Journal Homepage: - www.journalijar.com
INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)
Article DOI: 10.21474/IJAR01/8151
DOI URL: http://dx.doi.org/10.21474/IJAR01/8151
ISSN NO. 2320-5407

RESEARCH ARTICLE

ANTIDIABETIC AND WOUNDS HEALING ACTIVITIES OF *ECLIPTA PROSTRATA* (ASTERACEAE) LEAVES.

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Manuscript Info

Manuscript History
Received: 04 October 2018
Final Accepted: 06 November 2018
Published: December 2018

Keywords:
Eclipta prostrata, antidiabetic, wound, streptozotocin, rat.

Abstract

This work aims to evaluate the antidiabetic and wounds healing activities of the leaves extract of *Eclipta prostrata*. Antidiabetic activity was evaluated respectively in normal and hyperglycemic rats by overdose of glucose 10% at 3g/kg orally and, in the rat made diabetic of type II by intravenous injection of the streptozotocine 45mg/kg. The wound healing activity was evaluated by incision of approximately 1cm^2 on the beforehand shaven back of rat. The obtained results revealed that the aqueous extract (200, 400 and 800 mg/kg) descrease significantly the peak of glycemia compared to the obtained with distilled water in the rats, 30 min after the overdose of glucose. This decrease continues until the 5th hour and, the extract effect occurs at different hours according to the doses. In the same way, the ointment A and B, respectively with aqueous and hydro-ethanolic extract added with vaselin, significantly reduce the wounds diameters. The wounds were completely healed after 14 day by the ointment against 18 day for vaselin and cicatryl. These results justify the use of *E. prostrata* in traditional medicine against diabetes and wounds.

Introduction:-

Diabetes is a metabolic condition characterized by permanent hyperglycemia resulting from either lack of insulin secretion by endocrine pancreatic β cells, or insulin action or both (Hajzadeh et al., 2011). In Africa, the epidemiologic data of the diabetes are estimated at 14 million in 2011 and in 2030 this number will reach 28 million diabetic (Sambo, 2011). In the Republic of Congo, according to Diabaction Association of Congo, the prevalence is estimated at 7% (ADC, 2015). Unsupported diabetes leads to several complications (neurological, nephrological, metabolic, cardiovascular...), including various amputations due to difficulties in healing wounds, sometimes very negligible at first. These complications affect the patient life or lead to death if treatment does not occur in time. In developing countries, the prevalence is around 20 to 30% (Grimaldi, 1997), with the outlook for 2025 moving towards a global prevalence of 300 million adults with diabetes. The modern drug exists, but no treatment regimen is perfect, because in the long term, there are several side effects and a gradual decrease in the effectiveness of treatment (Nissen et al., 2007; Nissen et al., 2010). In response, WHO, in resolution AFR / RC50 / R3 of 31 August

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2000, encouraged African countries to develop regional strategies on traditional medicine to undertake research on medicinal plants in animal health delivery systems. Health care. In 2008, diabetologists came to the conclusion that a therapeutic supplement consisting of plant extracts is necessary to optimize the treatment of diabetes (WHO, 2008). Thus, *Eclipta prostrata*, a plant widely used in traditional African medicine has been studied. Several healing virtues have been attributed to it and some have been scientifically proven, including: analgesic activity (Dithala et al., 2012), the antioxidant effect (Mohamed and Nalini Devi, 2015), anti-inflammatory activity (Tewtrakul et al., 2011). In Congo, this plant is used for the treatment of diabetes and also very famous for the healing of wounds even the most serious (Ahombo et al., 2012). In addition, no scientific study has so far confirmed these effects. Therefore, this study was undertaken to justify its use in traditional medicine for the treatment of diabetes and wound healing.

Material and methods:-

Plant material

Fresh leaves of *Eclipta prostrata* were collected at Talangai (Brazzaville) in March 2017. The plant was authenticated by National Research in Exacts and Naturals Sciences Institute (IRSEN) and compare with the sample N^{o} 1556 collected by P. SITA on April 22, 1967. The collected leaves were previously washed and air dried at a temperature of 25° ± 1°C during 14 days in the laboratory of the Faculty of Science and Technology and, grounded into powder thanks to a Wood mortar.

Animal material

Wistar male rats of 3 months old weighing between 200 and 300g were used. They were kept under standard conditions (12 hours of lighting and 12 hours of darkness, water and food were given to them at will).

Preparation of extracts

Two types of extracts were preparing for experimentations. Respectively, 200 g of leaves powder were boiled for 20 minutes in 2000 ml of distilled water and, in 1600 ml of ethanol added to 400 ml of distilled water. After cooling, the mixture was filtered using a hydrophilic cotton and a filter paper. The filtrate was concentrated using a rotavapor and then dried at 40°C until the water was exhausted. 30 g of dry extract were obtained, corresponding to the yield of 15% with aqueous extract and 37.50 g of dry extract as a yield of 18.75% extraction.

Induction of type II diabetes

Type II diabetes was induced by injection of streptozotocin (SIGMA, Chemical-Co, USA), previously dissolved in 0.9% sodium chloride solution. Male rats were injected with streptozotocin through the dorsal vein of the penis after diethyl ether anesthesia at 45 mg/kg.72 hours after rats with moderate hyperglycemia ranging from 1.50 to 2.80 g/l after a 16 hours fasting were selected as type II diabetics (Tedong et al., 2007).

Anti-hyperglycemic effect of the aqueous extract in normal rats.

The method described by Metais et al., (1980) was used. Normal rats divided into five groups of five rats each one received orally respectively, 10 ml/kg of distilled water (negative control); 5 mg/kg of glibenclamide (positive control); 200, 400 and 800 mg/kg of aqueous extract of *E. prostata* 1 hour before the 10% glucose overdose. Glycemic level was taken 1h before glucose overdose and 1/2, 1, 2 and 3h after overdose. However, a level of blood glucose was performed just before the overdose (t = 0 hours).

Antidiabetic effect of the aqueous extract in rats made diabetic by injection of streptozotocin.

Diabetic rats were fasted for 16 hours and then divided into five groups of 5 rats each one and treated orally as follow: group 1 received 10ml/kg of distilled water (negative control); group 2, 5 mg/kg of glibenclamide (positive control); group 3, 4 and 5 respectively 200, 400 and 800 mg/kg of extract. Glucose intake in all rats was made before administration of the different products (t = 0 hours). After the administration of the products, the blood glucose levels were taken 1, 2, 3, 4 and 5 hours after.

Evaluation of the wound healing activity by ointments of aqueous and hydroethanolic extracts.

The incision wounds of around 1cm^2 were made with scissors and cleaned with ethanol at 96° before applying the ointments on the previously shaved back (Klotoe et al., 2014). The table 1 present the ointments composition. Wound dressing was done daily once a day with a precise dose of ointments (around 0.50 g) applied to wounds cleaned with 95° alcohols. The wound measurements were made every two days until complete healing. The percentage of cicatrization (% C) was given according to the following formula:

$$\text{percentage of Healing} = \frac{\text{Healed area}}{\text{Initial area}} \times 100$$

Table 1:-Ointments composition of aqueous and hydroethanolic extract

Product composition	Ointment A (aqueous extract)	Ointment B (hydroethanolic extract)
Number of extracts	5mg	5mg
Distilled water	5ml	5ml
Vaselin	45g	45g
Tween 80	5ml	5ml

Statistical analysis

The results expressed affected on average of the standard mistake are submitted to an analysis of the variance to a factor followed of Student-Newman-Keuls test. The limit of significativity is fixed at $p<0.05$.

Results:-

Evolution of blood glucose levels in normal rats subjected to the hyperglycemia test

The figure 1 present the evolution of glycemia level. The treated rats with the aqueous extract at 200 and 800 mg/kg do not show a reduction of average glycemia one hour afterwards. There are only those which received the glibenclamide and the extract at 400 mg/kg. 30 min after overdose of glucose, the groups of the rats having received the extract at various doses and glibenclamide, show a lower peaks of glycemia than those having received only distilled water.

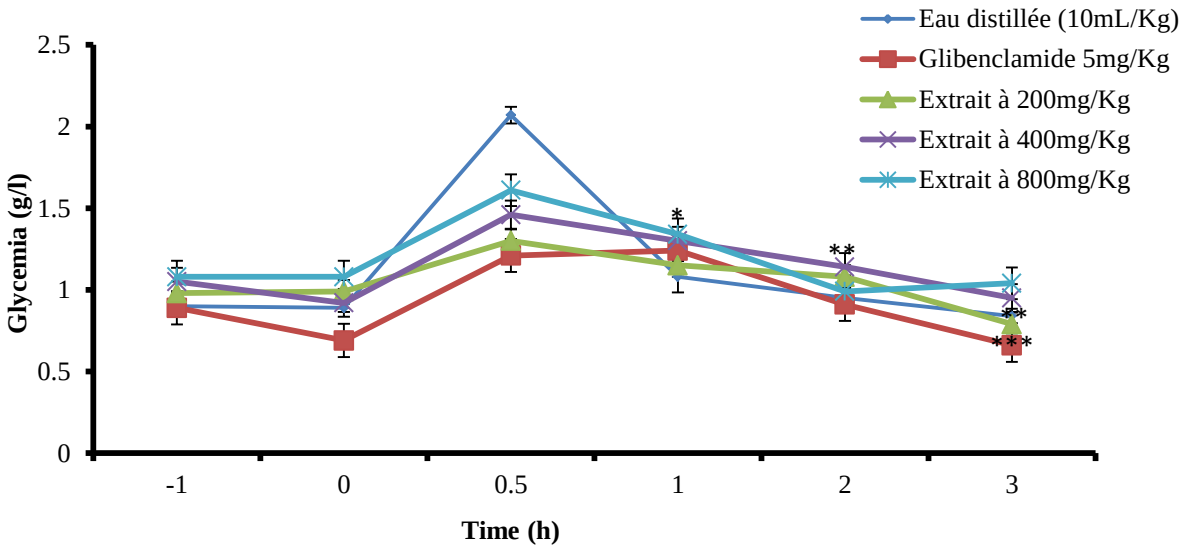

Figure 1: Evolution of glycaemia levels in normal rats tested orally induced hyperglycemia

Evaluation of antidiabetic activity

The table 2 show that the administration of distilled water significantly does not modify the basic glycemia of the rats after 5heures (1.59±0.01 with 0 hour and 1.29±0.05 after 5 hours, corresponding to the PRG of 18.86%). The glibenclamid provoke a significant reduction of glycemia in the diabetic rats at the 1th until the 5th hour (1.77±0.40 with 0.86±0.16, corresponding to a percentage of 51.41%). At 200mg/kg, the extract significantly reduces the average glycemia only from the 4th hour ($p<0.05$). At the 5th hour the reduction becomes very significant ($p<0.001$). At 800 mg/kg, the aqueous extract significantly reduced the average glycemia of the rats from the 2nd hour, but his activity remains weak ($p<0.01$) until the 5th hour, compared to the doses of 200 and 400mg/kg. The dose of 400 mg/kg appeared more effective from the 2nd until 5th hour.

Table 2:-effect aqueous extract of *Eclipta prostrata* on diabetic rats

	Average glucose blood values (in g/l) and percentage reduction in blood glucose					
Types of extracts	0 hour	1hour	2 hours	3 hours	4 hours	5 hours
Distilled water (10ml/kg)	1.59 ± 0.01	1.59 ± 0.06 **(00.00%)**	1.42 ± 0.21 **(10.69%)**	1.36 ± 0.10 **(14.46%)**	1.30 ± 0.06 **(17.61%)**	1.29 ± 0.05 **(18.86%)**
Glibenclamide (5mg/kg)	1.77 ± 0.40	1.01 ± 0.22 **(42.93%)***	0.95 ± 0.22 **(45.76%)****	0.76 ± 0.17 **(57.06%)****	**0.78 ± 0.10** **(55.93%)****	0.86 ± 0.16 **(51.41%)****
Aqueous extract (200mg/kg)	1.60 ± 0.10	1.50 ± 0.07 **(6.25%) ns**	1.51 ± 0.05 **(5.62%)ns**	1.44 ± 0.10 **(10%)ns**	1.37 ± 0.10 **(14.37%)***	1.21 ± 0.04 **(24.37%)*****
Aqueous extract (400mg/kg)	1.63 ± 0.12	1.51 ± 0.08 **(7.97%)ns**	1.44 ± 0.06 **(11.65%)***	1.38 ± 0.02 **(14.72%)****	1.28 ± 0.06 **(21.47%)*****	1.10 ± 0.02 **(32.51%)*****
Aqueous extract (800mg/kg)	2.77 ± 0.58	2.17 ± 0.34 **(21.66%)ns**	1.85 ± 0.15 **(33.21%)***	1.70 ± 0.15 **(38.62%)****	1.45 ± 0.11 **(47.65%)****	1.37 ± 0.10 **(50.54%)****

The results are expressed as mean ± standard deviation with n = 4; ns = not significant

* p <0.05; ** p <0.01; *** p <0.001 significant difference compared to controls.(...): percentage reduction in blood glucose

Evaluation of wood healing activity of aqueous and hydroethanolic extracts

The effect of the ointment of the aqueous (A) and hydroethanolic (B) extracts are presented by figure 4. One observe that the treatment with Cicatryl and the ointment (A and B) decreases the the wounds diameters of the animals compared to the wounds of the treated animals with vaselin. However, at the 8th day (J8), one notes a weak reduction in the treated animals with cicatryl that those treated with the ointment (A and B) compared to vaselin. At J8, these diameters are 100 % for vaselin, 100,5 % for Cicatryl, 73,75% for ointment A and 90,25 % for the ointment B. These results show also that the treated animals with ointment A heal better the wounds of incision that those treated with the pomade B, Cicatryl and vaselin. In addition, the wounds of the animals treated with the ointment A and B completely healed in J14 compared to those treated with cicatryl (J16) and vaselin (J18).

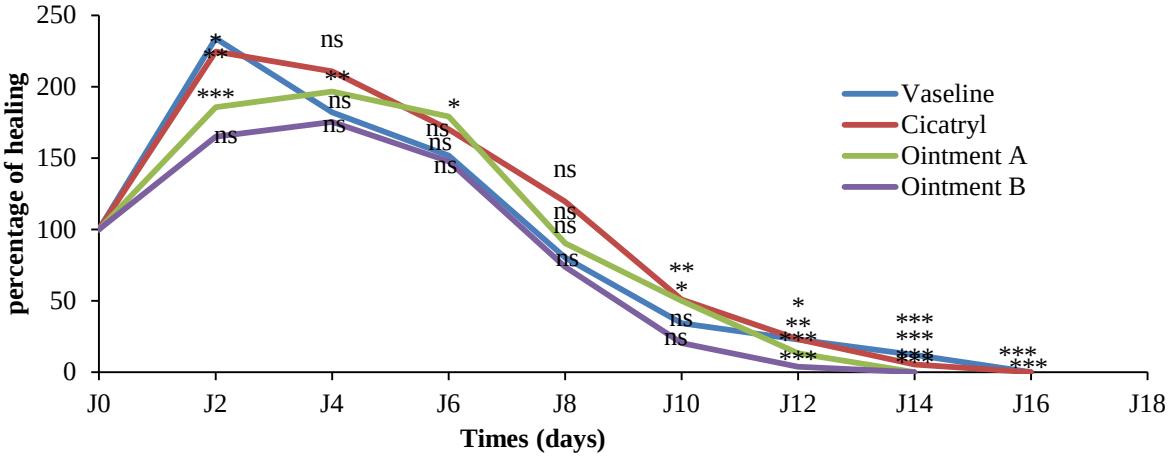

Figure 4:-Effect of the two ointments (A and B) on the diameter of the incision wounds in the rat. Each value represents the average plus or minus standard error of the percentage wounds healing (P).

Discussion:-

E. prostrata is a plant widely used in the treatment of several affections which the diabetes, wounds, arterial hypertension, diarrhoea and abdominal pains (Ahombo and al., 2012). At the date, no scientific data exists to support the antidiabetic and wound healing activities as claimed by rural populations; thus we proposed to evaluate the antidiabetic and wound healing properties extracts of the leaves in the rat.

The experimentation reveal that, the aqueous extract significantly reduced the glycemia as well in the rats hyperglycemic as in the rats made diabetic by injection of the streptozotocin. The glucose tolerance test by oral way, shows that the extract has a protective effect against occurred of the hyperglycemia due to the overdose of glucose. The figure 3 shows that the administration of the aqueous extract (200, 400 and 800mg/kg), one hour before the

overdose of glucose, prevents the increases of glycemia to the breaking values, compared to the animals of control group (distilled water) which make high hyperglycemia. Indeed, the animals having received the aqueous extract at various doses present 30 min after the overdose of glucose, the lower peaks of glycemia than those of the rats having received only distilled water. These results are similar to those obtained with other plants (Ampa et al., 2013 ; Ndomou et al., 2014 ; Ampa et al., 2018).

Table 2 shows that the aqueous extract (400 and 800 mg/kg) reduce significantly ($p<0.05$) the average of glycemia in the diabetic rats from the 2nd until the 5th hour where the effectiveness remains and becomes more significant ($p<0.01$ at 800mg/kg and $p<0.001$ at 400mg/kg). The dose of 200 mg/kg becomes significantly effective only from the 4th hour. These results suggest that the antidiabetic activity of the aqueous extract of *E. prostrata* is dose-dependent and the dose of 400mg/kg seems to be the better. These results let think that aqueous extract of *E. prostata* contain bioactives substances which would be responsible of this activity. Indeed, a former phytochimical study had already revealed the presence of polypeptids, sterols, triterpens, tanins, carotenoids, flavonoids, polypeptids, coumarins, polyuronids, mucilage and heterosids (Sissoko, 2012).Thus this antidiabetic activity of *E. prostrata* can probably be allotted to the flavonoids. This compound are known as a regulator of glycemia in rats (Ampa et al., 2017).

The second goal of this study was to evaluate the wound healing capacity. In oder to confirm the results, we use the ointments of aqueous and hydroethanolic extract ; and vaselin as excipient in the formulation for his skin lenitive property. The macroscopic observation of the wounds show that the process of cure proceeded in several phases : a phase of progressive disappearance of inflammation (wounds became less red), a phase of contraction (the wounds became hard and covered a little black crusts). The treatment allowed a complete cure of the wounds (figure 4). The significant difference between the treated group and the control group was obtained between J6 and J16 would be related to the healing effect of *E. prostrata.* This period would correspond to the proliferative phase and, epithelialisation of the cicatricial process which is characterized by the granulation and epithelialisation phenomenon (Bensegueni et al., 2007). The healing capacity of *E. prostrata* would be related to the effects of various chemical compounds (Korotimi, 2010 ; Klotoé et al., 2012)

Conclusion:-

The results of this study let conclude that the leaves of *E prostata* have antidiabetic and wound healing properties. These results would justify the use of this specie in the treatment of diabetes and wounds in traditional medicine. In the light of the results obtained, it would be judicious to research toxicity of the extracts.

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