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Alterations in sperm parameters of adult male *Wistar* rats following exposure to aqueous flower extract of *Aspilia africana*

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Abstract
In recent times, reproductive health has become a very key aspect of human health requiring a lot of attention. This is as a result of the various health and societal issues arising from deviations in reproductive health. Several individuals in the society today have one or more reproductive health concerns, including infertility, fertility regulation issues, sexually transmitted diseases, reproductive tract malignancies and many more. Medicinal plants have gained global acceptance and are now being used in the management of diseases, infections reproductive and public health concerns. This study was designed to investigate changes in the sperm parameters of adult male *wistar* rats exposed to aqueous flower extract of *Aspilia africana* including sperm count, morphology and motility. The results obtained revealed a significant (p<0.05) dose dependent reduction in the sperm count, progressive motility and morphological abnormalities in male rats given aqueous extract of *A. africana* flowers when compared with the control (74%). This shows that *A. africana* flowers may have herbal contraceptive effects and possible spermicidal potentials. Further study is therefore required to elucidate its mechanism of action.

Keywords: Medicinal plants, Infertility, Sperm count, sperm motility, sperm morphology, *Aspilia africana.*

Introduction
According to the world health organization (WHO), infertility is defined as lack of the ability to conceive and impregnate a woman in females and males respectively, who have had unprotected sex for up to a year [1]. The most outstanding causes of male infertility centers on defective spermatogenesis and sperm dysfunction which have been shown to be caused by several factors including chemical pollution, electromagnetic radiation, pesticides, stress, smoking, obesity, alcohol, and inflammatory diseases amongst others [2, 3, 4, 5]. Virtually all the above factors causes oxidative stress consequently which will in turn cause cell/tissue damage [6] The presence of large quantities of unsaturated fatty acids in the cell membrane of sperm cells makes them mostly susceptible to reactive oxygen species damage; as these fatty acids can easily be oxidized leading to lipid peroxidation. Lipid peroxidation if not controlled in these cells will eventually lead to loss of membrane integrity, DNA damage, and cellular apoptosis, increased permeability and the cellular enzymes may also get inactivated. Consequently, this results in general sperm dysfunction including abnormal morphology, low sperm count and motility [7, 8]. Medicinal plants are sources or precursors of most orthodox drugs as their active principles are extracted and used in the production of drugs that are used by people all over the world [9] Several medicinal plants have been reported as having the ability to interfere with fertility. Many researchers have investigated a number of plants in a bid to elucidate the mechanisms of their actions while others focus on confirming the veracity of the claimed effects of these plants on fertility. While some medicinal plants have been claimed to enhance fertility, others have antifertility principles. This study was carried out to elucidate the changes that may occur in sperm parameters of adult male wistar rats following exposure to aqueous flower extract of *A. africana* flowers in a bid to determine if it the plant possess contraceptive spermicidal properties.

Fig 1: Picture of *A. africana* showing the different parts, including the flowers.

Aspilia africana have been reported to have several uses and properties and is widely used in Africa and Asia. Reports have shown that this traditional herb is used as an anti-inflammatory agent; it has astringent properties, acts as a bactericide and is very effective in wound healing [10]. Gill [11] reported that the leaves of *A. africana* are potent in the relief of febrile headache as well as cure ailments such as stomach disorders, sciatica and lumbago. The ethanolic leaf extract of *A. africana* can increase vascular tone and also have *in vitro* gastro-protective properties [12]. Other abilities that have been credited to this plant include anti-bacterial, anti-fungal and anti- spasmodic properties. It is used to hasten delivery and it strengthens smooth muscle contraction [13, 14, 15, 16]. Reports from recent studies have revealed that the methanolic leaf extract administered intraperitoneally altered the estrus cycle and caused deleterious damages on uterine tissues [17, 18]. This plant is generally known as the hemorrhage plant which is borne out of its ability to arrest bleeding even when a major artery is severed [19], and is used in the treatment of anemia, corneal opacities and stomach ailments [20]. Studies carried out on the leaves, revealed the presence of procene1 in the purified aqueous extract of *A. africana* leaf, adding that sequitterpenes and monoterpenes are the major essential lipid (oils) constituents of the leaves of *A. Africana* [21]. *A. Africana* leaves have been studied and reported to be rich in both micro and macro elements which makes it relevant as a herbal remedy of some sort [22]

2. Materials and methods

Reagents/Chemicals: All reagents used were of analytical grade and products of British Drug House (BDH) England, E. Merck, Darmstadt, Germany and Aldrich Chemical Company.

2.1. Collection and Identification of Plants: The fresh flowers of *Aspilia africana* were collected between September and November 2019 from Iguomo village, near Okada, Benin City, Edo State. It was identified and authentication was done at the college of Natural and applied sciences, Igbinedion University, Okada.

3. Methods

3.1. Extraction of Plant material: The fresh flowers were destalked, pooled, thoroughly rinsed with distilled water and left to drain at room temperature. It was then air- dried, pulverized and stored in air tight containers for subsequent use. The powdered plant materials were macerated with distilled water for 24 hours. The mixture was then filtered using Whatman's (No.l) filter paper and then lyophilized to give the crude aqueous extracts.

3.2. Male animals study: A total number of twenty adult male rats of the wistar strain weighing between 250g to 300g were obtained from the central animal holding, Igbinedion University Okada, were used for this study. They were divided into four groups of five animals each (A, B, C and D) and acclimatized for a period of two weeks before the commencement of the experiment. The extract was administered to the animals for three months to enable us estimate a complete spermatogenetic cycle which takes about 56 days. Group A served as control and was given 1ml of distilled, while groups B, C and D were treated with 150mg, 200mg and 300mg per kilogram body of the rats respectively. At the end of the treatment period, the animals were allowed fast for a day and sacrificed the next day using chloroform anaesthesia. Sperm analysis was done to determine sperm count, sperm morphology and motility using samples from the caudal epididymis. Fertility potential test was carried out by introducing proestrus females to the treated males for mating at the ratio of one male: one female.

3.3. Sperm analysis

3.3.1. Sperm count

A modified method of Yokoi and Mayi, [23] was applied in counting the spermatozoa from the right caudal epididymis. The epididymis was minced with scissors and placed in 5ml of physiological saline, and allowed to incubate for 2mins. The resultant fluid was further diluted at 1:100 with a solution containing 5g sodium bicarbonate and 1ml of 35% formalin. Ten mililitres (10ml) of the fluid with sperm cells suspended in it was placed on the counting chamber and viewed under a microscope. The total number of spermatozoa was counted using the new improved Neubauer's counting chamber.

3.3.2 Sperm motility

This study was carried out in line with already studied and reported methods with slight modifications [24]. The fluid from the caudal epididymis was diluted with Tris buffer solution to 0.5ml. An aliquot of the solution was observed under the microscope at a magnification of x400. The mean motility of three estimates was used as final motility score.

3.3.3 Sperm morphology

This was determined using the original dilution for motility; the solution was diluted 1:20 with 10% neutral buffered formalin. A smear was done by using a drop of sperm suspension. After the smear had dried, the specimen was fixed with absolute ethanol for 5 minutes. Then, the specimen was immersed in Diff-Quik Stain I and II for 5 minutes each and viewed under the microscope. Findings were expressed as percentage of morphologically normal and abnormal sperm [25].

3.4 Statistical analysis

All statistical analysis of data obtained in the course of this study were carried out using the IBM Statistical Package for Social Sciences (SPSS version 20). The results are expressed as mean ± SEM. Statistical significance was determined by analysis of variance (ANOVA). Significant differences between groups were determined in ANOVA using Duncan post-hoc test at $p<0.05$.

4. Results

4.1. Changes in sperm parameters of extract treated male rats

Figures 1, 2 and 3 shows results of the effects of exposure of experimental rats to graded doses of aqueous flower extract of *A. africana* on sperm parameters including count, motility and morphology. Figure 1 reveals a dose dependent reduction in sperm count in male rats administered aqueous extract of *A. africana* flowers. The results in figure 2 reveals significant (p<0.05) reductions in the progressive motility of all the treated groups (38%, 36% and 38% for treated 150mg, 200mg and 300mg per kilogram body weight of the animals respectively) when compared with the control (74%). Non-progressive motility also increased across the treatment groups but was particularly significant at the dose level of 200mg/kg. The percentage of immotile sperm significantly (p<0.05) increased across the treatment groups (40%, 36% and 40% for 150mg, 200mg and 300mg per kilogram body weight of the animals respectively) against 10% recorded in the control. Figure 3 shows the morphological differences between the experimental animals exposed to graded doses of aqueous flower extract of *A. africana*. A seeming dose dependent significant (p<0.05) decline in the percentage of morphologically normal sperm cells was recorded with the attendant increase in morphologically abnormal sperm cells when compared with the control.

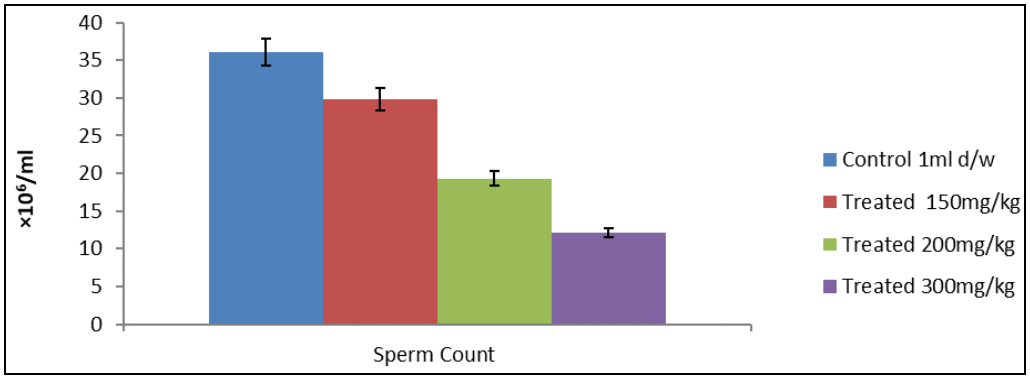

Fig 2: Sperm count for male rats exposed to aqueous extract of *A. africana* flowers. Values are Mean ± SEM.

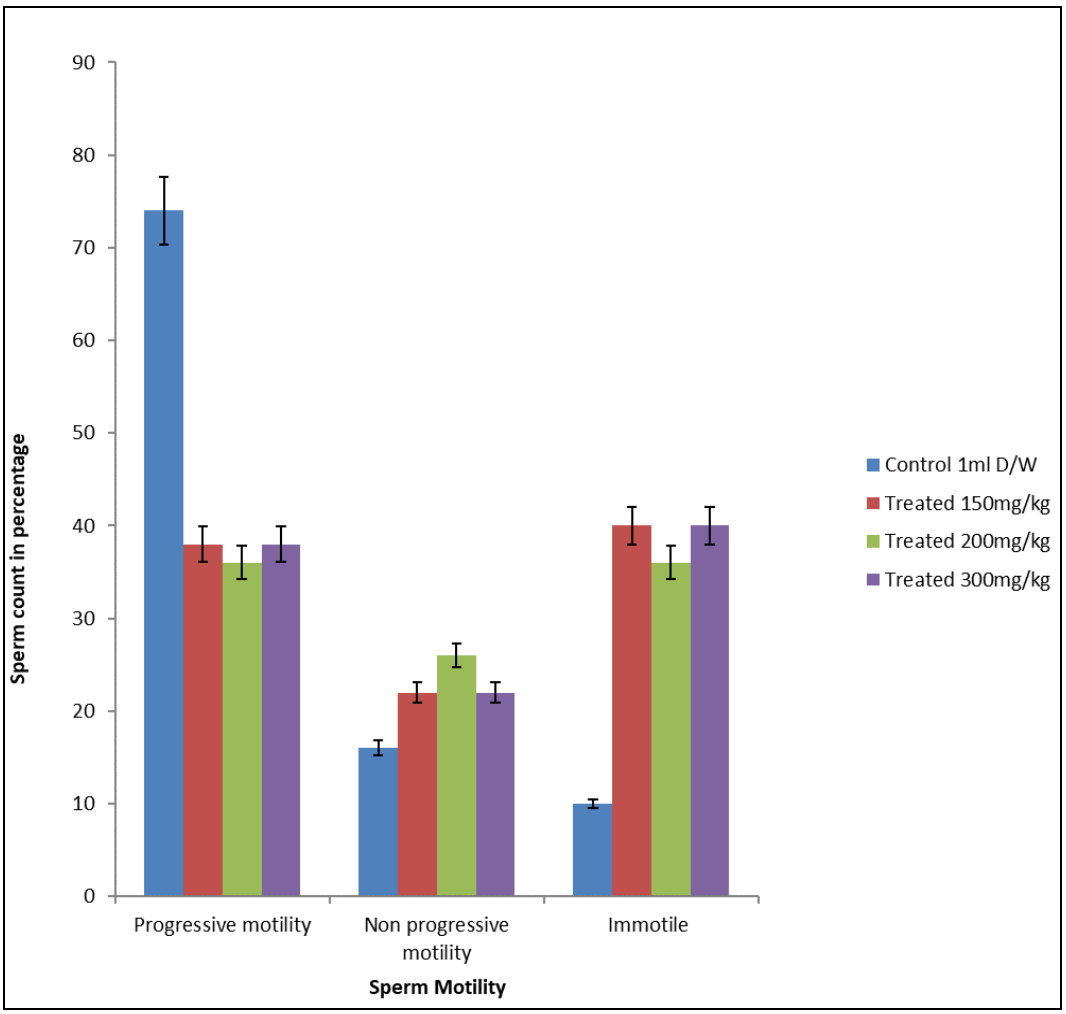

Fig 3: Sperm motility for extract-treated male rats. Values are Mean ± SEM

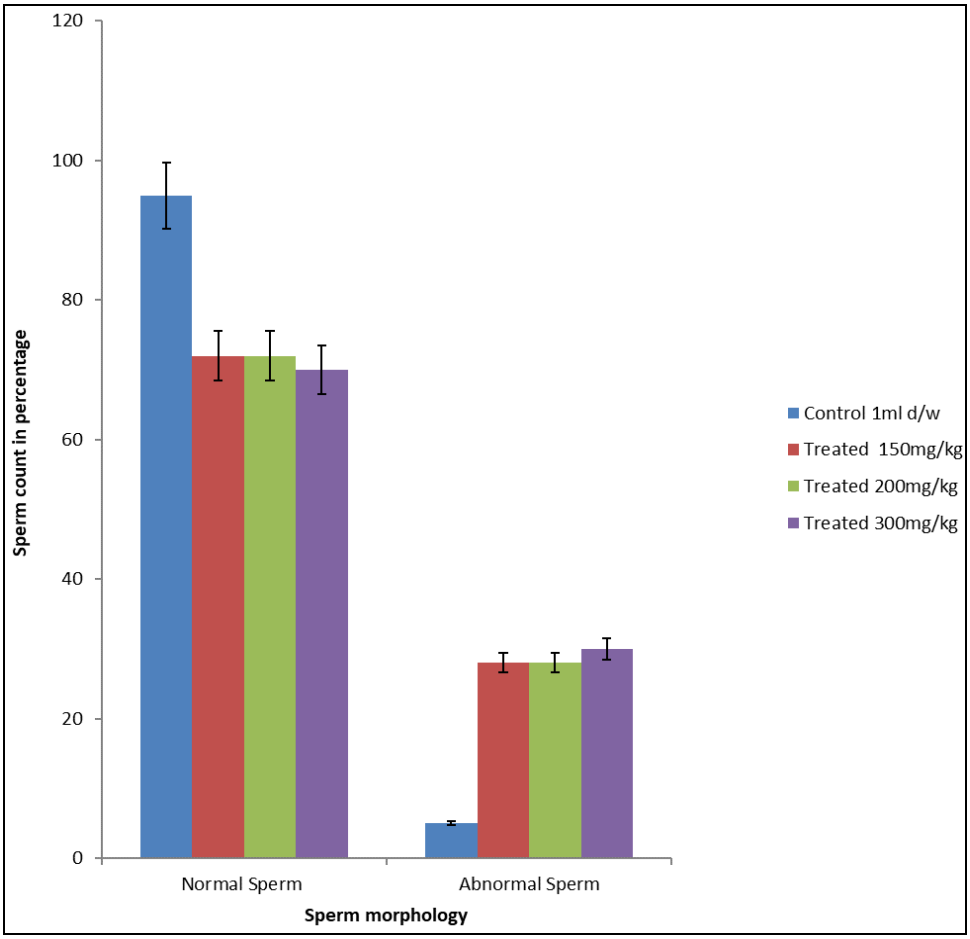

Fig 4: Sperm morphology for extract-treated male rats. Values are Mean ± SEM.

4.2. Sperm population

The photomicrographs of sperm count/population revealed a dose dependent reduction in the population of spermatozoa across the groups treated with 150mg/kg, 200mg/kg and 300mg/kg of the extract, with the high dose group showing the least population of spermatozoa compared to the control

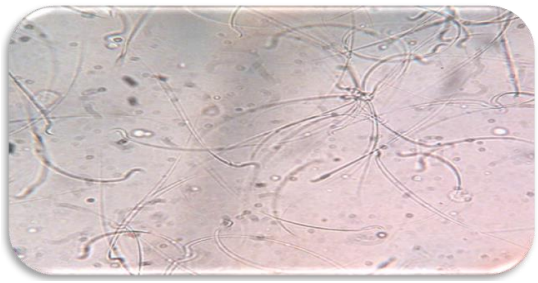

Plate 1: Photomicrograph showing spermatozoa population with 1ml distilled water (Control)

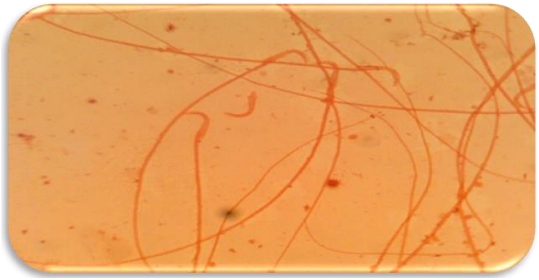

Plate 2: Photomicrograph showing spermatozoa population with 150mg/kg body weight

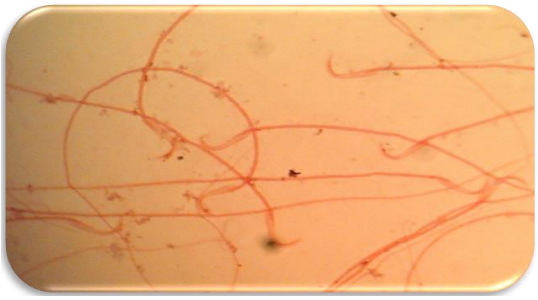

Plate 3: Photomicrograph showing spermatozoa population with 200mg/kg body weight

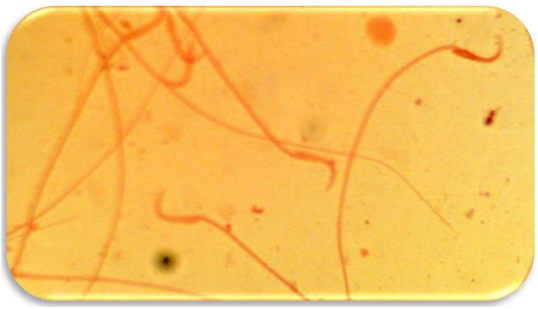

Plate 4: Photomicrograph showing spermatozoa population with 300mg/kg body weight.

5.0 Discussion

Sperm parameters have a leading role both in natural conception and assisted reproduction technologies (ART) outcomes. In this present study, the effects of the aqueous

flower extract of *A. africana* flowers on sperm and by extension fertility in male *Wista*r rats as well as some andrological parameters were evaluated. Sperm count, morphology and motility, doubles as the key parameters that determine the viability and functionality of the mature sperm cell. Decrease in sperm count and increased numbers of morphologically abnormal sperm indicates interference of the plant extract with testicular spermatogenesis [26]. These results compares favorably with reports of other studies on medicinal plants with similar objectives. It has been reported that benzene seed extract of *Carica papaya* exerted anti-fertility effects on male albino rats by reducing sperm testicular mass, count and motility [27]. Similarly, *Quassia amara* has been reported to possess the ability to reduce sperm count, decrease viability of spermatozoa and its motility when the chloroform extract of the bark was administered to albino rats [28]. These findings are at variance with the reports of the study that shows that *Chrysophyllum albidum* treatment caused increase in motility, morphology and sperm count [29], thus exerting a sperm-positive or pro-fertility effect.

Increase in sperm count was the outcome of exposing rats to the aqueous extracts of root, leaf or whole plant of *Withania somnifera dunai* [30]. It has been reported that most medicinal plants with contraceptive or antifertility properties usually bring about that effect by reducing sperm density or count [31]. The mechanism by which the extract reduced sperm count may be by androgen depletion, which may have been brought about by the high level of alkaloids present in the extract [32].

Motility is the percentage of sperm that moves during a sperm or semen analytical study. Total motility refers to any movement at all; while progressive motility is forward movement in a either a line or large circle. It is well established that active sperm motility is a prerequisite to achieve fertilization, such that for sperm cells to be able to fertilize mature ova, they must possess the ability to swim up the female reproductive tract [33]. Motility is considered to be normal when at least 40% of the sperm cells are moving and up to 32% has the ability to swim either in a line or a circle. Decreased sperm motility also suggests alteration of sperm maturation and these are leading causes of disturbed fertility [34]. The results from this study imply that the extract caused morphological changes in the sperm cells, thus making them immotile and incapable to bring about conception. Sperm motility depends greatly on acetylcholinesterase control which brings about the flagella movement, as well as energy derivation from utilization of fructose and oxidation of glucose [35]. It is therefore highly probable that decrease in sperm motility recorded in the present study may be because the plant extract has the potential to lower glucose and inhibit acetylcholinesterase. There is similar a finding from a study which show that *Morinda morindiodes* root bark caused a significant reduction in sperm motility, count and increased morphological abnormalities [36]. Again chronic consumption of honey appeared to have deleterious effects on sperm parameters and fertility potential of male *Wistar* rats in a reported study [37]. In their study, percentages of normal sperm, motile sperm and total sperm count were significantly reduced.

Several researchers have reported the sperm parameters enhancing effects of medicinal plants; for instance, kolaviron from the seed of garcinia kola has been confirmed to have the ability to prevent perioxidative changes in spermatozoa thus enhancing sperm quality and motility [38] Improvements in the process of spermatogenesis and sperm quality following the use of *Tribulus terrestris* have been reported, [39] and studies have also shown that serum testosterone level correlates positively with sperm concentration and motility [40] The significant decrease in the motility of spermatozoa that was obtained in the present study may simply be as a result of the presence of tanins in the extract [32], which has been implicated in the reduction of testosterone levels, as was the case in this study [41].

The morphological changes obtained in this study may be as a result of distortions in the process of spermatozoa maturation, which may have resulted in mature malformed sperm cells [42].

5.1 Fertility potentials test

The low sperm count affected the fertility potentials of the experimental animals in such a manner that only one out of all the females that had shown sperm positive index (sperm in vaginal smear) eventually got pregnant with highly reduced litter size of 2 Pubs. These pubs died three days after. This goes to show that the aqueous extract of *A. africana* also decreased the fertility potential of the male *Wistar* rats in this study thus corroborating the claim that the flowers may have antifertility properties, which are potent in males. Phytochemical analyses carried out on the crude and aqueous extract of *A. africana* flowers revealed that it contains anthraquinones as one of its abundant secondary metabolites [32]. This phytochemical have several isolates which have the propensity to inhibit the growth of the malaria parasite plasmodium falciparium. Antimalarial agents are generally believed to have antifertility potentials and in line with above claim, it may be probable that the antifertility effects recorded in this present study may have been a result of the presence of anthraquinones in the aqueous flower extract of *A. africana*. Secondly, the extract may possess spermatotoxic agents, which caused regression of the maturing spermatozoa and the eventual reduced number of litters in the females mated with the extract-treated males in this study [43]. The reduced fertility potential elicited by this plant material could be attributed to the reduced population of mature spermatozoa and the regressed percentages of motile sperms, which may have been caused by the reduced levels of testosterone. The plant extract may have had a direct effect on the hypothalamus, thus inhibiting the secretion of the gonadotropin-releasing hormone required for the pituitary gonadotropins to be released which in turn modulates the release of testosterones at the level of the testis.

Conclusion

There was a clear demonstration in this study that the aqueous flower extract of *A. africana* produced significant alterations in the sperm parameters of male rats. Whilst it can be utilized in the development of herbal contraceptives or possible spermicides, those desiring pregnancy should avoid its use. We therefore recommend further studies on the mechanism of action of the aqueous flower extract of *A. africana* on spermatogenesis.

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