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EFFECTS OF *PIPER UMBELLATUM* LINN. (PIPERACEAE) LEAVES EXTRACT ON SOME OXIDATIVE STRESS AND REPRODUCTION PARAMETERS IN RESTRAINT STRESS MODEL OF MALE INFERTILITY IN RAT

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ABSTRACT

Background: Oxidative stress plays an important role in the pathogenesis of many diseases including male infertility and the use of antioxidants tends to become popular in modern society. The present study aimed to investigate effects of the aqueous extract of *Piper umbellatum* on male infertility induced by restraint stress. **Methods:** Rats were immobilized for 6 hours daily for 6 weeks. At the end of this period, animals were treated with *Piper umbellatum* aqueous extract at the doses of 150 and 300 mg/kg, distilled water (10 mg/kg) and vitamin C (30 mg/kg) for 60 days. The 61st day, rats were sacrificed and sexual organs weighted. Testicular volume and oxidative stress parameters were determined. Besides, *in vitro* antioxidant of the extract against DPPH radical and some biochemical and fertility parameters were evaluated. **Results:** The extract stimulated antioxidant system *in vivo* and inhibited lipid peroxidation. *Piper umbellatum* extract exerted *in vitro* antioxidant activity against the DPPH radical and the IC_{50} was 331.60 µg/mL. The total content of flavonoids of the extract measured was 145.70 ± 3.51 rutin equivalent. Oxidative stress parameters and sperm characteristics were ameliorated with an increase of testicular volume in plant extract groups. **Conclusions:** The extract had *in vivo* and *in vitro* antioxidant properties and restored fertility parameters evaluated in the current study. The plant could be therefore useful in the management of male infertility.

KEYWORDS: *Piper umbellatum*, oxidative stress, male infertility, antioxidant, chronic stress.

INTRODUCTION

About 5 to 15 % of couples are infertile, and the male factor is responsible for 50 % of these causes[1,2]. Male infertility is caused by many factors like psychological stress, exposure to high heat for prolonged periods, genetic abnormalities, heavy use of alcohol, impotence and infections of the testes or epididymis[3]. Sometimes, no identifiable cause can be found in over 25 % of infertile males[4]. These recent years, oxidative stress has been implicated in the pathogenesis of many diseases and disorder such as atherosclerosis, cancer, diabetes, lung diseases, liver damage, inflammatory bowel disease and central nervous system disorders[5]. Oxidative stress results from the production of oxygen radicals in excess of the antioxidant capacity, and any oxidizing radical is a potential agent of oxidative stress. Oxidative stress as a potent physiological factor can lead to sperm damage and deformation and male infertility[6]. Indeed, in tissues like testis with high rate of metabolism and cell replication, oxidative stress can be especially damaging. Testicular oxidative stress appears to be a common feature in much of what underlies male infertility[7]. Exposure to free radicals has led organisms to develop series of defense mechanisms. Enzymatic antioxidant defenses include superoxide dismutase, catalase and glutathione peroxidase, they are endogenous. Beside these endogenous antioxidants, there are exogenous antioxidants like vitamin A, vitamin C, vitamin E, flavonoids and carotenoids found in food[8,9]. Administration of antioxidants in men can improve the antioxidant status in the testis and maintain steroidogenesis and spermatogenesis[10]. It is well-known that most of the plants used in the folk medicine exert antioxidant activity. In addition, traditional medicine is still being used nowadays in all parts of the world and has been growing in economic importance particularly by the use of medicinal plants. They have a respectable position today, especially in developing countries, where modern health services are limited and represent the only accessible treatment[11]. *Piper umbellatum* is widely used in many countries like Cameroon as vegetable or condiment, besides its medicinal uses[12]. Its leaves have been shown effective in the treatment of several ailments. Studies conducted on the leaves and the fruits of *Piper umbellatum* indicated that these parts of the plant had antioxidant activity[13]. The leaves of this plant

are used by some traditional healers of the Centre region of Cameroon to manage male infertility. From what said above, it might be beneficial not only to promote the use of medicinal plants but also to develop better antioxidant therapies in case of infertility. Therefore, the present study was designed to investigate the effects of *Piper umbellatum* aqueous extract on testicular oxidative stress on restraint stress model of male infertility in rats.

MATERIAL AND METHODS

Collection of plant material and extraction

Piper umbellatum leaves were collected in a rural area, Mbele village, near Obala town in the Centre Region of Cameroon. The plant was authenticated at the national herbarium under the voucher number 10391SRF/Cam by comparison with the sample of the collector J.F. Breteler 429. Fresh leaves were shade dried and crushed in a mortar. 220g of leaves powder were soaked in a volume of 6 liters of tap water for 12 hours. After this time a first filtration was performed with a sieve of 0.5 mm meshing and a second filtration with Whatman N°3 paper. The solution was then lyophilized and a powder of 34.29 g was obtained for an extraction yield of 15.58 %.

Experimental animals

The study was done with healthy male rats weighing 175-190 g and at least 10 weeks old of age. Animals were obtained from the animal house of the Laboratory of Animal Physiology of University of Yaoundé I. The animals were housed in clean cages placed in well-ventilated housed conditions. Rats were given free access to food and tap water, and received a multivitamin complex (KELAVITASOL®) every fortnight. The study was conducted according to the guidelines of the Cameroon National Ethical Committee on the use of laboratory animals for scientific research (Ref No. FW-IRB00001954).

Chemicals

Assay kit supplied by *Fortress diagnostics*, United Kingdom was used to determine proteins levels. The reference product used during the test was vitamin C (VITASCORBOL®, *COOPER* France) usually sold in pharmacy. Different buffers and solutions used were prepared in laboratory in flasks with distilled water.

METHODS

Induction of infertility and animal grouping

Immobilization stress was induced with the help of the modified protocol of Almeida *et al.*, [14]. For this section of the study, 35 rats were used. They were divided in sets of 10 and 25 rats each. For 6 weeks, 25 rats (St group) were stressed through immobilization and 10 remained unstressed.

Apparatus

Rats were immobilized in cone-shaped plastic tubes of 15.80 centimeters each. The apex of the tube had an opening of 2.20 centimeters diameter and the basis an opening of 5.80 centimeters diameter. The basis was recovered by a wire-netting with square meshes of 4.00 millimeters each side. Each tube had a neck of 4.20 centimeters of diameter, 5.5 centimeters from the apex of the tube, which impeded rats to turn up or to change their position once installed. Rats were introduced in these tubes 6 hours per day and different tubes were arranged in plastic cages covered with wooden shaving.

After the restraint period, 5 unstressed rats (group Normal 1) and 5 stressed rats (group St/ negative control 1) were randomly chosen and sacrificed. The 25 remaining rats were divided in five groups of five rats each treated for 60 days as follows:

-group Normal 2 unstressed which received distilled water (DW), 10 mg/kg;
-group St + DW (negative control 2) stressed which received DW, 10 mg/kg;
-group St + VC stressed and treated with vitamin C (VC), 30 mg/kg;
-group St + *Pu*AE 150 stressed and given *Piper umbellatum* aqueous extract (*Pu*AE) at the dose of 150 mg/kg;
-group St + *Pu*AE 300 stressed treated with *Pu*AE at the dose of 300 mg/kg.

Sacrifice and sample collection

Initial and final body weights of the animals were recorded. Sacrifice of rats was carried on the 61st day. Rats were anesthetized by intraperitoneal injection of diazepam. Under anesthesia, the neck was cleared to expose jugular veins. The jugular veins were cut and the rats were made to bleed into clean and dry centrifugation tubes. The blood was centrifuged at 3000 rpm for 15 minutes and different sera were stored at -20° C for further biochemical analysis. Thereafter, the rats were quickly dissected, the testes and the epididymis removed. These organs were cleaned of superficial fatty layer and weighed for the determination of each organ/body weight ratio.

Collection of epididymal sperm

Shortly after, the cauda of the right epididymis measuring 6 mm length was dissected out and used for the estimation of the number of spermatozoa. The cauda was cut into small pieces in a stemmed glass containing 10 mL of NaCl 0.9 % solution and incubated in a water bath at 34° C temperature. This sperm was further used for determination of some sperm characteristics.

Evaluation of sperm characteristics

Sperm count

A volume of 20 µl of the sperm suspension was aspirated and deposited on a Malassez cell. It was observed in a photonic microscope (OLYMPUS JAPAN), X400 and the number of spermatozoa was rapidly counted in four areas. The number of spermatozoa per mL of sperm (N) was estimated.[15]

Hypo-osmotic swelling test (HOS test)
Sperm's flagella membrane integrity was assessed by hypo-osmotic swelling test[16]. Assay was performed by incubating 50 µL sperm suspension with 1 mL hypo-osmotic solution for 45 minutes, and observed for coiling tails under the microscope. The percentage of coiling was determined.

Determination of testicular volume
The length (L) and the width (W) of the left and the right testis were measured with a millimetric ruler (*Ward's Natural Science*) and the testicular volume (V) was calculated [17].

Preparation of homogenates
The testes were homogenized at 20 % in phosphate sodium buffer, pH =7.4. The epididymis was homogenized in phosphate potassium buffer, pH = 6.8, at 20 %. The homogenates were centrifuged at 3000 rpm for 45 minutes. The supernatants were stored at -20° C and were further used for biochemical assays. Seminal vesicles were homogenized at 25 % in distilled water and centrifuged at 3000 rpm for 15 minutes. The supernatant was taken and stored at -20 °C for the dosage of seminal fructose.

Biochemical analysis
The concentrations of testicular proteins were determined according to the principle of the Biuret reaction. Superoxide dismutase (SOD) and catalase activities were determined in the testis[18,19]. Reduced glutathione (GSH) and malondialdehyde (MDA) were evaluated of Ellman method[20,21]. Alpha-glucosidase activity in the epididymis and seminal fructose concentration were evaluated according to WHO [22] protocols. Antioxidant capacity of the extract was measured by the DPPH scavenging method as described by Molyneux[23]. Total flavonoids content of the extract was determined[24].

Histology
The left testis of each animal was fixed using the Bouin solution for two weeks. Thereafter, transversal sections of the testis measuring about 3 mm were made. Fixed tissues were then transferred to graded series of alcohol (50°, 70°, 95° and 100°), cleared in xylene and infiltrated in molten paraffin wax at 55°C. Sections of 5 µm thickness were obtained using a microtome from solid paraffin blocks of tissue, fixed on clean slides, cleared and stained with haematoxylin-eosin (HE) stains. The observations were made under a light microscope.

Statistical analysis
Results were expressed as the mean ± Standard Error of the Mean (S.E.M.). The data were analyzed using ANOVA followed by Tukey post-test to compare control and test groups with GraphPad Prism software version 5.03. Values of $p<0.05$ were considered significant.

RESULTS

Effects of *Piper umbellatum* extract on the relative weight of some reproductive organs
The testis, epididymis, ventral prostate and the seminal vesicles relative weight were lower ($p < 0.05$) in St group when compared to normal groups (Table 1). Treatment with the plant extract for 60 days induced an increase of these organs. The increase was not significant ($p > 0.05$) in extract treated groups in comparison to St + DW group (Table 1).

Table 1: Effects of *Piper umbellatum* extract on the relative weight (g/100 g bw) of the testis, epididymis, ventral prostate and the seminal vesicles.

Groups	Testis	Epididymis	Ventral prostate	Seminal vesicles
Normal 1	1.40 ± 0.04	0.50 ± 0.01	0.36 ± 0.00	0.43 ± 0.02
St	0.70 ± 0.11^a	0.29 ± 0.03^a	0.20 ± 0.03^a	0.31 ± 0.02^a
Normal 2	1.17 ± 0.05	0.45 ± 0.01	0.31 ± 0.03	0.34 ± 0.04
St + DW	0.96 ± 0.03	0.30 ± 0.00	0.27 ± 0.01	0.28 ± 0.03
St + VC	1.04 ± 0.02	0.35 ± 0.00	0.39 ± 0.04	0.32 ± 0.01
St + *Pu*AE 150	1.09 ± 0.02	0.33 ± 0.00	0.32 ± 0.01	0.32 ± 0.01
St + *Pu*AE 300	1.05 ± 0.00	0.37 ± 0.02	0.30 ± 0.02	0.30 ± 0.02

Data values represent mean ± SEM; n=5. ^{a}p < 0.05 significant difference when compared with normal 1 group.

Effects of *Piper umbellatum* extract on some testicular antioxidant parameters
Chronic stress induced an elevation of testicular MDA level, a decrease of reduced glutathione level and SOD and catalase activities ($p < 0.05$) in St and St + DW groups in comparison to normal 1 and normal 2 groups (Table 2). *Piper umbellatum* leaves extract provoked a decrease of MDA concentration, an increase of GSH level and, a stimulation of SOD and catalase activity when compared to rats submitted to stress and treated with DW (Table 2).

Table 2: Effects of *Piper umbellatum* extract on testicular malondialdehyde, reduced glutathione, SOD and catalase activities.

Groups	MDA (mmol/gx10^{-6})	GSH (nmol/g)	SOD (µmole/mg of protein)	Catalase (µmole H_2O_2/min/mg of protein)
Normal 1	3.09 ± 0.37	6.25 ± 0.22	0.50 ± 0.15	0.24 ± 0.04
St	7.46 ± 0.55 ^{a}	4.28 ± 0.20 ^{a}	0.20 ± 0.08 ^{a}	0.13 ± 0,06
Normal 2	2.15 ± 0.10	4.75 ± 0.51	0.54 ± 0.11	0.77 ± 0.07
St + Dw	7.40 ± 0.30	2.64 ± 0.15	0.26 ± 0.09	0.74 ± 0.05
St + VC	1.82 ± 0.17 ^{u}	3.50 ± 0.25	0.65 ± 0.25	0.62 ± 0.08
St + *PuAE* 150	2.04 ± 0.04 ^{u}	3.06 ± 0.40	0.44 ± 0.20	0.97 ± 0.02
St + *PuAE* 300	1.70 ± 0.13 ^{u}	4.70 ± 1.21^{u}	0.58 ± 0.30	0.78 ± 0.08

Data values represent mean ± S.E.M.; n=5. $^{a}p < 0.05$: significant difference when compared with normal 1 group; $^{u}p < 0.05$ significant difference when compared with St + DW group.

Effects of *Piper umbellatum* extract on testicular proteins concentration and vesicular fructose

Restraint stress induced a decrease of testicular proteins in St group when compared to normal 1 group (Figure 1). This depression of testicular proteins was still observed in St + DW in relation to normal 2 group. Treatment with the plant extract at the doses of 150 and 300 mg/kg provoked a non-significant elevation of testicular proteins concentration when compared to St + DW (Figure 1).

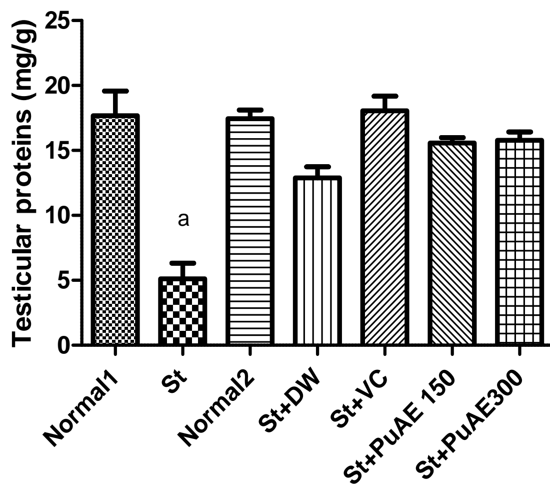

Figure 1: Effects of *Piper umbellatum* extract on testicular proteins concentration.

Data bars represent mean ± S.E.M.; n=5. $^{a}p < 0.05$: significant difference when compared with normal group in stress group.

Effects of *Piper umbellatum* extract on vesicular fructose level

Chronic stress caused a reduction of vesicular fructose in St group when compared to normal 1 group (Figure 2). The decrease persisted in St + DW in comparison to normal 2 group. Treatment with the *Piper umbellatum* leaves extract at the doses of 150 and 300 mg/kg induced a non-significant elevation of seminal fructose level when compared to St + DW (Figure 2).

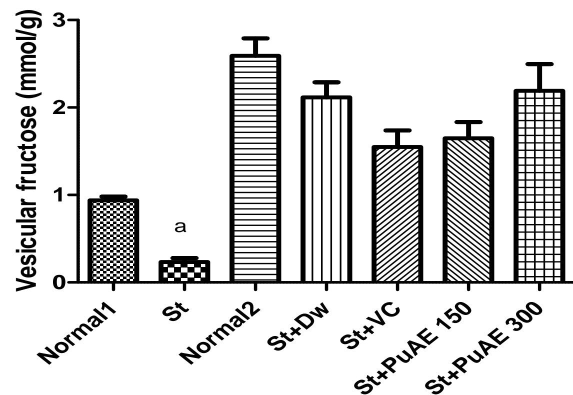

Figure 2: Effects of *Piper umbellatum* extract on vesicular fructose concentration.

Data bars represent mean ± S.E.M.; n=5. $^{a}p < 0.05$: significant difference when compared with normal group in stress group.

Effects of *Piper umbellatum* extract on acid phosphatase and alpha-glucosidase activities

From the results obtained immobilization stress induced a decrease ($p < 0.05$) of prostatic acid phosphatase activity in St group in comparison to normal 1 group (Figure 3A). This activity remained low in St + DW group in relation to unstressed rats treated with DW. Administration of plant extract at the doses of 150 and 300 mg/kg caused an elevation of acid phosphatase activity when compared to St + DW rats (Figure 3A). The increase was significant ($p < 0.05$) only at the dose of 150 mg/kg.

As for alpha-glucosidase activity, restraint stress lowered this parameter, in St and St + DW group when compared respectively to normal 1 and normal 2 groups. The reduction was reversed by a 60-days treatment period with *Pu*AE at the doses of 150 and 300 mg/kg.

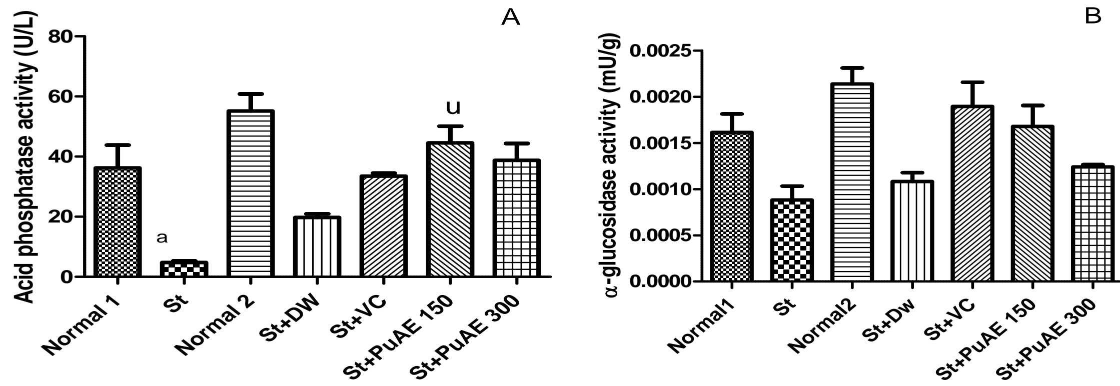

Figure 3: Effects of *Piper umbellatum* extract on acid phosphatase activity (A) and alpha-glucosidase activity (B). *Data bars represent mean ± S.E.M.; n=5. [a]p < 0.05 significant difference when compared with normal 1 in stress model; [u]p < 0.05 significant difference when compared with St + DW group.*

Effects of *Piper umbellatum* extract on testicular volume

Testicular volume was reduced (p < 0.05) in St group when compared to the normal 1 group (Figure 4). The decrease persisted in stressed group of rats treated with DW when compared to unstressed rats of normal 2 group. The extract provoked at the two doses, an increase (p < 0.05) of the testicular volume in immobilization stress induced infertility in comparison to St + DW group.

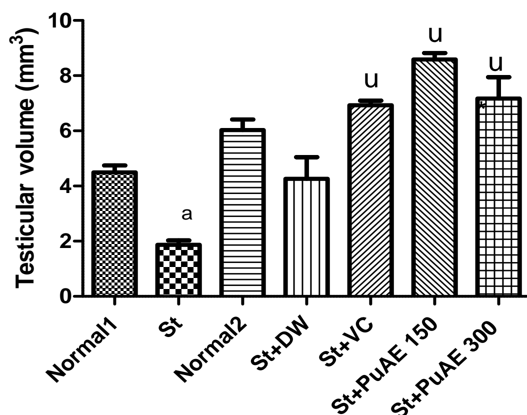

Figure 4: Effects of *Piper umbellatum* extract on testicular volume.

Data bars represent mean ± S.E.M.; n=5. [a]p < 0.05 significant difference when compared with normal group; [u]p < 0.05 significant difference when compared with St + DW group.

Effects of *Piper umbellatum* extract on some sperm characteristics and histomorphometry

According to the results, sperm count and the percentage of spermatozoa with coiled tail were lower in St and St +DW when compared respectively to normal 1 and normal 2 groups. These sperm characteristics were significantly (p < 0.05) increased after 60 days of treatment with *Pu*AE at the two doses (Table 3).

Stress caused a reduction of testicular epithelium thickness and the diameter of epididymal tubules, in St and St +DW groups, when compared respectively to normal 1 and normal 2 normal groups. On the contrary epididymal epithelium thickness was increased in St and St + DW groups when compared to normal groups (see supplemental Figure 6). *Pu*AE induced an increase of testicular epithelium thickness and the diameter of epididymal tubules. Different values became similar to those of normal groups in rats treated with *Pu*AE (Table 3).

Table 3: Effects of *Piper umbellatum* aqueous extract on sperm count, membrane integrity, testicular and epididymal morphometric parameters.

Groups	Sperm count[C] ($x10^6$)	HOS test (%)[C]	Testicular epithelium thickness (µm)	Epididymal epithelium thickness (µm)	Diameter of epididymal tubules (µm)
Normal 1	3.77 ± 0.11	78.75 ± 0.60	80.40 ± 5.63	15.02 ± 0.84	140.35 ± 20.51
St	0.51 ± 0.24 [a]	14.50 ± 7.03 [a]	43.63 ± 2.80	34.72 ± 2.34	98.62 ± 12.02
Normal 2	3.34 ± 0.27	89.00 ± 0.55	79.20 ± 6.30	17.42 ± 1.14	137.50 ± 13.20
St + DW	1.98 ± 0.11	72.50 ± 2.11	76.91 ± 2.60	32.60 ± 1.30	112.70 ± 10.91
St + VC	3.50 ± 0.36 [u]	90.00 ± 0.63[u]	70.30 ± 6.40	20.43 ± 0.57[u]	145.00 ± 13.62
St + *PuAE* 150	3.38 ± 0.10 [u]	92.25 ± 1.11[u]	87.23 ± 8.54	17.83 ± 0.84[u]	122.30 ± 10.84
St + *PuAE* 300	3.68 ± 0.18 [u]	91.00 ± 0.83[u]	71.90 ± 5.70	22.00 ± 0.74[u]	100.13± 9.94

Data values represent mean ± S.E.M.; n=5 ([C]) or n=10. [a]p < 0.05: significant difference when compared with normal 1 group [u]p < 0.05 significant difference when compared with St + DW group.

In vitro antioxidant activity of *Piper umbellatum* leaves extract against DPPH radical and total flavonoids content

The inhibiting concentration (IC_{50}) of *Pu*AE against DPPH radical was determined from the regression curve illustrated by the figure 5. Results indicated that the IC_{50} of the extract was 331.60 µg/mL. The IC_{50} value of *Pu*AE was 5.23 higher than vitamin C (VITASCORBOL®) IC_{50} used as reference antioxidant. Concerning total flavonoids content, the dosage indicated that our extract contained 145.70 ± 3.51 µg rutin equivalent.

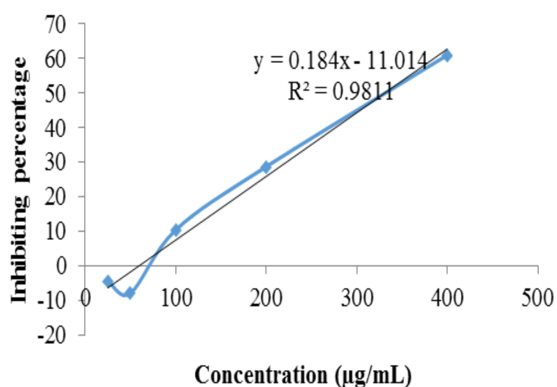

Figure 5: Regression curve of *in vitro* antioxidant activity of *Piper umbellatum* aqueous leaves extract against DPPH radical.
Each point represents the mean ± S.E.M. n = 3.

Effects of *Piper umbellatum* extract on testicular histology

Microphotography of the testis of rats of all normal groups exhibited a normal tissue architecture, with an ongoing spermatogenesis process in the seminiferous tubules (Figures 6A and 6B). In the rats of negative group, spermatogenesis was remarkably and visibly impaired. Sections revealed cell degeneration. Lumen of seminiferous tubules were devoid of spermatozoa (Figure 6C). In St + DW group, spermatogenesis was partially restored and few germ cells were observed in the lumen of seminiferous tubules (Figure 6D). Treatment with vitamin C and plant extract at the doses of 150 and 300mg/kg re-established the testis architecture. Different sections were similar to those of normal rats (Figures 6E, 6F and 6G).

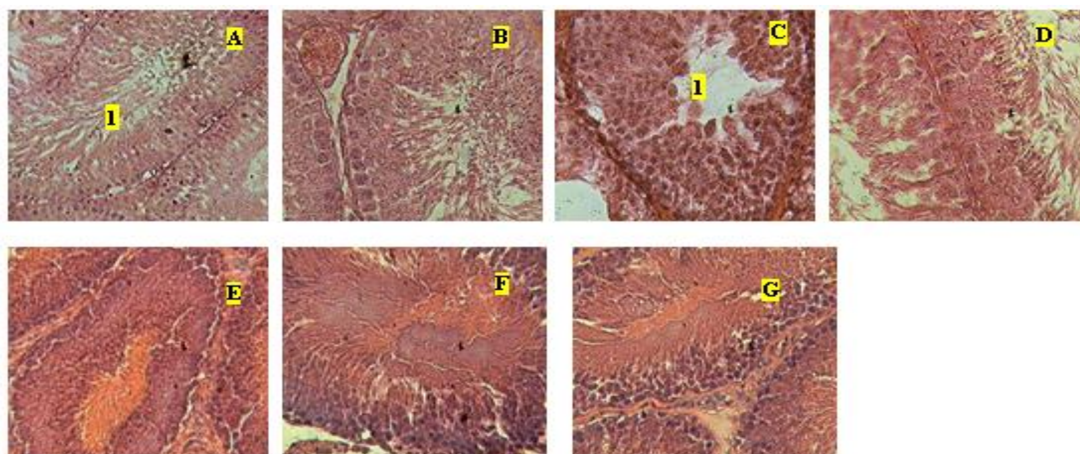

Figure 6: Microphotography of testis sections of rats: normal 1 (A), normal 2 (B), St (C), St + DW (D), St + VC (E), St + *Pu*AE 150 (F) and St + *Pu*AE 300 (G). (H&E., 400X). 1: lumen.

DISCUSSION

The main objective of the present study was to evaluate the effects of *Piper umbellatum* aqueous extract on chronic stress model of male infertility. In fact, psychological stress is common in the current modern society [25]. In addition, stress is considered a major factor contributing to male infertility and several previous studies noted that stress depressed male fertility [26]. Restraint stress is widely utilized in many studies to mimic the psychological stress. Stressful conditions lead to excessive production of free radicals that cause an imbalance in the oxidant/antioxidant system [27]. In previous studies, it has been shown that chronic stress enhanced oxidative stress, oxidative damage, and reduction in the total antioxidant levels in the testes of rats [27, 28]. In consistent with these results, restraint stress induced oxidative stress in the testis of rats in our study marked by an elevated MDA, reduced GSH and antioxidant enzymes SOD and catalase. Administration of *Pu*AE reversed that state of oxidative stress in the testis, suggesting that the extract might possess antioxidant potentials. *In vitro* efficacy of *Piper*

umbellatum extract against DPPH radical was in favor of the previous argument. Also, the total content of flavonoids in the extract indicated that it possesses these specific phenolic compounds which have a known antioxidant activity. Indeed, flavonoids exert their antioxidant activity by inhibiting the production of reactive oxygen species or through prevention of lipid peroxidation [29]. Thus, these findings suggest that the plant extract might protect the testicular function through its antioxidant properties.

The negative impact of testicular oxidative stress might be first seen on the relative weight of androgen-dependent organs like epididymis, prostate and seminal vesicles. Indeed, elevation of testicular MDA indicates an enhancement of lipid peroxidation of cell membrane. Thus, oxidative stress is responsible of testicular cell death. Interstitial Leydig cells synthesize androgens during steroidogenesis from cholesterol. These hormones stimulate the functioning of androgen-dependent organs [30]. Due to oxidative stress chronic stress may have inhibited the synthesis of androgens in the testis, leading to its depletion. Leydig cells are reported to be particularly susceptible to oxidative damage *in vivo* due to their close proximity to ROS-producing testicular interstitial macrophages [31]. This perturbation of steroidogenesis is probably responsible of the decrease of the relative weight of androgen-dependent organs due to reduced male hormones. This fact might cause the reduction of testicular volume register after chronic stress. Increase of relative weight of androgen-dependent organs or testicular volume following treatment of rats with *Piper umbellatum* aqueous extract might suggest that antioxidant capacity of the latter protected and/or restored Leydig cells function.

Testicular proteins levels were reduced in negative groups. Likewise, seminal fructose, α-glucosidase and acid phosphatase activities were also lower in negative groups than in normal ones. The observed reduction in the total protein level may be a result of accelerated mRNA degradation [32], and thus the inability of the testis to synthetize proteins properly. The different results could also be explained by the fact that oxidative stress affected the Leydig cells functioning which became unable to secrete testosterone. As the synthesis and the secretion/or activity of the parameters previously evoked are under the regulation of androgens, testosterone depletion may responsible of the failure of androgen-dependent organs in different negative groups. The increase of testicular proteins and seminal fructose levels, and, the stimulation of α-glucosidase and acid phosphatase activities in plant extract groups might strongly suggest that the *Pu*AE antioxidant activity facilitated the recovery of Leydig cells function.

Sperm count was negatively affected by chronic stress. Our results also indicated according to hypo-osmotic swelling test that more damaged spermatozoa were produced in negative groups. These results were associated to a reduction of testicular germinal epithelia. Observation of microphotographs of the testis sections corroborated these findings. A previous study reported that adult male rats exposed to prolonged immobilization exhibit a decrease in spermatozoid production and sperm density, in addition to lower plasma testosterone concentration [33]. Spermatogenesis takes place in the testis under the influence of androgens. Spermatozoa possess a membrane rich in polyunsaturated fatty acids and their cytoplasm contains few antioxidant enzymes to fight against lipid peroxidation. An increase of free radicals can damage sperm cell membrane and is negatively correlated to the sperm count and sperm motility [34]. Stress caused a great impairment of male rat fertility and results are features of androgen deprivation probably secondary due to oxidative stress. As the treatment with the *Piper umbellatum* extract induced a visible relief of different studied signs and parameters of infertility in male rats, beneficial effects may be attributed to its antioxidant capacity.

CONCLUSIONS

Piper umbellatum extract exerted *in vivo* and *in vitro* antioxidant properties and contained flavonoids. These results may explain its ability to restore fertility and may justify the empirical use of this plant to treat male infertility in Cameroon.

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