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Evaluation of the antioxidant activity of the aqueous extract of *Catharanthus roseus* (Apocynaceae) on hypertensive rats induced by 60% fructose

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Abstract

Catharanthus roseus is a shrub from the Apocynaceae family, which is used in the treatment of many diseases including hypertension. The triphytochemistry of the aqueous extract of the leaves of this plant revealed the presence of numerous molecules including alkaloids, flavonoids, tannins, saponins and polyterpenes. The present study showed that the consumption of 60% fructose by rats of the wistar strain during the first ten days of the experiment, very significantly increased the activities of catalase and malondialdehyde in the kidney and heart, then reduced them by highly significant at the end of the experiment. On the other hand, the activities of superoxide dismutase in the kidney and heart, and of nitric oxide in the aorta, increased significantly during the first two weeks before gradually decreasing until the end of the experiment. Treatment of 60% fructose-induced hypertension with aqueous extract of *Catharanthus roseus* leaves increased serum levels of catalase, superoxide dismutase, malondialdehyde, kidney and heart, and oxide nitric in the aorta. This increase was observed at doses ranging from 200 to 1000 mg/kg bw compared to the batch of patients treated. Compared to nifedipine and metformin, the aqueous extract of the leaves of *Catharanthus roseus* appears to have interesting antioxidant activity implicated in the treatment of hypertension induced by fructose.

Keywords: Antioxidant activity, Aqueous extract, *Catharanthus roseus*, Induced hypertension, Fructose

1. Introduction

Plants, because of their many therapeutic virtues, are widely used around the world and particularly in Africa. They represent nearly 80 to 85 % of the health coverage of populations [1, 2]. Also, the secondary metabolites that they contain give them invaluable biological properties and they are also available, cheaper and accessible to everyone [3, 4]. Some of these plants have been shown to be effective in treating many diseases including high blood pressure. In fact, high blood pressure is now the cause of many cases of death around the world (14,000 deaths per day or one death every seven (7) seconds) [5]. One of the causes of this disease is said to be eating precisely foods high in sucrose. Recent studies have shown that a diet high in glucose is the cause of high blood pressure through oxidative stress [6]. Likewise, the consumption of 33% fructose by rats is thought to be the cause of arterial hypertension [7]. Therapeutic interventions via the suppression of free radicals and/or the increase of endogenous antioxidant enzymes would attenuate myocardial dysfunctions [8]. *Catharanthus roseus* is one of the routes indicated in the treatment of hypertension and diabetes thanks to its hypotensive and antidiabetic properties. The many secondary metabolites available to this plant may prove effective in suppressing the generation of free radicals. The objective of this work is to evaluate the antioxidant activity of the aqueous extract of *Catharanthus roseus* leaves in hypertensive rats by induction of 60 % fructose.

2. Material and methods

2.1 Material

2.1.1 Plant material

The leaves of *Catharanthus roseus* were collected in the common of Cocody (Abidjan). These leaves were sorted, washed, dried at room temperature and pulverized using an IKA-MAG type machine. The resulting powder was used for aqueous extraction.

2.1.2 Animals

The animals used for the manipulations are adult rats, of the wistar strain, aged eight (8) to twelve (12) weeks and weighing on average between 160 and 200g. These animals come from the animal facility of the Higher Normal School (ENS) of Abidjan. These animals were previously acclimatized for two weeks. During this period, the animals were fed with food from Ivograin-CI (granules, proteins and fat) and watered with water. They were maintained at a temperature of $22^{\circ} \pm 2^{\circ}\text{C}$ and subjected to a cycle of 12/12h. These animals were classified into groups of six animals for the rest of the experiments.

2.2 Methods

2.2.1 Triphytochemical

The characterization of the different chemical groups present in the aqueous extract of this plant was carried out using the protocols presented by the Pharmacognosy-Botanic Cryptogamy laboratory of the Pharmacy Training and Research Unit of the Felix Houphouët Boigny University of Cocody.

Sterols and polyterpenes have been demonstrated by the Liebermann reaction. Polyphenols have been characterized by reaction with ferric chloride (FeCl_3). For the detection of flavonoids, we used the reaction to Cyanidin. Tannins were characterized by Stiasny reagents and Ferric Chloride. Quinonic substances were searched from the Bornstraëgen reagent. The alkaloids have been demonstrated by the reagents of Burchard and Dragendorff. Saponins were characterized by the foam index. The results obtained have been recorded in a table.

2.2.2 Preparation of the aqueous extract

After drying and pulverizing the leaves of *Catharanthus roseus*, eighty grams (80g) of this powder was dissolved in two liters (2L) of distilled water. The whole thing is brought to the boil for 20 minutes. After cooling the mixture obtained, filtration on cotton wool and then on watman paper was carried out. The filtrate obtained is dried in an oven at a temperature of 50°C . The crude aqueous extract of dark brown color constitutes the aqueous extract [9].

2.2.3 Induction of arterial hypertension by 60 % fructose.

To demonstrate the role of fructose in the induction of arterial hypertension, forty-eight (48) rats (male and female) of the wistar strain, with a body weight of between 160g and 195g and divided into two (2) groups have been used. The control group (T) consisted of six (6) rats and the test group of forty-two (42) rats. The animals had free access to water and food. Animals in control batch received running water while animals in test batch received 60% fructose solution for thirty (30) days. Every five (5) days for the duration of the experiment, the cardiovascular parameters (Systolic Blood Pressure, Diastolic Blood Pressure, and Heart Rate) were determined by the non-invasive method.

2.2.4 Organ sampling and enzymatic assays

At the end of the manipulation, the rats were anesthetized with chloroform (94 %) after 16 hours of fasting and sacrificed by decapitation. The liver, heart, kidneys and aorta were carefully dissected, rinsed with physiological fluid, and then stored in 10 % formalin. These organs were subsequently crushed and centrifuged at 3000 rev/min for 15 min. The homogenate obtained was used for the determination of the

parameters of oxidative stress.

2.2.5 Enzymatic assays

➤ Determination of catalase activity

The method used for the determination of the enzymatic activity of catalase is that of Clairborne [10], whose principle is based on the decrease in absorbance at 240 nm which is due to the decomposition of hydrogen peroxide (H_2O_2) in the presence of catalase.

➤ Determination of superoxide dismutase activity

Heart and kidney superoxide dismutase activity was determined using the Marklund method [11]. The principle is based on the ability of the self-oxidation of pyrogallol to be inhibited by superoxide dismutase.

➤ Indirect determination of nitrites / nitrates

The determination of nitric oxide was carried out by the Griess method which is based on two diazotization reactions. Acidified nitrite produces a nitrosating agent which reacts with sulfanilic acid to produce iondiazonium. The latter is coupled to naphthylethylene diamine to form an azotechromophoric derivative which absorbs at 570 nm [12].

➤ Determination of malondialdehyde

Malondialdehyde levels (heart or kidney) were assessed using the Ohkawa method [13]. The assay is based on the formation in an acidic and hot medium (100°C) between malondialdehyde and thiobarbituric acid of a colored pigment absorbing at 530 nm, extractable by organic solvents such as butanol.

2.2.6 Evaluation of the antioxidant activity of the aqueous extract of the leaves of *Catharanthus roseus* (apocynaceae) in rats made hypertensive by induction of 60 % fructose.

The forty-two (42) male and female rats made hypertensive were divided into 7 groups of 6 rats each. The batches are processed every day for the seven (7) days of the experiment.

→ Batch 1: hypertensive control rats that received no treatment

→ Batch 2: positive control rats (rats made hypertensive and which continued to receive 60 % fructose as a drink from day 34 until day 40). This batch will not be processed.

→ Batch 3: rats made hypertensive and treated by gavage with 200 mg/kg bw of the aqueous extract of *Catharanthus roseus*

→ Batch 4: rats made hypertensive treated by gavage 500 mg/kg bw of the aqueous extract of *Catharanthus roseus*

→ Batch 5: rats made hypertensive treated by gavage 1000 mg/kg bw of the aqueous extract of *Catharanthus roseus*

→ Batch 6: rats made hypertensive and treated with nifedipine (reference product) by gavage at a dose of 10 mg/kg bw

→ Batch 7: rats made hypertensive and treated with nifedipine by gavage at a dose of 20 mg/kg bw.

2.2.7 Statistical analysis

The results obtained are presented in the form of the mean \pm SEM ($n = 6$), the results were processed using the Graphpad Prism software (version 7) by One-way Anova analysis. The comparison of treatments was performed using the Tukey test.

$P > 0.05$: no significant difference between the values

$P < 0.05$: the difference between the values is insignificant

$P < 0.01$: the difference between the values is significant

$P < 0.001$: the difference between the values is very significant

$P < 0.0001$: the difference between the values is highly significant [14]

3 Results and discussion

3.1 Triphytochemical

The phytochemical screening of *Catharanthus roseus* leaves enabled us to demonstrate the presence of molecules whose pharmacological activities are the best known. The results obtained are shown in Table I. These results revealed the presence of polyterpenes, sterols, polyphenols, flavonoids, tannins, alkaloids, and saponosides in the aqueous extract of this plant. No quinone substance has been revealed. Our results are in agreement with the work of Shohel *et al.*, (2015)

[15] which showed the presence of polyterpenes, polyphenols, tannins, saponosides; alkaloids and flavonoids in the aqueous extract of *Catharanthus roseus*. On the other hand, our results do not corroborate those of Mohammed *et al.*, (2011) [16] who showed an absence of flavonoids, tannins and saponins at the level of the same species. The active principles demonstrated by the phytochemical screening in the extracts of the plants analyzed would partly explain the therapeutic indications of traditional preparations based on these plants. This is evidenced by the results of the scientific work carried out by N'Guessan *et al.* (2009) [17]; Yinyang *et al.* (2014) [18]; Ngene *et al.*, (2015) [19].

Table I: Triphytochemical of the aqueous extract of the leaves of *Catharanthus roseus*

Molecules sought	Reactions	Coloration	Results
Sterols/ Polyterpenes	Liebermann's reaction	Purple purple ring	+
Polyphenols	Reaction to iron chloride	Blackish blue	+
Flavonoids	Reaction to cyanidin	Orange rose	+
Tannins	Stiasny's reagent - Iron Chloride reagent	Intense black blue	+
Substances quinoniques	Bornstraëgen's reagent	Red to purple	-
Alkaloids	Dragendorff's reagent	Orange	+
Saponosides	Vigorous agitation of the water solution	Persistent moss	+

+: Presence; -: Absence

3.2 Effect of fructose on cardiovascular parameters

After thirty (30) days of experimentation, the rats made hypertensive by consuming 60 % fructose all had systolic blood pressure (SBP) greater than 140 mmHg and diastolic blood pressure (DBP) greater than 115 mmHg. SBP values ranged from 125.7 ± 0.50 (control value) to 169.80 ± 0.70 mmHg, DBP values from 105.05 ± 1.15 (control) to 140.00 ± 2.04 mmHg and heart rate (HR) of 250.51 ± 3.38 (control value) at 360, 80 ± 1.50 beats / min. Rats of the wistar strain fed a fructose-enriched diet found a link between hyperinsulinemia and high blood pressure. These two parameters are risk factors associated with cardiovascular disease [20]. The results of the work showed that the administration of 60 % fructose to rats of the wistar strains

produced hyperglycemia, hyperinsulinemia and arterial hypertension. These results are in harmony with the work of Sowers *et al.*, (2001) [21], Guo *et al.*, (2005) [22], Mellouk, 2013 [23] and Madani *et al.*, 2012 [24] who showed that the consumption of fructose led to high blood pressure throughout the experiment. The change in values observed at the level of cardiovascular parameters could be one of the consequences of the decrease in tissue concentrations of nitric oxide either to the attenuation of their production, or to their inactivation by the superoxide anion or insulin resistance [23].

3.3 Effect of fructose on oxidative stress parameters

3.3.1 Effect of fructose on catalase

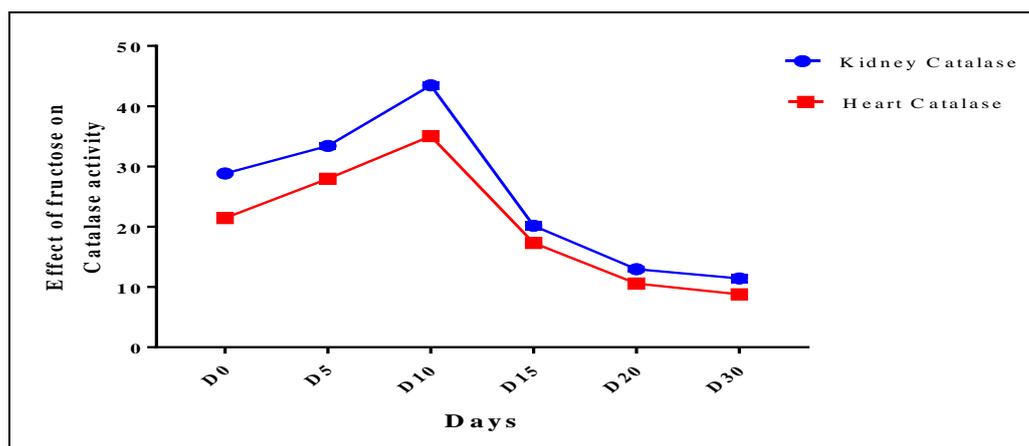


Fig. 1: Effect of fructose on catalase activity in the kidney and heart

The results of the evolution of the activities of catalase at the level of the kidney and the heart during the induction of arterial hypertension by 60 % fructose are represented by Figure 1. This figure shows that the activities of catalase in the kidney and heart increase significantly from D0 to D10 then decrease very significantly ($P < 0.001$) from D10 to D30. The control values for catalase activities in the kidney and heart are respectively 29 ± 0.2 mmol for the kidney and 21.00

± 0.7 mmol for the heart. From D0 to D10, these values increase significantly during the induction of arterial hypertension by 60 % fructose and go from 29 ± 0.2 mmol (Control value at D0) to 45.02 ± 0.5 mmol (value at D10) for the kidney, and from 21.00 ± 0.7 mmol to 38.00 ± 0.01 mmol for the heart; then decrease very significantly from D10 to D30. The values of catalase activities in the kidney vary respectively from 45.02 ± 0.5 mmol (value at D10) to $10.5 \pm$

0.80 mmol and at the level of the heart, they go from 38.00 ± 0.01 mmol (value at D10) at 9.5 ± 0.4 mmol (value at D30) at the heart.

Oxidative stress is one of the causes of degenerative diseases such as arterial hypertension [25, 26] which are normally controlled by the antioxidant defense system such as catalase present in the body (kidney and heart for example). When the level of synthesized free radicals is no longer controlled by the defense system, it results in overproduction in the blood. Thus, the results of our work have shown an increase in the activity of catalase in the kidney and heart. This could be due

to an integrity of the cells of these two organs (kidney and heart) due to moderate oxidative stress. On the other hand, an alteration of the cells of these organs through intense oxidative stress would be the cause of the decrease in the activity of catalase observed subsequently. Our results are in agreement with those of Mellouk, 2013 [23] and Madani *et al.*, 2012 [24] who showed in their work that the consumption of fructose increased oxidative stress at the origin of hypertension while decreasing serum catalase levels.

3.2.2 Effect of fructose on superoxide dismutase

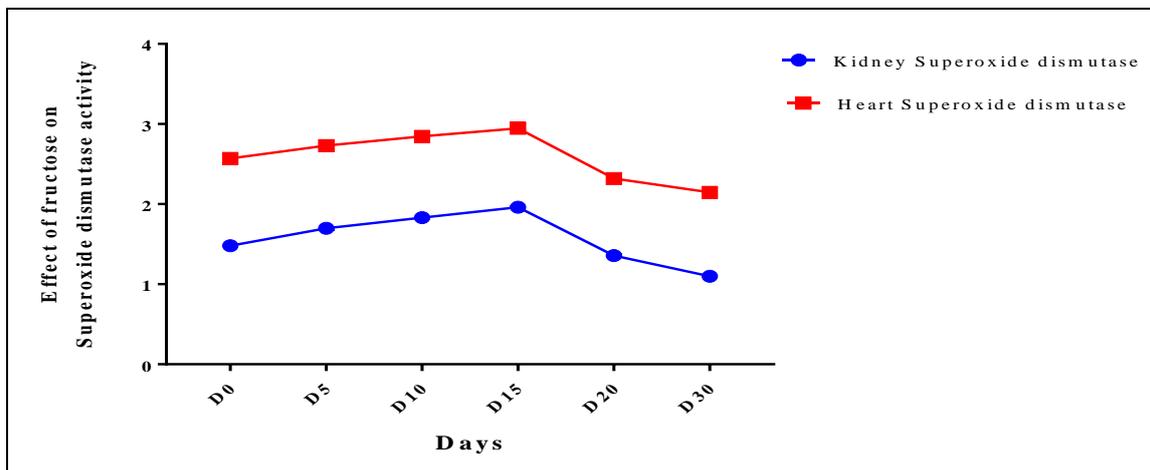


Fig. 2: Effect of fructose on superoxide dismutase activity in the kidney and heart

Figure 2 shows the results of the change in kidney and heart superoxide dismutase activities during the induction of high blood pressure by fructose 60 %. The control values for superoxide dismutase activities in the kidney and in the heart are 1.5 ± 0.8 U/mg in the kidney and 2.6 ± 0.3 U/mg in the heart, respectively. These values increase little significantly from D0 to D15 at the level of the kidney and the heart. They vary from 1.5 ± 0.8 U/mg to 1.8 ± 0.6 U/mg in the kidney, then from 2.6 ± 0.3 U/mg to 2.95 ± 0.04 U/mg at heart level. Beyond D15 to D30, the activities of superoxide dismutase in the kidney and the heart decrease little significantly varying respectively from 1.9 ± 0.7 U/mg to 1.01 ± 0.4 U/mg then from 2.95 ± 0.04 U/mg to 2.23 ± 0.05 U/mg. Superoxide dismutase is an enzyme produced by living animal and plant organisms whose role is to scavenge oxygenated free radicals by converting them into non-toxic molecules. It is therefore a regulator of oxidative stress. A decrease in the activity of this enzyme leads to severe muscle disorders and heart disease [27].

The results of our work showed an insignificant increase in the activity of superoxide dismutase in the heart and kidney during the first fifteen days of the experiment. This increase is thought to be due to the cardioprotective role exerted by superoxide dismutase [28, 29]. Then an insignificant reduction in superoxide dismutase activity in the kidney and heart compared to the normotensive rats towards the end of the experiment. This decrease in the activities of these enzymes is thought to be due to intense oxidative stress in these two organs following the induction of arterial hypertension. Our results are in agreement with those of Cash *et al.*, 2007 [30], of Madani *et al.*, 2012 [24] and of Mellouk, 2013 [23] who showed in their work that the consumption of fructose reduced the levels serum antioxidant defense players. This results in an overproduction of radical compounds and therefore an installation of oxidative stress.

3.2.3 Effect of fructose on the activity of nitric oxide

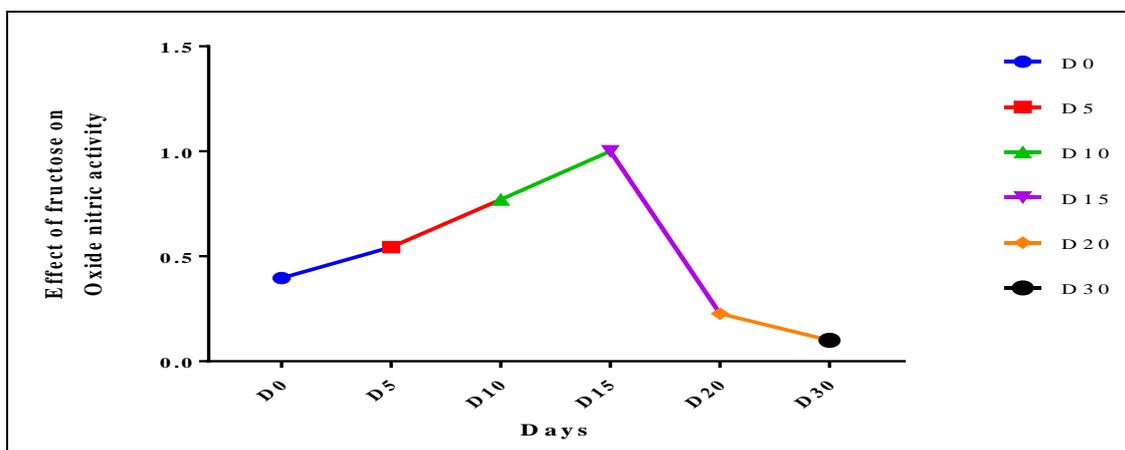


Fig. 3: Effect of fructose on nitric oxide in the aorta

Figure 3 shows the results of the change in nitric oxide levels in the aorta of rats during the induction of arterial hypertension by 60 % fructose. The control value of the nitrogen monoxide level in the aorta of normotensive rats is $0.4 \pm 0.8 \mu\text{M}$ on D0. This value increases very significantly ($P < 0.001$) from D0 to D15. It varies from $0.4 \pm 0.8 \mu\text{M}$ to $1.01 \pm 0.5 \mu\text{M}$, then decreases very significantly ($P < 0.001$) from D15 to D30, to reach the value of $0.12 \pm 0.6 \mu\text{M}$. Nitric oxide is a free radical compound synthesized from L-arginine using nitric oxide synthases. It is found in the liver, endothelial cells, macrophages, brain, white blood cells and neurons. It is involved in the dilation of the coronary arteries, acts as a neurotransmitter and regulator of blood pressure and blood insulin levels. Low levels of nitric oxide increase the risk of high blood pressure. The results of our work showed first an increase in serum nitric oxide levels. This increase would imply low blood pressure, which would reduce the risk of

heart attacks. Then a decrease in the concentration of nitric oxide in the aorta which could be perceived as a consequence of endothelial dysfunction. This dysfunction is characterized by an alteration of endothelium-dependent vasodilator responses and an overproduction of radical compounds and therefore an installation of oxidative stress. Our work is consistent with that of Mellouk, 2013 [23] who showed that the consumption of fructose reduced the serum nitric oxide level. According to Cash *et al.*, 2007 [30], the overproduction of radical compounds leads to arterial hypertension due to prolonged vasoconstriction and lumen narrowing of certain vessels. This prolonged vasoconstriction due to the formation of superoxide anions (O^{2-}) can cause a decrease in their bioavailability by reacting with nitric oxide. This leads to a decrease in vasodilation [31].

3.2.4 Effect of fructose on the activity of malondialdehyde

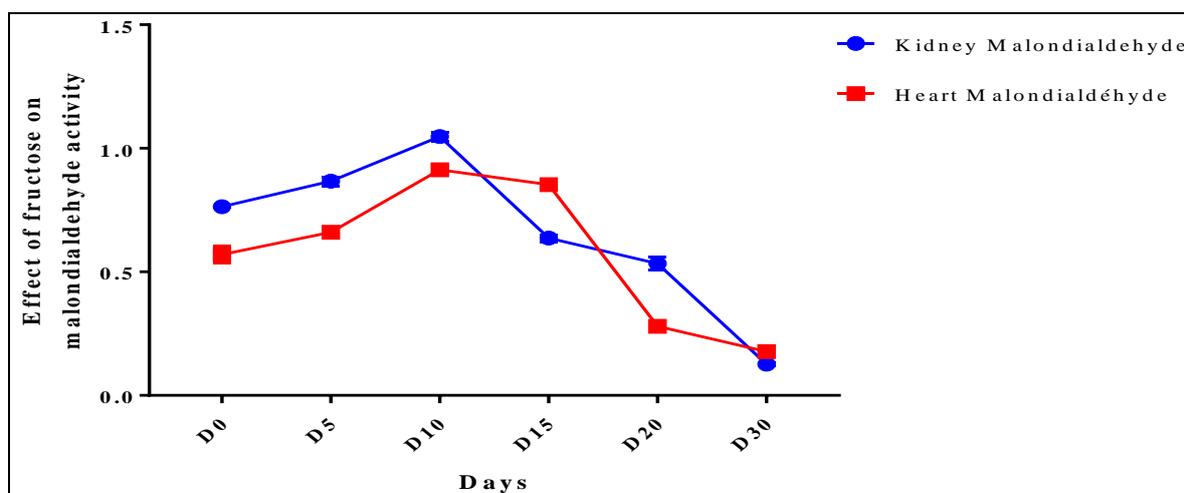


Fig. 4: Effect of fructose on malondialdehyde in the kidney and heart

The results of the variation over time of the serum level of kidney and heart malondialdehyde during the induction of hypertension by 60 % fructose are shown in Figure 4. The values of malondialdehyde at the kidney level and the heart before the induction of high blood pressure are 0.76 ± 0.02 mmol/g protein for the kidney and 0.57 ± 0.03 mmol/g protein for the heart, respectively. During induction of arterial hypertension by 60 % fructose, these levels increase significantly and pass in the kidney from 0.76 ± 0.02 mmol/g protein to 1.02 ± 0.06 mmol/g protein (D0 to D10). At the heart level, from D0 to D10, the serum malondialdehyde levels vary from 0.57 ± 0.03 mmol/g of protein to 0.90 ± 0.01 mmol/g of protein. Beyond D10 in the kidney and heart, these levels drop very significantly ($P < 0.001$), going from 1.02 ± 0.06 mmol/g of protein to 0.12 ± 0.00 mmol/g of protein from D10 to D30 for the kidney, and from 0.90 ± 0.01 mmol/g of protein to 0.18 ± 0.01 mmol/g of protein from D15 to D30 at the heart level. Malondialdehyde is a derivative of lipid oxidation. Its presence in tissues explains the state of degradation of cells and implies a state of oxidative stress. The results of our work have shown that the administration to rats of 60 % fructose leads to an increase in Malondialdehyde activity in the heart and kidney followed by a significant decrease in this activity until the end of the period experimentation at the level of these two organs. This increase is believed to be due to an alteration of membrane fatty acids by reactive oxygen species in these organs. The results obtained are similar to that of Nayeemunisa and kumuda,

(2003) [32] who showed in their work that rats made hypertensive to glucose had a high level of malondialdehyde in the heart and kidney. These alterations lead to a modification of the structure and permeability of these organs.

3.3 Effect of treatment with aqueous extract of *Catharanthus roseus* and nifedipine on cardiovascular parameter, catalase, superoxide dismutase and nitric oxide.

3.3.1 On cardiovascular parameters

The treatment of hypertensive rats with the aqueous extract of *Catharanthus roseus* at doses of 200, 500 and 1000 mg/kg bw and nifedipine at doses of 10 and 20 mg/kg bw significantly reduced ($P < 0.001$) the systolic blood pressure (SDP), diastolic blood pressure (DBP) and heart rate (HR) values. The SBP goes from 169.80 ± 0.70 mmHg (hypertensive control) to 126 ± 1.6 mmHg when the animals are treated with the aqueous extract of the leaves of *Catharanthus roseus* at 1000 mg/kg bw, and 125.7 ± 0.45 mmHg if treated with nifedipine at 20 mg/kg bw. The DBP varies from 140.00 ± 2.04 mmHg (HT) to 102.50 ± 0.8 mmHg then from 140.00 ± 2.04 mmHg to 106 ± 0.2 mmHg, if the animals are respectively treated with 1000 mg/kg bw of the aqueous extract of the leaves of *Catharanthus roseus* and 20 mg/kg bw of the nifedipine. The heart rate (HR) changes from 360.80 ± 1.50 beats/min (HT) to 251.4 ± 1.50 beats/min, then from 360.80 ± 1.50 beats/min to 250.9 ± 0.85 beats/min, when the animals are treated with 1000 mg/kg bw of the

aqueous extract of the leaves of *Catharanthus roseus* and nifedipine at 20 mg/kg bw. The aqueous extract of *Catharanthus roseus* leaves at different doses of the extract brings the cardiovascular parameters to values close to those of the control. The aqueous extract appears to be effective

than nifedipine, a benchmark drug used in the treatment of high blood pressure.

3.3.2 On catalase

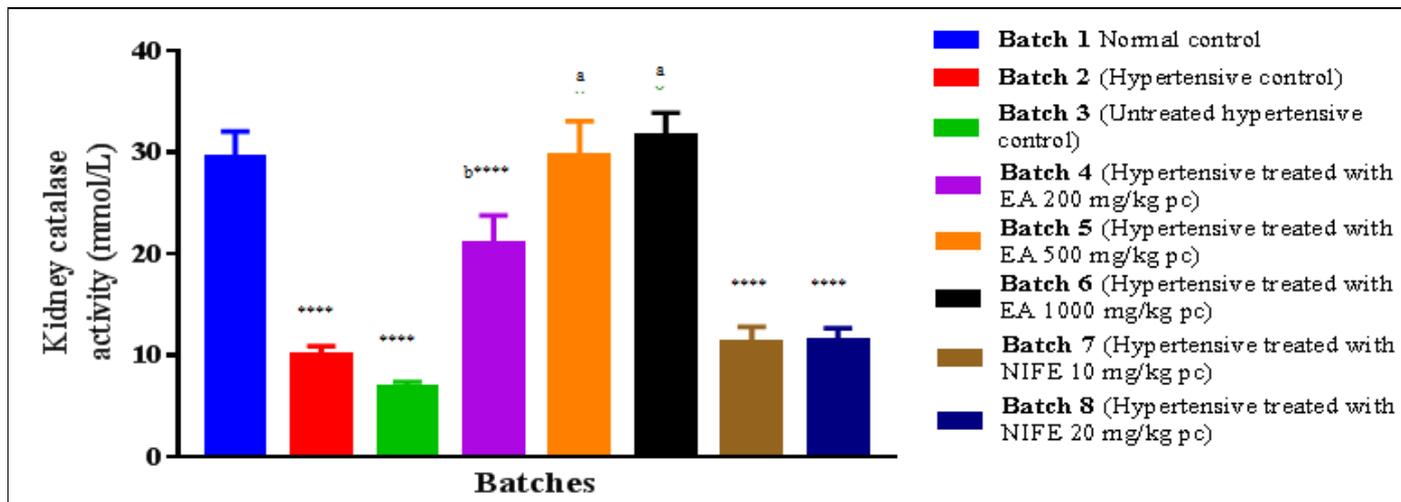


Fig. 5: Effect of the aqueous extract of the nifedipine on catalase activity in kidney

**** : P<0,0001 = highly significant difference compared to the control ; . a : P<0,0001 = highly significant difference compared to the hypertensive control ; b : P<0,001 = very significant difference compared to the hypertensive control. The means are in the form ± SEM (n=6) ; NIFE: Nifédipine ; EA = Aqueous Extract ; mmol/L = millimole per liter

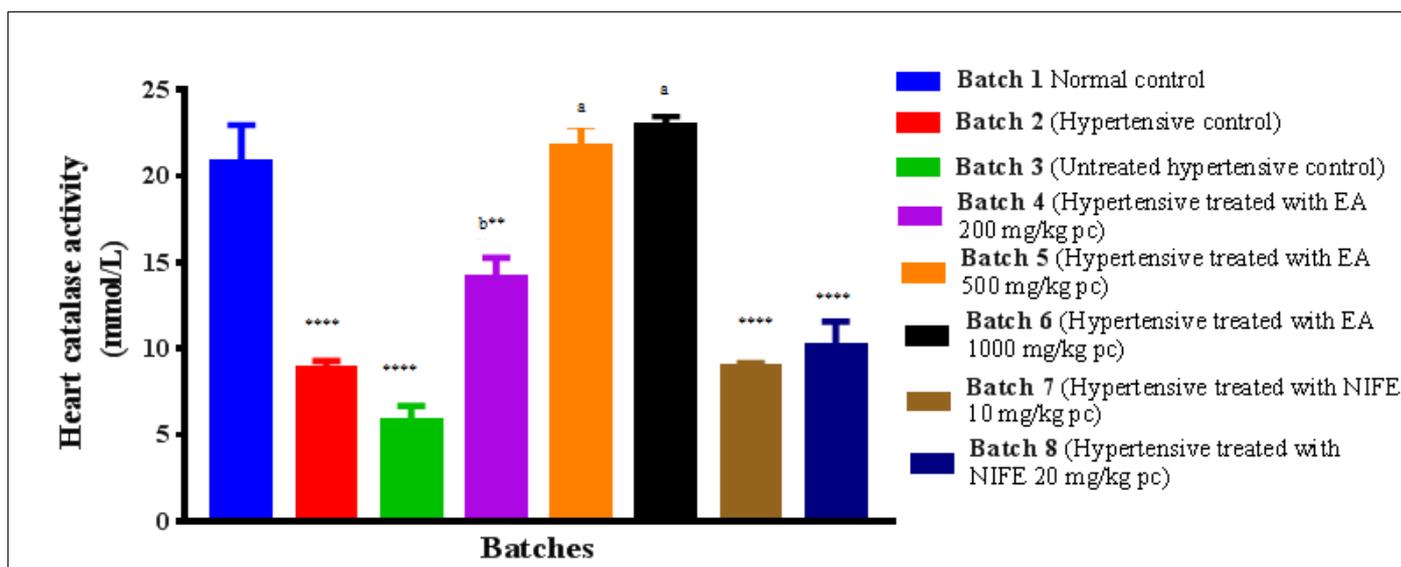


Fig. 6: Effect of the aqueous extract and nifedipine on catalase activity in heart

**** : P<0,0001 = highly significant difference compared to the control ; ** : P<0,01 = significant difference compared to control. a : P<0,0001 = highly significant difference compared to the hypertensive control ; b : P<0,001 = very significant difference compared to the hypertensive control. The means are in the form ± SEM (n=6) ; NIFE: Nifedipine ; EA = Aqueous Extract ; mmol/L = millimole per liter

Figures 5 and 6 show the results of the effects of in vivo treatment with the aqueous extract of the leaves of *Catharanthus roseus* and nifedipine on catalase activity in the kidney and heart of hypertensive rats.

Treatment of hypertensive animals with the aqueous extract of *Catharanthus roseus* leaves at different doses (0, 200, 500 and 1000 mg / kg bw) caused a highly significant increase (P <0.0001) in catalytic activity. Catalase in the kidney and heart until normalization. These activities varied for the kidney from 10.08 ± 0.48 mmol/L to 31.55 ± 1.36 mmol/L, when the animals are treated with 1000 mg/kg bw the aqueous extract of *Catharanthus roseus* and for the heart from 8.79 ± 0.28 mmol/L to 22.92 ± 0.3 mmol/L, when the animals are treated

with 1000 mg/kg bw the aqueous extract of *Catharanthus roseus*. Nifedipine even at 20 mg/kg bw does not normalize the catalytic activities of catalase. This increase in catalase activity could be explained by the presence of secondary metabolites in the aqueous extract. In fact, the aqueous extract of *Catharanthus roseus* contains polyphenols, tannins and flavonoids which have significant antioxidant activity and therefore able to fight against certain factors that promote arterial hypertension [33, 34, 35]. Treatment of these hypertensive animals showed no significant difference with nifedipine at doses of 10 and 20 mg / kg bw. The aqueous extract is therefore found to be active at a dose of 500 mg / kg bw than nifedipine. Our results are similar to those of Wu *et al.*, 2001

[36]; Hornig, 2002 [37] who showed that treating animals with different antioxidants suppressed high blood pressure.

3.3.3 On superoxide dismutase

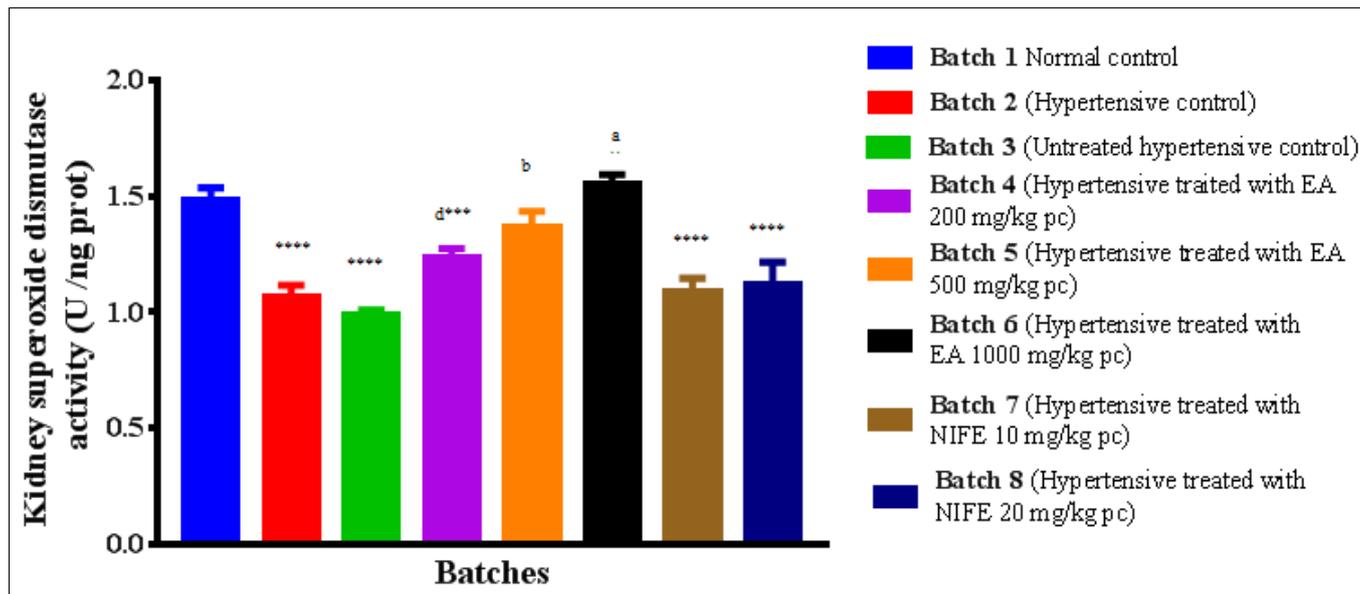


Fig. 7: Effect of the aqueous extract and nifedipine on superoxide dismutase activity in kidney

**** : P<0,0001 = highly difference significant compared to control ; *** : P<0,001 = very difference significant compared to control. a : P<0,0001 = highly difference significant compared to hypertensive control ; b : P<0,001 = very difference significant compared to hypertensive control. d : P<0,05 = insignificant difference compared to hypertensive control. The means are in the form ± SEM (n=6) ; NIFE: Nifedipine ; EA = Aqueous Extract ; U/ng/prot = Unit per nanogram per protein.

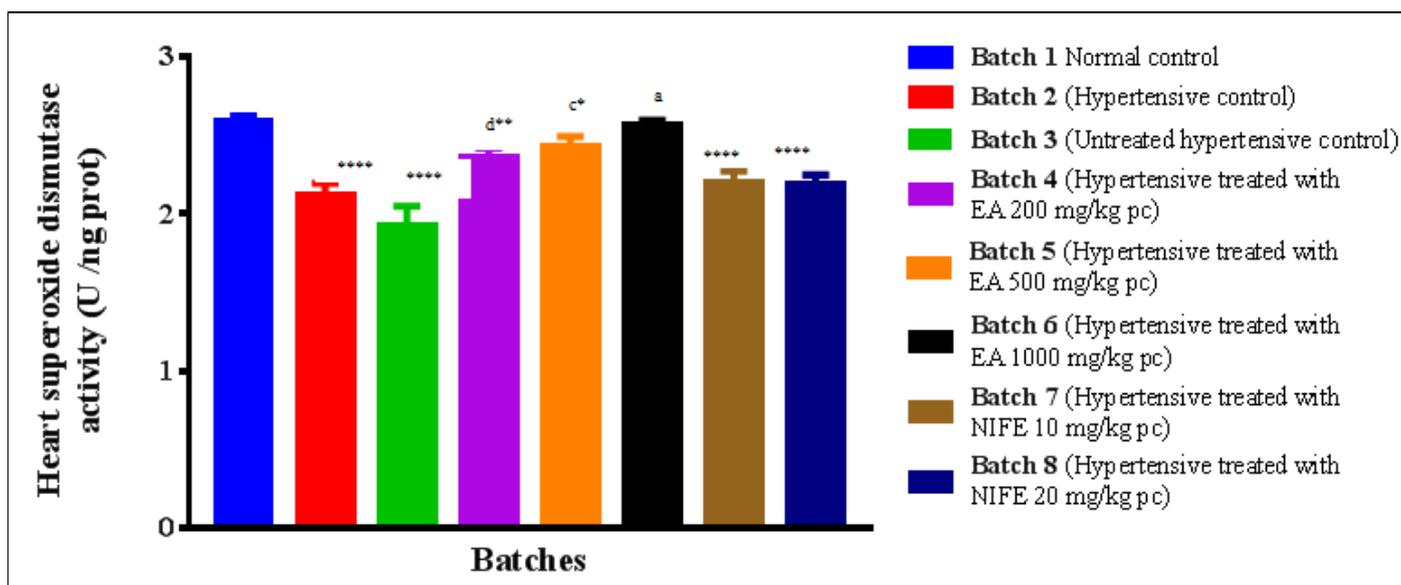


Fig. 8: Effect of the aqueous extract and nifedipine on superoxide dismutase activity in heart

**** : P<0,0001 = highly difference significant compared to control ; ** : P<0,001 = significant difference compared to control * : P<0,05 = difference peu significative rapport au témoin. a : P<0,0001 = highly difference significant compared to hypertensive control ; c : P<0,01 = significant difference compared to hypertensive control; d : P<0,05 = insignificant difference compared to hypertensive control. The means are in the form ± SEM (n=6) ; NIFE: Nifedipine ; EA = Aqueous Extract; U/ng/prot = Unit per nanogram per protein

The results of the influence of the treatment with the aqueous extract of the leaves of *Catharanthus roseus* at different doses and with nifedipine at 10 and 20 mg / kg bw are shown in Figures 7 and 8. These results show that arterial hypertension caused a highly significant decrease (P <0.0001) in the catalytic activity of SOD in both the kidney and the heart. Treatment of hypertensive animals with the aqueous extract of *Catharanthus roseus* at doses of 200, 500 and 1000 mg / kg bw and with nifedipine at 10 and 20 mg / kg bw resulted in a highly significant increase (P < 0.0001) superoxide dismutase in the heart and kidney. This increase continues until

normalization, when the animals are treated with 1000 mg / kg bw of the aqueous extract of *Catharanthus roseus*. nifedipine at 20 mg / kg bw does not fully normalize superoxide dismutase activities. This increase is believed to be due to the antioxidant properties of the aqueous extract of this plant [38]. Treatment of these hypertensive animals showed no significant difference with nifedipine at doses of 10 and 20 mg / kg bw. The aqueous extract appears to be effective at a dose of 1000 mg / kg bw than nifedipine. Our results are similar to those of Négishi *et al.*, 2004 [39]; Haddy *et al.*, 2006 [40] who also showed that the treatment of

hypertensive animals with antioxidants of various kinds suppressed arterial hypertension.

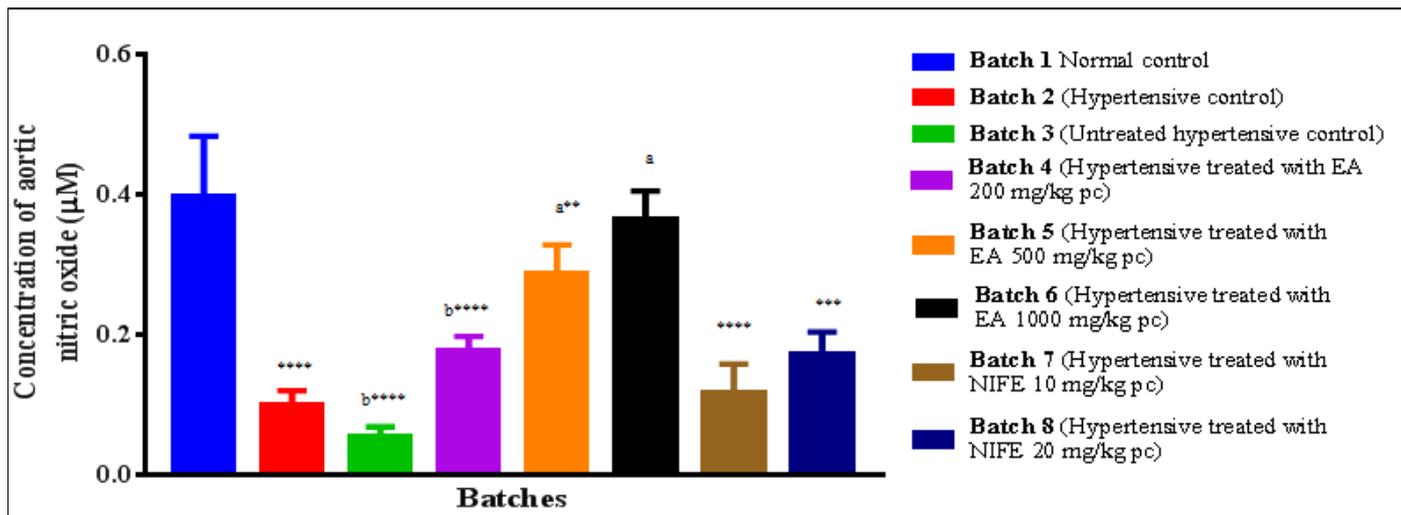


Fig. 9: Effect of the aqueous extract and nifedipine on nitric oxide activity in aorta

**** : P<0,0001 = highly difference significant compared to control ; *** : P<0,001 = very difference significant compared to control. a : P<0,0001 = highly difference significant compared to hypertensive control ; b : P<0,001 = very difference significant compared to hypertensive control. The means are in the form ± SEM (n=6) ; NIFE: Nifedipine ; EA = Aqueous Extract ; µM = micromole

Figure 9 shows the results of the effects of in vivo treatment with the aqueous extract of the leaves of *Catharanthus roseus* and nifedipine on the variation of the serum concentration of nitric oxide of the aorta of hypertensive rats. Treatment with aqueous extract of *Catharanthus roseus* at doses of 200, 500 and 1000 mg / kg bw and with nifedipine at 10 and 20 mg / kg bw of animals hypertensive with 60 % fructose resulted in a highly significant increase (P < 0.0001), when the animals are treated with 1000 mg / kg bw of the aqueous extract of *Catharanthus roseus*. Nifedipine at 20 mg / kg bw does not normalize serum nitric oxide concentration. The increase in serum nitric oxide levels may be due to the hypotensive properties of the aqueous extract of *Catharanthus roseus*. Indeed, the aqueous extract of this plant contains alkaloids which are molecules capable of lowering high blood pressure

[41]. The increase in the rate of oxidative stress parameters could be due to the presence of these molecules. Treatment of these hypertensive animals showed no significant difference with nifedipine at doses of 10 and 20 mg/ kg bw. The aqueous extract is therefore found to be active at a dose of 1000 mg / kg bw than nifedipine. Our results are similar to those of Hornig, 2002 [36]; Négishi *et al.*, 2004 [39] who have shown that when animals are treated with antioxidants of various kinds, it allows the functioning of the endothelium to be restored. The increased nitric oxide level appears to be effective in decreasing endothelium-dependent vasodilation in hypertensive animal arteries [42, 43].

3.4 Comparative effect of the aqueous extract of *Catharanthus roseus*, metformin on malondialdehyde

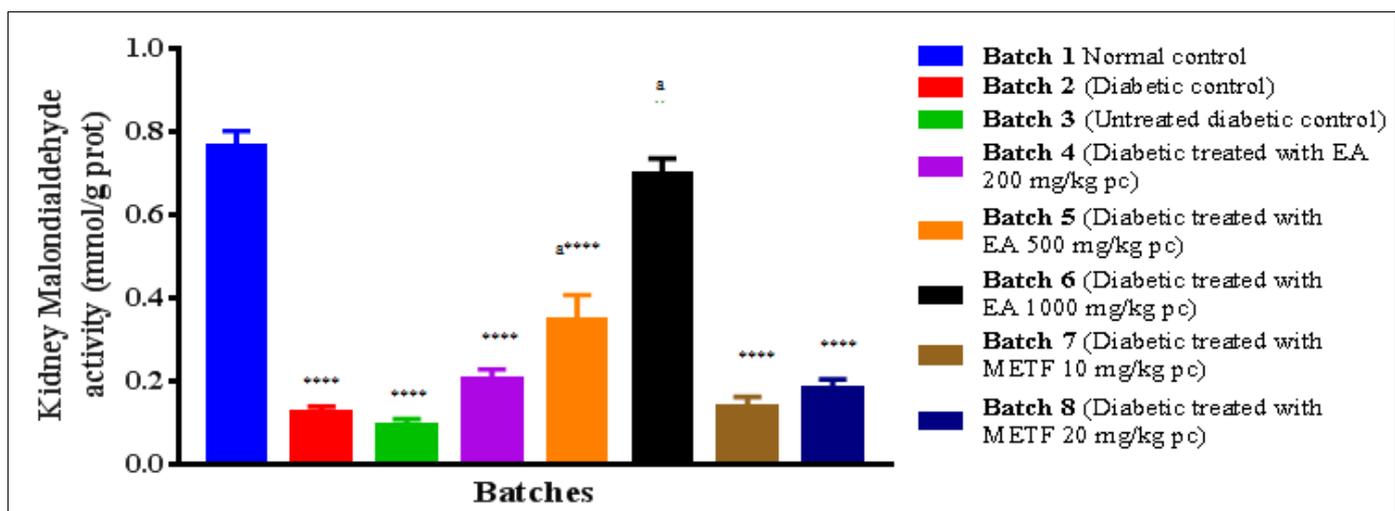


Fig. 10: Effect of the aqueous extract and metformin on malondialdehyde activity in kidney

**** : P<0,0001 = highly difference significant compared to control ; *** : P<0,001 = very difference significant compared to control. a : P<0,0001 = highly difference significant compared to hypertensive control. The means are in the form ± SEM (n=6). EA = Aqueous Extract ; METF = Metformin ; mmol/g prot = millimole per gram of protein

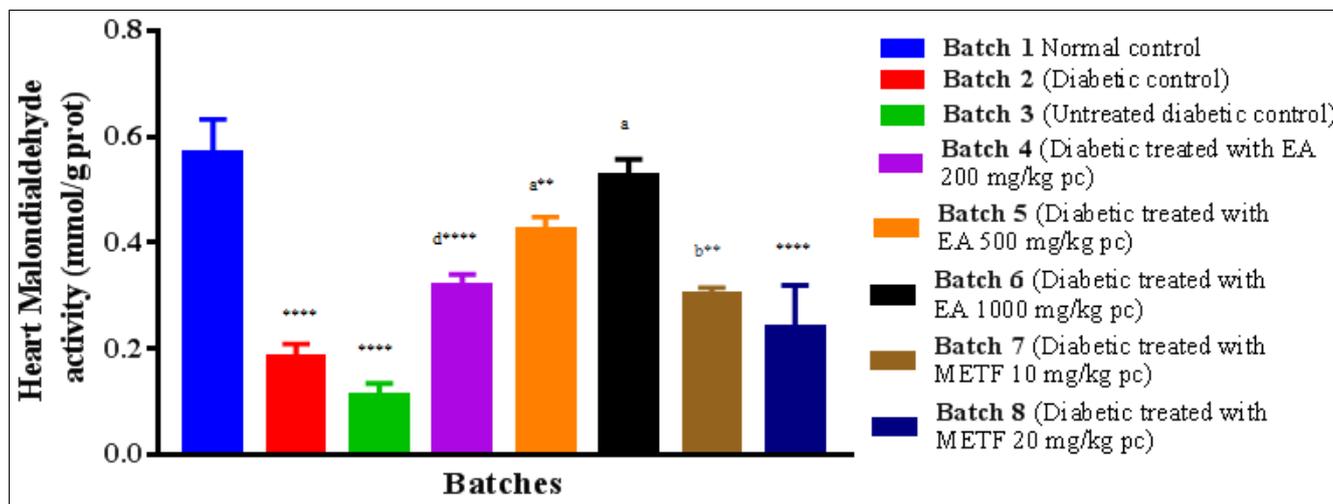


Fig. 11: Effect of the aqueous extract and metformin on malondialdehyde activity in heart

**** : $P < 0,0001$ = highly difference significant compared to control ; ** : $P < 0,01$ = significant difference compared to control. a : $P < 0,0001$ = highly difference significant compared to hypertensive control ; b : $P < 0,001$ = very difference significant compared to hypertensive control. d : $P < 0,05$ = insignificant difference compared to hypertensive control. The means are in the form \pm SEM (n=6) EA = Aqueous Extract ; METF = Metformin ; mmol/g prot = millimole per gram of protein

Figures 10 and 11 show the results of 7 days of treatment with the aqueous extract of *Catharanthus roseus* and with metformin on the change in serum malondialdehyde levels in diabetic rats. Treatment of diabetic rats with the aqueous extract of the leaves at doses of 200, 500 and 1000 mg / kg bw and with metformin at 10 and 20 mg/kg bw resulted in a gradual and highly significant increase in serum levels of metformin compared to rats in the control group. The serum level of malondialdehyde in the kidney of diabetic rats varies from 0.12 ± 0.00 mmol/g of protein (Diabetics Treated) to 0.2 ± 0.12 mmol/g of protein; then at 0.34 ± 0.03 mmol/g of protein and finally at 0.69 ± 0.02 mmol / g of protein when the animals are treated with 200, 500 and 1000 mg / kg bw of the aqueous extract of the leaves of *C. roseus*. In the heart, this rate varies from 0.18 ± 0.01 mmol/g of protein (Diabetics Treated) to 0.32 ± 0.01 mmol / g of protein, then to 0.42 ± 0.07 mmol / g of protein, and finally to 0.52 ± 0.03 mmol / g of protein. Similar results were obtained when sick animals were treated with metformin at 10 and 20 mg/kg bw. The increase in the serum metformin level at a dose of 1000 mg/kg bw compared to the treated control batch shows that the aqueous extract would have prevented the reduction of lipid peroxidation in the kidneys and the heart. Treatment of these hypertensive animals showed no significant difference with metformin at doses of 10 and 20 mg / kg bw. The aqueous extract is therefore found to be active at a dose of 1000 mg / kg bw than metformin.

4. Conclusion

Like all plants, the aqueous extract of *Catharanthus roseus* contains many molecules. Some of them have antioxidant properties that play an important role in the fight against oxidative stress and therefore against high blood pressure.

5. Conflict of interests

The authors claim that there is no conflict of interests

6. References

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