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Gomphrena celosioides (Amaranthaceae) aqueous extract effects on vascular reactivity of rats subjected to myocardial ischemia for 7 and 14 days

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ABSTRACT

In this study, we evaluated *Gomphrena celosioides* aqueous extract effects, a plant used in high blood pressure traditional treatment in human patients, on isolated vascular rings contractility of rat aorta submitted to myocardial ischemia created by coronary ligation for 7 days and 14 days. Vascular reactivity measurement for rings subjected to injections of *Gomphrena celosioides* aqueous extract at increasing doses from 10^{-9} µg/mL to 10^{-2} µg/mL indicated dose-dependent relaxation and dependent endothelium on rat aorta preparations IDM D7 and IDM 14D. Obtained rates are comparable to those of Ach. Indeed, the GC relaxation rates on EP contraction do not give significantly different values compared to Ach which is the reference substance. Relaxation maximum value and Ec50 in IDM 7D are 585 ± 1.10 and $1.89.10^{-9}$ µg/mL versus Ach, 567 ± 6.69 and $1.89.10^{-9}$ µg/mL, respectively. On the other hand the same proportions were noted in rats IDM 14D; GC gives as maximum values: 591.33 ± 10.77 and Ec50 of $2.73.10^{-9}$ and for the Ach one has: 647 ± 9.54 and Ec50 $1.34.10^{-9}$ µg/mL. A significant effect was not also note in SHAM compared to NOP. Experimentation does not result in a change in endothelial function and therefore any change noted would be due to ischemia effects. In conclusion our results are due to the fact that the model ischemia does not cause a significant alteration to modify endothelial function. A long duration of ischemia would be necessary to better mimic pathological situations often observed in patients.

Keywords: *Gomphrena celosioides*, chronic ischemia, vascular reactivity, phytotherapy.

INTRODUCTION

Gomphrena celosioides (GC) is an annual herb of Amaranthaceae family frequently used by traditional healers for hypertension treatment. It is a hairy grass with stems and twigs contracted at nodes. Its leaves are petiolate and very acute. It has small white flowers. It is a widespread species in sub-Sahelian Africa and used in Burkina Faso, for several diseases treatment such as jaundice, asthma, arterial hypertension, body aches, dysmenorrhoea, and certain infectious diseases (Nacoulma / Ouedraogo, 1996). It is also used in Benin and Togo to treat malaria, jaundice and constipation (Sangare et al., 2012). Preliminary work has shown that *Gomphrena celosioides* contains various potentially active compounds, such as

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alkaloids, betalaines (gomphrenin), tripenoids and steroids (Nacoulma / Ouedraogo, 1996). On the other hand, in one of our previous publications, it is confirmed to contain compounds with antihypertensive effects (Sanou et al., 2009). This study purpose was to evaluate GC effects on the susceptibility of cardiac muscle subjected to chronic ischemia for 7 and 14 days. Indeed, it is well known that many African plants contain active compounds in various diseases treatment (Traore et al., 2004 ; Lagnika et al., 2005; (Sawadogo et al., 2017). Some of these herbs are effective in high blood pressure treatment because they contain antihypertensive compounds relaxing vessels via endothelium that releases compounds such as nitric oxide (NO), and in ischemia case, cardiac or arterial muscles may be impaired in endothelial function (Varin et al., 1999); there is sometimes a reduction in vascular tone. Elliot et al.; Rodman et al. (1989). Chronic ischemic hypoxia is sometimes associated with depression of α1 receptor-induced contractile response and that of endothelium-dependent relaxation (Yokoyama et al., 1996; Fields et al., 1998 ; Furchgott et al., 1980). This may be due to vessels structural modification and thus influence their sensitivity to relaxing compounds (Andry et al., 2008). To evaluate GC action on heart failure in rats, a left coronary artery ligation was performed. This inevitably leads to the zone insufficient infusion and will create oxidative stress. Myocardium inadequate perfusion is responsible for endothelial function alteration as a result of changes in the vessels smooth muscle structure. Vasomotricity in these animals will be evaluated and compared to that of control animals. Thus any ischemic endothelial function alteration (Andry et al., 2008 ; Elliot et al., 1989 ; Rodman et al., 1989) could be noted. It is well known that chronic hypoxia induces depression of vascular contractility and its dependent endothelium relaxation by reducing blood vessels elasticity and distensibility during the bloodstream, which results in high blood pressure (Martin, 1995). This study aimed at viewing what kind of change endothelial function has undergone during chronic ischemia and how it behaves, when it is subjected to GC aqueous extracts action.

MATERIALS AND METHODS

Gomphrena celosioides crude aqueous extract preparation

The entire plant is harvested in Ouagadougou city, cleaned and dried in the laboratory of Animal Physiology. It is then grinded with mortar and the sieved powder is brewed for 24 hours at a rate of 40 grams per liter of distilled water. The solution obtained is filtered on whatman paper, frozen and lyophilized. The maroon powder obtained is used as *Gomphrena celosioides* raw extract for our experiments.

Animals

Normal rats

Male Wistar strain rats (Charles River or Harlan) with a body weight between 200 and 300 grams were used for the vascular reactivity experiments. The rats, under constant temperature and humidity conditions, with a standard day-night cycle (12h/12h), had free access to water and food in the Joseph Fourier University laboratory of Grenoble. They had received care in accordance with the European Community directive on laboratory animals use (L358-86/609.EE).

Rats subjected to heart muscle chronic ischemia

Cardiac ischemia is achieved by left coronary artery ligation in anesthetized rat and after a left thoracotomy. Rib cage is opened at the fourth rib and gently pressing on both opening edges we make the heart and its coronary arteries appear. Thanks to fine forceps a wire is passed under coronaries to perform ligature. The heart is returned to ribcage, closed with a suture, and the rats are returned to their cage and monitored for 7 and 14 days. To properly evaluate changes in vasomotility due to ischemia, one rat batch underwent surgery without coronary ligation (SHAM rat). Their vasomotility will be

compared to that of heart failure rats to see simple surgery effect on endothelial function.

Aorta rings contractility registration

Rats are anesthetized by intraperitoneal injection for 1: 1 mixture of ketamine-xylazine (50 mg and 10 mg, respectively), followed by heparin intravenous (saphenous vein) injection (100 IU/100 g body weight). After heart removal, a descending thoracic aorta segment of about 5 cm is rapidly removed and placed in a physiological solution of Krebs-Hepes with the following composition (in mM): NaCl, 133.8 ; KCl, 4.75; $MgSO_4$,7 H_2O, 1.19 ; Hepes, 5 ; D-glucose, 11 ; $CaCl_2$, 2.4 ; $2H_2O$; pH, 7.4. It is rinsed, cleaned of all connective and adipose tissue and cut into transverse segments of about 2 mm thick. The experiments are carried out in parallel in 4 identical perifusion tanks containing 150 mL of Krebs-Hepes thermostated at 37 °C and oxygenated continuously.

The device for measuring contractility consists of two needle points, one of which is fixed, and the other connected to an isometric transducer, itself connected to an amplifier connected to a computer acquisition system. Each ring is placed horizontally on the 2 needle points of the recording device in the perifusion tank.

The ring is stretched a first time at 2 g (2×10^{-2} N), then left to stabilize for one hour before being stretched again to 2 g and stabilized again for 15 minutes. The ring then undergoes an integrity test to evaluate the functional quality in its smooth muscle component and its endothelial component.

Smooth muscle component integrity is reflected in its ability to contract in response to various agonists, notably epinephrine (EP). If smooth muscle component is normal, EP addition, at 10^{-6} M final concentration, should induce a ring contraction of at least 10^{-3} N. After obtaining EP contraction plateau, Endothelium integrity results in at least 50% relaxation by adding 10^{-6} Ach to the infusion tank.

Experimental protocols

Vascular contractility measurement

After mounting the rings in tanks and stabilizing for 45 to 60 minutes, each ring undergoes integrity test. Rings which are intact, will undergo, after a new stabilization, a precontraction with EP 10^{-6} M and then a concentration-dependent relaxation with doses of 10^{-9} µg/mL at GC 10^{-2} µg/mL, obtained from a stock solution of 100 g/L of powder diluted 10^{-3} to 10^{-9} in distilled water. A comparative study with concentrations of 10^{-9} to 10^{-3} M acetylcholine is carried out under the same conditions. The different GC and Ach induced relaxation variations on EP contraction are recorded in unoperated rats (NOP), non-ligated operated rats (SHAM), rats with ischemia for 7 and 14 days (IDM). Ach and GC Vasorelaxations are evaluated as a percentage of EP contraction.

Statistical analysis

Data are expressed as mean ± standard error to mean (EMS) ; n is the number of experiments and average effective concentration and p EC 50 = - log EC50, is expressed in concentration in µg/mL in the solution which enables us to obtain 50% reduction of EP contraction with 95% confidence intervals. Applied statistical analysis is t-student. Difference of $P < 0.05$ is considered significant: (Computer program GRAPH PAD PRISM).

RESULTS

GC effects in NOP and SHAM rats

Mean maximal values of GC relaxation on EP vascular contraction in non-operated rats (NOP) GC give 539 ± 5.50 for n = 4 compared to Ach maximal effect which is 481.02 ± 6.6 with n = 3 and GC has a pEC50 of -8.81 ± 2.82 and Ec50 of $1.54 .10^{-9}$ µg/mL against a pEC 50 of -8.96 ± 2.63 and Ec50 of $1.54 .10^{-9}$ for acetylcholine. Differences in these values were not significant (Figure 1). The same measurements are performed in rats

(SHAM) who underwent thoracic surgery without coronary ligation. This evaluation purpose is to note the thoracic surgery effect on myocardium sensitivity. There is also no significant change in vascular motility. GC has mean maximum values of 700 ± 6.49, PEC50 of -8.81 ± 3.73 and EC50 of 1.54. 10^{-9} against 558 ± 12.6; pE50 of -8.962 ± 2.62, EC50 of 1.54 .10^{-9} (Figure 2).

GC extract effects in 7D IDM and 14D IDM rats

GC inhibitory effects at cumulative doses 10^{-9} to 10^{-3} of M EP contraction at 10^{-6} observed in 7D IDM and 14D IDM rats compared to Ach inhibition effects which is the Reference substance does not affect dose-response curve significantly, although there is a slight increase in GC relaxation in endothelium presence and even more in endothelium absence (Figures 3 and 4). The maximal relaxations obtained in response to 10^{-6} M EP contraction in 7D IDM are as follows: GC, mean value of 585 ± 11.10 ; pEC50 = -8.92 ± 2.04 and EC50 = 1.89. 10^{-9} against Ach which has an average value of 567 ± 6.69; pEC50 -8.92 ± 0.40 and EC50 of 1.189. 10^{-9}. It is noted that GC relaxation has not been significantly modified compared with that of Ach in endothelium presence. It is the same in aorta rings group in endothelium absence, relaxation in this group is greater compared to the first two, mean value is 685 ± 9. This confirms other vessel relaxation pathways existence. The findings on previous curves shape are found in 14D IDM group. Relaxation rates in control batch and GC groups remain very close and do not give a significant difference. 14D IDM submitted to GC have a maximum relaxation rate of 591.33 ± 10.77 versus 647 ± 9.59 for Ach. The pEC 50 is 5.56 ± 0.39 and EC50 is 2.73. 10^{-9} µg/mL against Ach which has a pEC50 of 5.87 ± 0.36 and EC50 of 1.34. 10^{-9} µg/mL. GC relaxation on rings without endothelium also gives values very similar to those of GC with endothelium, but always higher. The maximum average is 631 ± 3.38. pEC50 of -8.85 ± 3.12 and EC50 of 1.22. 10^{-9} µg/mL.

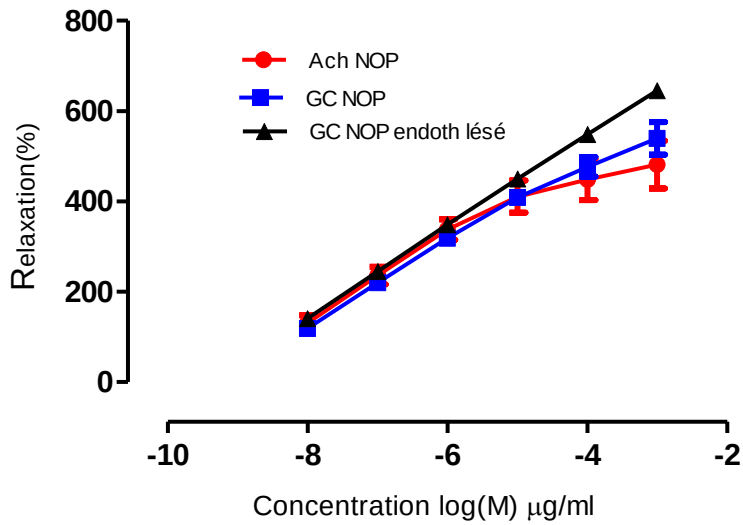

Figure 1: GC and Ach inhibition effects at cumulative doses of 10^{-9} to 10^{-3} µg/mL in endothelium presence and GC in endothelium absence for contraction induced by EP on normal rat aorta rings, not subject to chronic ischemia (NOP).

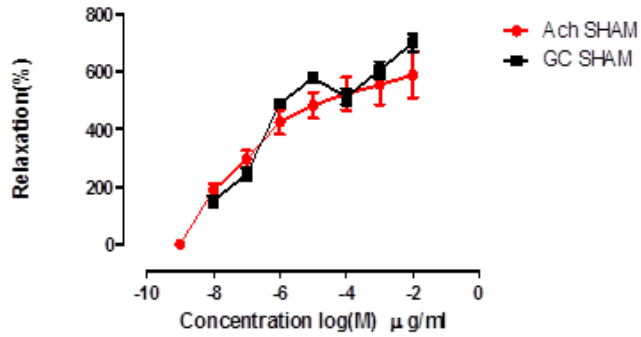

Figure 2: GC and Ach inhibition effects at cumulative doses of 10^{-9} and 10^{-3} µg/mL in endothelium presence for EP-induced contraction on rat aorta rings subjected to chronic thoracic surgery without ligature (SHAM).

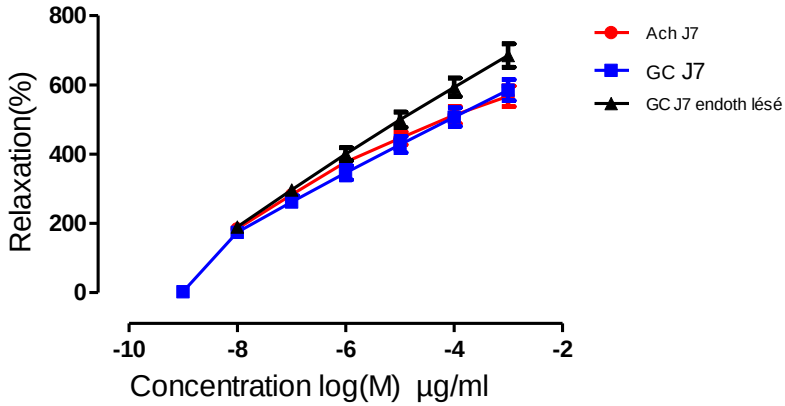

Figure 3: GC and Ach inhibition effects at cumulative doses of 10^{-9} and 10^{-3} µg/mL in endothelium presence and GC in endothelium absence of EP induced contraction on rat aorta rings subjected to 7 days' chronic coronary ischemia (7D IDM).

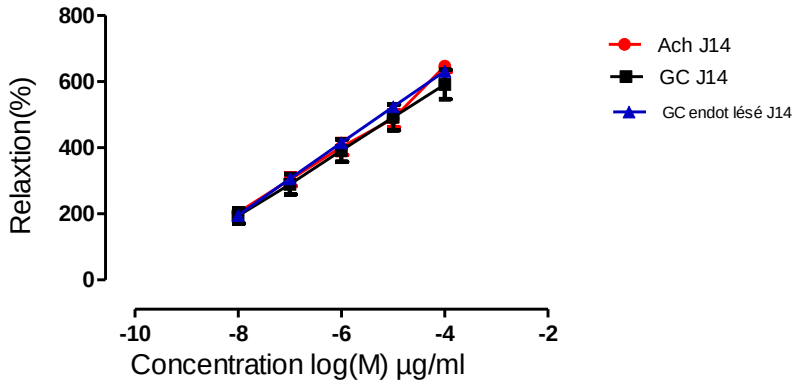

Figure 4: GC and Ach inhibition effects at cumulative doses of 10^{-9} and 10^{-3} µg/mL in endothelium presence and GC in endothelium absence of EP induced contraction on rat aorta rings subjected to 14 days' chronic coronary ischemia (14D IDM).

DISCUSSION

This study is carried out in order to test GC hypotensive effects in heart failure case due to 7 days and 14 days' chronic ischemia by coronary ligation in rat. 7D IDM and 14D IDM groups' aortic rings contracted by EP 10^{-6} M are subjected to GC increasing doses of 10^{-9} to 10^{-3} µg/mL. These relaxation rates values are analyzed in relation to those obtained in normal rats (NOP), rats which underwent surgery without ligature (SHAM) and compared to values obtained with Ach, which is the reference substance. The different values gave concentration-dependent curves similar to that of control, which is the Ach, which confirms that GC contains compounds acting as Ach, results similar to those found by Sanou et al. (2009). Curves in GC NOP in endothelium presence and absence compared to that obtained with increasing concentration of Ach are similar. GC relaxation rates in both presence and absence are slightly elevated with Ach without a significant difference. The highest relaxation rate is noted in rings without endothelium. This shows that the extract contains other compounds whose relaxation pathway is independent from endothelium. The presence of polyphenolic compounds such as flavonoids in *Gomphrena celosioides* extract has been reported by Nacoulma/Ouedraogo (1996) and Adeoti et al. (2016). Polyphenols and flavonoids in particular are involved in the synthesis of NO and EDHF which are implicated in the relaxation of vascular smooth muscle cells (Dal-Ros, 2009; Donzo et al., 2014). Our results could be related to this molecule presence in *Gomphrena celosioides* extract. Relaxation rates measured in NOP and SHAM are similar and do not differ significantly from control levels with Ach. Results obtained in 7D IDM and 14D IDM give concentration-dependent curves that follow the same pattern as curve obtained with the control substance, acetylcholine. Nevertheless, there is a decrease in GC relaxation rate in 14D IDM compared to Ach; which is not the case in NOP, SHAM and 7D IDM. Can these results be interpreted as due to heart failure progressive course that could have greater effects on endothelial function if the development took longer? Indeed, chronic hypertension is a slow and progressive phenomenon that causes a change in cardiovascular system structure over time depending on tissue nature and most often the animal species. To better evaluate vascular structure adaptation to chronic ischemia alterations, a longer heart failure development would be necessary, as it is done in patients.

Conclusion

The 7D IDM and 14D IDM rats developed chronic ischemia, but its development was sufficient to induce structural alterations that could significantly modify endothelial function, the main exploration pathway of our study. An in-depth study after an ischemia which development lasts longer could enable us to better evaluate GC action mechanism in people with heart failure.

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