosis, and retinopathy [7].

On the basis of several criteria, 5968 patients were classified as type 1 in 11.8% of cases, type 2 without excess body weight in 48.7% and type 2 with excess body weight in 39.5%. [8]. Among the declared diabetes, around 90% are suffering from type 2 diabetes. The treatment of diabetes is based on parenteral insulin and oral antidiabetic drugs. The most common conventional oral antidiabetic agents such as glibenclamide, metformin chlorpropamide, etc, are widely used for the management of diabetes [9].

Although these hypoglycemic drugs are effective in managing blood sugar level, they are quite expensive and have serious side effects and deleterious contraindications, such as weight gain, hypoglycemia, abdominal cramping, flatulence, diarrhea, hepatotoxicity, hypersensitivity, and skin ulceration [1, 10]

Most common alternative diabetes management strategies are stimulation of insulin production and release, inhibition of dipeptidyl peptidase-4, lens aldose reductase, oxidative stress protection and inhibition of advanced glycation [11].

Existing pharmacotherapy is still far from achieving optimal blood sugar control in such patients, as an effect of dysfunction in insulin secretion, action, or both.

Therefore, several reviews from different countries have highlighted the significance of medicinal plant application to control diabetes [12-14]. In response to this global health challenge, the WHO Expert Committee on diabetes mellitus recommended further evaluation of the folkloric methods of managing the disease because of high mortality and morbidity arising from its attendant complications and drawbacks associated with using conventional antidiabetic drugs [14]. In pursuit of this goal, several medicinal plants are being investigated for their hypoglycemic efficiency.

The present study was undertaken to experimentally study the anti diabetic effects of aqueous extracts of

Stachytarpheta indica leaves on hyperglycaemic rabbits in order to provide scientific justification basis of the traditional use of the plant, as an effective antidiabetic.

Material and Methods
Vegetable material
Stachytarpheta indica (L.) Vahl (Verbenaceae) is an erect Woody shrub with long prominent tip inflorescences. The sessile flowers are arranged terminal spike. The salver-shaped flowers, blue to purple, have a white throat and 5 lobes. Opposite leaves are elliptical to oval with serrated edge.

The Woody steam has beige bark with linear shaped lenticels. Ethnobotanical studies have revealed the uses of *Stachytarpheta indica* in traditional medicine, all plant parts such as the whole plant, leaves, and root are used to cure various human diseases. The leaves are consumed as soup and as a spice. Additionally, the leaves are used to treat diabetes and fever. In West Africa, the juice of the plant is used to treat cataracts and sores on the ears of children. Leaf extracts have been shown to protect liver cells from toxic chemical [15]. The level of protection was comparable to that of standard hepatoprotective drug called Liv-52. (https://www.nparks.gov.sg/florafaunaweb /flora/2/4/2475 consulted on 08/22/2021)

Stachytarpheta indica: Leaves were collected from the forest in Dabou (Southern region of Côte-d'Ivoire) rinsed then dried for 3 weeks under the shade, in a well-ventilated place. The leaves were crushed in an electric grinder (type RETSCH) and powder was obtained. Two hundred (200) g of the dry powder were mixed with 500 ml of distilled water. The mixture was spun on magnetic stirrer for 30 minutes, then poured through a neat square cloth, filtered twice on absorbent cotton and on Whatman 3 mm paper successively. The volume of the filtrate obtained was first concentrated under vacuum type BÜCHI at 60°C and then the different pastes obtained, of dark brown color, were lyophilized. The total aqueous extract, was kept in sterilized glass bowls, hermetically closed, in a fridge and then used for experiments.

Animal material
we used rabbits (*Oryctolagus cuniculus*, Leporidae) brought from Pasteur Institute of Abidjan (Côte-d'Ivoire). They were twenty four [24], males and females. Rabbits age between 6 to 14 weeks old and weighed between 88 and 206 grams. They were placed in ventilated metal cages containing litters of shavings which are regularly renewed. They were acclimatized to the conditions of the animal house, for 3 days before the treatment and fed with the pellets produced by the Ivorian Poultry Food Manufacturing Company (F.A.C.I.). They were given tap water ad libitum. The rabbits were grouped into 8 batches of 3, as follows:

- **Batch 1:** untreated rabbits sample with normal glycaemia
- **Batch 2:** hyperglycaemic rabbits sample treated with glucose (4 g/l)
- **Batch 3:** hyperglycaemic rabbits treated with glibenclamide (0.25 mg/ml)
- **Batch 4:** hyperglycaemic rabbits treated with herbal medicine at 40 mg/ml
- **Batch 5:** hyperglycaemic rabbits treated with herbal medicine at 10 mg/ml

- **Batch 6:** hyperglycaemic rabbits treated with herbal medicine at 2.5 mg/ml
- **Batch 7:** normal glycaemic rabbits treated with glibenclamide (0.25 mg/ml)
- **Batch 8:** normal glycaemic rabbits treated with herbal medicine at 40 mg/ml.

Technical material
Pasteur Pipettes were used to collect blood samples in hemolysis tubes. Electrical scales were used for weighing powders. The glycaemia level measuring device used was a spectrophotometer of KENZA type.

Chemicals
Surgical spirit was necessary to treat injured rats. The saccharose, a glucidic agent, known to cause hyperglycaemia, was used. For the treatment of induced hyperglycaemic rats in the control group, we used glibenclamide (DAONIL tablet, 5mg), the reference product having hypoglycaemic effect. Oxalate of sodium and sodium fluoride was needed in order to stabilize the process of glycolysis in the blood. To carry out the phytochemical screening, we used solvents (ether of oil, methanol and distilled water) and various classic reagents [16]. Classical methods described in the works of [17, 18, 19, 20] were used to characterize the chemical groups.

Induction of hyperglycaemia
We used glucose which hyperglycaemic property is established in rabbits [21, 22]. The glucose administration depends on the body weight of the animal: 0.6 ml of glucose for 20 g weight of rat. Except rabbits of batches 1, 7 and 8, all the animals received glucose overload at time T=0, after basal glycaemia determination. The administration of glucose (4 g/l) was done through oral administration, with an intubation nozzle.

Treatment of hyperglycaemic rabbits with glibenclamide and herbal medicine
The rabbits of batch 3 were treated with glibenclamide (5 mg/20 ml distilled water, that is to say 0.25 mg/ml), one hour, after the cramming by glucose. The rabbits of batches 4 and 5 were treated with the herbal medicine at different doses, one hour, after glucose overload. The hyperglycaemic rabbits received, orally, 0.6 ml of glibenclamide and herbal medicine of 20 g of body weight.
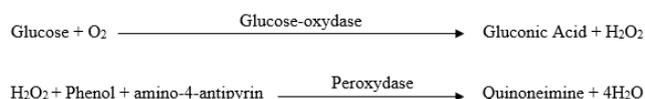
Rabbits' blood sampling
Pasteur pipettes were used to collect blood samples obtained by retro-orbital puncture in the eye of the animal where blood goes up by capillarity inside the pipette. The blood was collected in hemolysis tubes containing an anticoagulant (oxalate of sodium and sodium fluoride) in order to stabilize the process of glycolysis in blood. One hour before treatment, blood of all the animals were taken, for determination of basal glycaemia. Thereafter, blood samplings were made, every 1 hour after the treatment, according to the constituted batches. On the whole, 7 blood tests were carried out on each animal, for 6 hours.

Rats' glycaemia determination
Principle of determination
The enzyme related method was used [23]. It consists in oxidizing glucose by the glucose oxidase, enzyme with

production of gluconic acid and hydrogen pyroxyde (H_2O_2). The hydrogen pyroxyde reacts with phenol and 4-amino-antipyrine in the presence of peroxydase to form a compound of red brick, quinoneimine and water. The optical density of quinoneimine to 500 nm is proportional to the concentration of glucose in the sample. Below we present the following reaction of blood sugar level determination.

$$\text{Glucose} + O_2 \xrightarrow{\text{Glucose-oxydase}} \text{Gluconic Acid} + H_2O_2$$

$$H_2O_2 + \text{Phenol} + \text{amino-4-antipyrin} \xrightarrow{\text{Peroxydase}} \text{Quinoneimine} + 4H_2O$$

Method

A milliliter of enzymatic solution works in 10 microliters of serum. The blood was centrifuged at 3500 rpm for 10 min and then the serum was collected. After a water bath at 37 ° C, the serum was immediately analyzed with a spectrophotometer the KENZA type. A reading was made with a spectrophotometer at 500 nm against the white background of enzymatic solution. The glycaemia was then determined at every 1 hour during experiment. Below, we present the formula used to calculate the glucose level:

$$\text{Glucose level (g/l)} = \frac{D_0 \, \text{sample}}{D_0 \, \text{standard}} \times n, \text{ with } n = \text{standard value.}$$

The glycaemia level was measured in a Medical Analysis Laboratory located (L.A.M.C.A) in Cocody (Abidjan, Côte-d'Ivoire).

Statistical analysis

Data on the variations of glycaemia were expressed in the form Mean ± SEM of 3 observations; the curves were drawn using the STATISTICA software. Data were analysed statistically by one-way analysis of variance ANOVA statistical test using STATISTICA version 6.05 (Windows XP) to test for significance. $P < 0.05$ was considered significant. We used Mauchley test to verify the condition of sphericity and Newman Keuls test for the comparison of the means ($\alpha = 5\%$).

Results and Discussion

Effects of glucose overload on rabbits with normal glycaemia level

The level of blood glucose in normal, hyperglycaemic control and experimental groups of rabbits are reported in figures 1-6. Before treatment, all the animals had a basal glycaemia of about 1.10 g ± 0.08. The basal glycaemia recorded in rabbits deprived of food overnight confirms work of [22, 24]. After administration of glucose to all the animals, the glycaemia rise gradually to reach 1.40 g/l ± 0.10. The rabbits showed a significant increase in level of blood glucose. The glucose overload induces in rabbits a hyperglycaemia, after its administration. That confirms the property of glucose as a product able to create hyperglycemia. This result confirms that of [22, 24] on rabbits' glycaemia.

Evolution of glycaemia after glucose overload

Figure 1 shows result on rabbits of batches 1 and 2. The rabbits of batch 1 are the rabbits sample not treated with glucose (4 g/l). Their glycaemia fluctuates between 1.10 and 1.18 g/l: glycaemia remains basically stable, during the

experiment. We noticed two stages in the evolution of glycaemia in the rabbits of batch 2 (rabbits sample induced with glucose overload but not treated): an increase phase from basal glycaemia (1.15 g/l) to the peak (1.40 g/l) during which the rabbits showed a significant level of blood glucose and a decreasing phase during which glycaemia goes down from 1.40 to 1.12 g/l. A drop of 28 % was noted, after the peak glycaemic level. At the end of the experiment, the blood glucose value (1.12 g/l) was close to the normal level. The induced hyperglycaemia is transitory: the organism is able to come back to normal glycaemia, after a glycaemic overload.
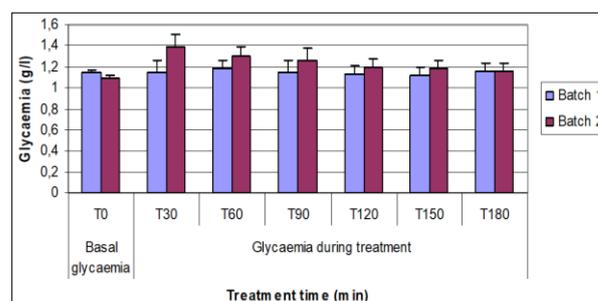

Fig 1: Glycaemia variation histogram for untreated rabbits sample with normal glycaemia and hyperglycaemic rabbits sample treated with glucose (4 g/l) Batch 1: untreated rats sample with normal glycaemia Batch 2: hyperglycaemic rats sample treated with glucose (4 g/l)

Effects of glibenclamide on hyperglycaemic rabbits

The administration of glibenclamide to hyperglycaemic rabbits of batch 3 shows a significant decrease in the level of blood glucose that was reduced from the peak 1.50 g/l to 0.85 g/l. A drop of 58 % was observed, 3 hours later (fig. 2): glibenclamide exerts a hypoglycemic effect, in accordance with the results of [22]. The administration of glibenclamide therefore treated the hyperglycaemia created by glucose overload. The fixing of glibenclamide to its receptor allows the entry of glucose into the cell, preventing the accumulation of glucose in the blood, which explains the hyperglycaemia reduction. The glibenclamide induces a significant hypoglycaemic effect, two hours, after glucose overload. This result is in line with that of [25] but not in line with that of [22] on Wistar rats; in their study, glibenclamide exerts significant hypoglycaemic effect, in 30 minutes after administration. The glycaemia state of rabbits treated with the reference product decreases significantly to its normal glucose value, three [3] hours after administration.
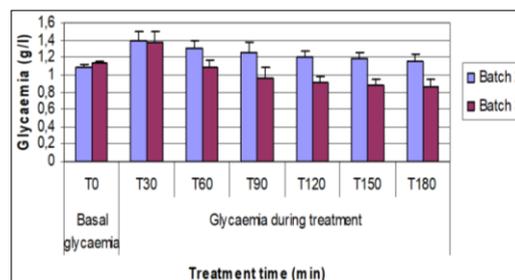

Fig 2: Glycaemia variation histogram for hyperglycaemic rabbits sample treated with glucose and hyperglycaemic rabbits sample treated with glibenclamide; Mean ± SEM, n =3, P < 0.05. Batch 2: hyperglycaemic rabbits sample treated with glucose (4 g/l) Batch 3: hyperglycaemic rabbits sample treated with glibenclamide (0.25 mg/ml)

Effects of herbal medicine on the glycaemia of hyperglycaemic rats

Figure 3 shows the level of blood glucose in hyperglycaemic control and experimental groups. We noticed that the herbal medicine has a significant hypoglycaemic effect. The glycaemia of the rabbits of batch 4, treated with the herbal medicine at 40 mg/ml, decreases gradually to normal glucose level. The drop (40%) is comparable to that of glibenclamide (58%); thus at a dose of 40 mg/ml, the herbal medicine has a glucose-lowering effect. Herbal medicine and glibenclamide treatment significantly reversed the level of blood glucose in hyperglycaemic rabbits. However, the effect of herbal medicine is not more prominent when compared to glibenclamide (fig. 4). The use of the aqueous extract of leaves of *Stachytarpheta indica* revealed that it has glycaemic properties, which vary from one dose to another (Figure 5). The glycaemia of rabbits in batch 5, treated with the herbal medicine at 10 mg/ml, decreases from the peak of 1.50 g/l to 1.20 g/l, three hours after treatment. From this value, the glycaemia increases gradually and at the end of the experiment, its normal value of approximately 1.10 g/l was not restored. There was a light stabilization of glycaemia to its normal value at the end of experiment. The glycaemia of rabbits in batch 5 experienced an evolution which is not similar to that of the other hyperglycaemic treated rabbits. Herbal medicine exerts a significant hypoglycaemic effect three hours after administration. The administration of the herbal medicine at lower doses (10 mg/ml) induces a light hypoglycemic effect; at lower doses (subliminal doses < 10 mg/ml), the herbal medicine would have normoglycemic activity. The administration of the herbal medicine at 40 mg/ml highlights the hypoglycaemic effect and restoration of normal glycaemia, after 3 hours of treatment. The effect of the herbal medicine at 40 mg/ml is similar to that obtained with glibenclamide at 0.25 mg/ml.

This experiment was conducted in order to study the antidiabetic activity of *Stachtarpheta indica* leaves in rabbits as well as to provide an introductory approach for the evaluation of its therapeutic activity in order to scientifically validate the traditional uses of the plant in the treatment of diabetes. The results showed that the effect of the herbal medicine at 40 mg/ml can be compared to glibenclamide (0.25 mg/ml). The effect of herbal medicine was more pronounced when the dose used is higher: the herbal medicine exerts dose-dependent hypoglycemic effect and acts like an antidiabetic.
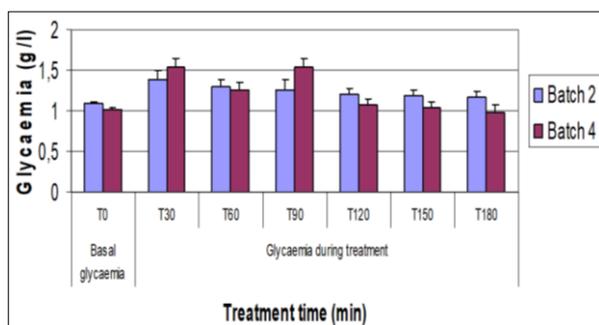

Fig 3: Glycaemia variation histogram for hyperglycaemic rabbits sample treated with glucose and hyperglycaemic rabbits treated with herbal plant at 40 mg/ml; Mean ± SEM, n =3, P < 0.05. Batch 2: hyperglycaemic rabbits sample treated with glucose (4 g/l) Batch 4: hyperglycaemic rabbits sample treated with herbal plant at 40 mg/ml
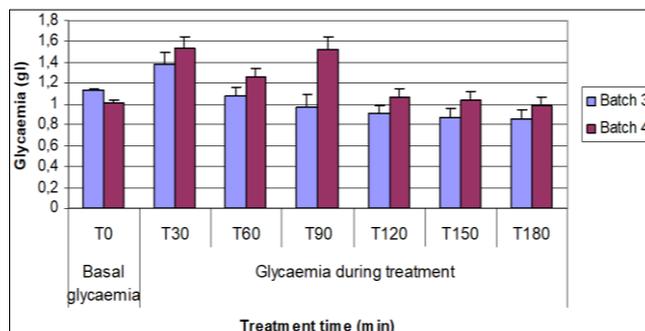

Fig 4: Glycaemia variation histogram for hyperglycaemic rabbits treated with glibenclamide and hyperglycaemic rabbits treated with herbal plant ; Mean ± SEM, n =3, P < 0.05. Batch 3: Hyperglycaemic rabbits treated with glibenclamide (0.25 mg/ml) Batch 4: Hyperglycaemic rabbits treated with herbal plant at 40 mg/ml
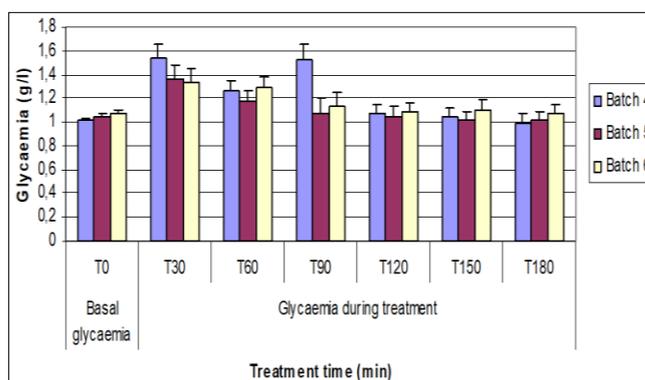

Fig 5: Glycaemia variation histogram for hyperglycaemic rabbits treated with herbal plant at 40 mg/ml, 10 mg/ml and 2.5 mg/ml; Mean ± SEM, n =3, P < 0.05. Batch 4: Hyperglycaemic rabbits treated with herbal plant at 40 mg/ml Batch 5: Hyperglycaemic rabbits treated with herbal plant at 10 mg/ml Batch 6: Hyperglycaemic rabbits treated with herbal plant at 2.5 mg/ml

Effect of glibenclamide and herbal medicine on basal glycaemia of rabbits

Figure 6 shows the level of basal blood glucose in normo-glycaemic experimental groups treated with the glibenclamide 0,25 mg/ml (rabbits in batch 7) and with the herbal medicine 40 mg/ml (rabbits in batch 8), at 40 mg/ml. The reference product exerts a significant glucose-lowering effect unlike herbal medicine. The two substances would not exert the same effect on the insulin secretion. The noninsulin treatment of diabetes utilizes oral hypoglycaemias' type of sulphamides and biguanides. Sulphamides to which belonged glibenclamide act by stimulating the secretion of insulin [26]. The biguanides reinforce the peripheral use of glucose and appear to inhibit the gluconeogenesis.

The herbal medicine would have an extra-pancreatic action by stimulating peripheral use of glucose, similar to that observed with the biguanides. The antidiabetic effect of herbal medicine would be due to an increase in the membrane permeability to blood glucose similar to that observed after biguanides administration. Administration of herbal medicine significantly increased the activities of membrane enzymes for glucose utilization in hyperglycaemic rabbits.
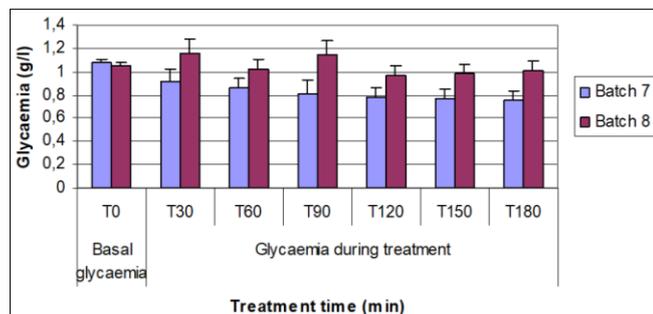
Fig 6: Glycaemia variation histogram for normal glycaemic rabbits treated with glibenclamide and herbal medicine; Mean ± SEM, n =3, P < 0.05. Batch 7: normal glycaemic rabbits treated with glibenclamide (0.25 mg/ml) Batch 8: normal glycaemic rabbits treated with herbal plant at 40 mg/ml.

Experimental evaluation of the medicinal activity of the plant using phytochemistry

We performed a primary evaluation of the traditional medical practices, by looking for the chemical constituents that could explain the antidiabetic effect of the herbal plant. Thus, *Stachytarpheta indica* leaves are chemically screened and alkaloids, quinone substances, saponosides, sterols and triterpenes were identified. Among these compounds, sterols and triterpenes can be linked to the antidiabetic activity of the plant. Sterols and triterpenes are known for their blood glucose reducing properties [27, 28]. Therefore, Sterols or triterpenes present in the leaves of the plant would be responsible for the observed antidiabetic effect.

Conclusion and Prospect

The aqueous decoction of *Stachytarpheta indica* leaves exerts a dose-dependent hypoglycemic effect. It has a normoglycaemic activity at lower doses (< 12 mg/ml) and a hypoglycaemic activity, at higher doses (≥ 12 mg/ml). The dose of 66 mg/ml is the most effective among the administered doses. Indeed, at 66 mg/ml, the herbal medicine reduces hyperglycaemia but does not bring back glycaemia to its normal value, after 6 hours of experiment. The plant proves to be an antidiabetic. Further studies are needed to identify the mechanism of action, specify the potential toxicity and identify the active compound of *Stachytarpheta indica* leaves which has a significant hypoglycaemic effect and acts like an antidiabetic due to chemical components like sterols and triterpens.

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