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Date Palm Pollen Grains as a Potential Manager for Male Sub-fertility: A Clinical Trial

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Authors' contributions

This work was carried out in collaboration among all authors. Author HSMS performed the experimental part, statistical analysis and discussed the results. Authors BO and TMHEH designed the study and wrote the protocol. Authors MSM and WJAO wrote the first draft of the manuscript. Authors IHA and RAM managed the literature searches and revised and edited the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Medicinal plants are identified and used throughout human history; it has a great economic value especially in drugs discovery. Date palm pollen (DPP) is used traditionally in Sudan for treating sub-fertile male patients. Male infertility is heterogeneous group of disorders, most of them are idiopathic. This study is aimed to investigate the role of pharmaceutical preparation of DPP in amelioration of male sub fertility with detection of any possible adverse effects on the major body system functions, through blood picture, liver enzymes and kidney function. This study is a single group pretest-posttest experimental prospective comparative self-control. Sub-fertile men with Idiopathic oligoasthenozoospermia or azoospermia were received 500 mg capsules of DPP twice daily for three months after conducting their safety profiles to detect any toxic effects on hematological, hepatological and nephrological functions, Blood samples were taken from the patients for serum level of FSH (for azoospermic patients), FSH and Testosterone (for

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oligoasthenozoospermic patients). Finally, Semen sample have been obtained for computerized assisted semen analysis (CASA) report I and II. DPP administration induced significant increase ($p \leq 0.001$) in testosterone level (in oligoasthenozoospermic patients) and FSH level (in azoospermic patients). DPP induced significant changes ($p \leq 0.001$) towards improvement in the total and progressive sperm motility percentages measured in oligoasthenozoospermic patients by CASA dynamic analysis report I and II. The toxicological studies for DPP approved their safety use in human.

Keywords: DPP; subfertile male; oligoasthenozoospermic; azoospermic patients.

1. INTRODUCTION

Infertility is defined as the failure to conceive after regular unprotected intercourse for two years in the absence of known reproductive pathology [1], reflected by defective spermatogenesis due to pituitary disorders, testicular cancer, germ cell aplasia, varicocele, and environmental factors, or due to defective sperm transport resulting from congenital abnormalities, immunological or neurological factors or could be caused by increased incidence of genetic disorders and apoptosis [2].

Oligoasthenozoospermia is characterized by abnormal semen quality with multiple sperm defects [3], while azoospermia is characterized by absent of sperm in the semen [4,5]. There are a number of drugs that can contribute to infertility, but Idiopathic Oligoasthenozoospermia is the commonest cause of male subfertility [6]. No data exist about infertility in Sudan, the big country in Africa [7]. There are few studies on infertility in sub-Saharan Africa, which tend to lack of research due to economic reasons and, possibly, the psychological denial of the problem [8,9].

Most empiric therapies in male sub fertility are hormonal and based upon the theory that increasing the amount of circulating testosterone and\or FSH will improve testicular function, specifically spermatogenesis [10,11]. Non endocrine treatments aimed to improve the quality of semen have been tested or are currently being evaluated; these treatments are varied and include those possible benefits and those without proved effect.Thyroxine, argenine [12], corticosteroids [13], antibiotics [14], methylyxanthines [15] and vitamins (as vitamin B_{12}, A,E and C) have all been studied [16-18]. Non pharmacological treatments (Elimination of toxic factors and thermal stress, surgical treatments, assisted reproductive technology) are also used [19].

*Phoenix dactylifera Linn (*family Arecaceae) is native to tropical Asia and Africa; it is good source of protein, amino acids, vitamins, dietary fiber, fatty acids, enzymes, hormones and minerals [20]. It also contains carbohydrates, alkaloids, steroids, flavonoids, vitamins and tannins [21,22]. Dates contain at least six vitamins including small amount of vitamin C, vitamin B1, B2, nicotinic acid (niacin) and vitamin A [23]. Applications and uses of DPP grains in traditional and herbal medicine have been recorded throughout history; it has been used in the treatment of sexual incapacity and weakness in the Arab world [24].

Sudan is one of the Arab countries that cultivate date palm trees and rank fifth in production among Arab date producing countries and third in Africa, date cultivation has been confined to Northern and River Nile States of Sudan [25].

2. MATERIALS AND METHODS

2.1 Plant Material, Patients and Study Design

Date palm pollen (DPP) was prepared as 500 mg capsules in the research and development department of Amipharma laboratories for drugs manufacturing in Khartoum North with quality assurance approval (Appendix 1). Freshly pods of DPP were obtained from Elgolid Locality near Dongola, Northern State, Sudan.

The study is single group pretest-posttest experimental prospective comparative self-control; it has been conducted in andrology clinic in the Reproductive Health Care Centre (RHCC), Khartoum-Sudan, from October 2010 to September 2013. Trial was done on subfertile men with age range from 20-60 years with Idiopathic oligoasthenozoospermia or azoospermia, after isolation of known causes and failure of the conventional reproductive therapeutic measures. Patients received 500 mg capsules of DPP twice daily for three months

after conducting their safety profiles to detect any toxic effects on heamatological, hepatological and nephrological functions (complete blood picture, Serum alkaline phosphatase enzyme and Serum creatinine).

Blood samples were taken from the patients for serum level of FSH (azoospermic patients), FSH and Testosterone (oligoasthenozoospermic patients). Finally, Semen sample have been obtained by masturbation into sterile containers after an abstinence period of 3-5 days to be sent for testing in the laboratory. Samples were allowed to liquefy at 37°C for 30 minutes and analysed within 1 h of collection for computerized assisted semen analysis (CASA). Analysis was performed according to the WHO strict criteria for motility detailed patterns (WHO 2010), the system follows WHO CASA for Sperm count, motility (total and progressive: report I) and dynamic parameters: report II. After three months the same samples were taken again for analysis and data collection [26].

2.2 Assessment of FSH and Testosterone Changes

The hormonal evaluation of FSH and Testosterone was done by radioimmunoassay using special Bio kit .FSH (N.R= 2.1 -18.6 µIU/ ml), while Testosterone (N.R =262-870 ng/dl).The procedure and normal range mentioned by the manufacturing company (CIS bio international) were followed.

2.3 Assessment of Immunologic, Hematologic and Biochemical Changes

Serum was analysed for the activities of thealkaline phosphatase (ALP) according to Chemiemethod [27]. Serum creatinine concentration was measured by a colorimetric method using commercial kit (Randox Laboratories Ltd., U.K.). Haemoglobin concentration, red blood Cells count, white blood cells count, and platelets count were estimated by (Diogon 60 Cell Computerized Haematology System), as tests for any haematological toxicity.

2.4 Statistical Analysis

Paired Student T-test through mean and standard deviation were used to demonstrate the significant differences between pre and post treatment values via determination of P value. Data were statistically analysed by the software computerized program SPSS Version 16. Data were presented in form of tables [28,29].

3. RESULTS

3.1 Effect of DPP Treatment on Different Parameters in Oligoasthenozoospermic Patients

FSH and Testosterone levels, CASA dynamic parameter analysis report I and II for per-post treated Oligoasthenozoospermic Patientsare shown in Tables (1, 2, 3 and 4).

3.2 Effect of DPP Treatment on Different Parameters in Azoospermic Patients

FSH level and CASA dynamic parameter analysis report I for pre-post treated Azoospermic Patients are shown in Tables 5 and 6.

3.3 Effect of DPP Treatment on Major Organ Body Systems in Both Oligoasthenozoospermic and Azoospermic Patients

The pre-post values of serum levels of: alkaline phosphate, creatinine, hemoglobin, RBC, WBC and platelets count for both Oligoasthenozoospermic and Azoospermic Patients are shown in Tables 7, 8, 9 and 10.

4. DISCUSSION

As shown in Table 1, The oligoasthenozoospermic patients treated with DPP had pre-treatment FSH mean level of 16.71 µIU/ ml which was increased to 16.90 µIU/ ml in the Post-treatment measurement [FSH normal range (N.R) :2.1-18.6 µIU/ml], this changes were with no statistically significant differences. The mean serum level of FSH among oligoasthenozoospermic patients was within normal range in the pre-treatment values and remains normal in the post treatment values Table 1. The result in the current study is contrary to other studies, which found that, DPP causes an increase in serum FSH level in patients with subfertility [30]. Pre-treatment measurement of FSH in azoospermic patients who received DPP was 28.76 µIU/ ml which changed to 28.93 µIU/ ml in the Post-treatment measurement, the change were with no statistically significant differences (Table 5). The

mean serum level of FSH among azoospermic patients was above normal range in the pre-treatment values and this high serum FSH level in men with azoospermia indicates damaged seminiferous tubule [31,32].

The pre-post treatment values of Testosterone [Testosterone N.R:262-870 ng/dl], in oligoasthenozoospermic patients show an increased level of Testosterone with a statistically significant difference ($p < 0.001$), where pre-treatment measurement of Testosterone 493.26 ng/dl has changed to 692.28 ng/dl in the Post-treatment measurement Table 1. A studies show that DPP increased the plasma level of testosterone significantly [30,33-35], and this comes in agreement with the findings of the current research.

DPP contains small amount of vitamin C [36-38], which maintain pituitary-gonadal axis and testicular structure within normal and alleviate detrimental effects of stress. However, vit.C supplementation maintains the soundness of pituitary-gonadal axis keeping the hormonal pattern within normal ranges and the testicular structure healthy, activating release of both Follicle Stimulating Hormone and Luteinizing Hormone from the anterior pituitary gland which could be a factor in the elevation of testosterone [39,40].

Sperm's count does not change significantly in oligoasthenozoospermic patients after DPP treatment, sperm's count percentage in pre-treatment measures has decreased from 12.37 to 7.59 million\ml in the post treatment measure Table 2 and this comes in agreement with other study that attributes this to the presence of phytoestrogen, as a steroidal component of DPP, which may have influenced sperm parameters negatively [41], because *Phoenix dactylifera*

Table 1. The pre-post treatment values of FSH and Test osteronein oligoasthenozoospermic patients after DPP treatment

Group	Measurement group	Mean ±SD	
		FSH µlU/ ml	Testosterone ng/dl
1	Pre-treatment measurement	16.71± 10.34	493.26±176.77
2	Post-treatment measurement	$^{N.S}$16.90± 11.03	***692.28±172.64

*n=21 ***= p < 0.001 (Significant differences) N.S = not significant*

Table 2. The pre-post treatment values of general semen parameters (sperm count, total motility percentage and progressive motility percentage) measured in oligoasthenozoospermic patients after DPP treatment

Group	Measurement group	Mean ±SD		
		conc. (Mill\ml)	Total motility (%)	Progressive motility(%)
1	Pre-treatment measurement	12.37± 18.36	25.08± 21.81	16.26± 18.36
2	Post-treatment measurement	7.59± 7.25$^{N.S}$	***48.48± 23.59	***38.38± 20.36

*n=21 ***= p < 0.001 (Significant differences) N.S = not significant*

Table 3. The pre-post treatment details and percentages of sperm motility measured (CASA dynamic parameter analysis report I) in oligoasthenozoospermic patients after DPP treatment

Group	Measurement group	Mean ±SD				
		PR(A)%Class A:Rapid progressive motility	PR(B)%Class B:Slow or sluggish	NP(C)% Class C:Non progressive motility	IM(D)% Class D: Immotile	PR(A+B)%
1	Pre-treatment measurement	9.15± 13.47	7.08± 12.37	8.84± 10.87	70.13± 26.49	16.26± 18.36
2	Post-treatment measurement	***25.54± 18.78	***12.84± 10.84	$^{N.S}$10.10± 13.17	***51.52± 23.61	***38.38±20.36

*n=21 ***= p < 0.001 (Significant differences) N.S = not significant*

Table 4. The pre-post values of sperm tracks and velocities (CASA dynamic parameter analysis report II) after DPP treatment measured in oligoasthenozoospermic patients

Group	Measurement group	Mean ±SD		
		VCL (µm/s) Track velocity	VSL(µm/s) Progressive velocity	VAP(µm/s) Path velocity
1	Pre-treatment measurement	29.59± 21.96	14.94±13.86	18.30± 15.11
2	Post-treatment measurement	46.92±19.47***	***29.09±14.30	***33.45± 15.01

*n=21 ***= p < 0.001 (Significant differences) N.S = not significant*

Table 5. The pre-post treatment values of FSH measured in azoospermic patients after DPP treatment

Group	Measurement group	Mean ±SD
		FSH µIU/ ml
1	Pre-treatment measurement	28.76± (11.90)
2	Post-treatment measurement	28.93± (10.45)N.S

n=19 N.S = not significant

(Date) showed an estrogen like activity with different degrees that may be attributed to the presence of sterols. Estrogen regulates the reabsorption of luminal fluid in the head of the epididymis, and presence of excess amount of such compound lead to disruption of this essential function causes the sperm to enter the epididymis in diluted form and reduces the sperm cell's count [42].

As shown in (Table 2), both total motility and progressive motility percentages of sperm motility measured in oligoasthenozoospermic patients stated by CASA dynamic parameter analysis report I after DPP Administration for three months has increased in a statistically significant value (p< 0.001), while (Table 3) shows an increased progressive motility PR type A percentage, progressive motility PR type B percentage and progressive motility combined PR (A+B) percentage with a significant decrease in the percentage of immotile spermatozoa percentage (IM% Class D) after DPP Administration for three months in a statistically significant value (p < 0.001).

In the current study, it is found that total motility percentage in pre-treatment measures has increased significantly from 25.08 to 48.48%, while it is a progressive motility percentage from 16.26 to 38.38% in the post treatment values (Table 2). According to the improvement in progressive motility percentages in the post treatment value (Table 3), the partitions of progressive motility stated by CASA dynamic parameter analysis report II, has increased significantly as follows: progressive motility PR type A percentage has increased from 9.15 to 25.54%, progressive motility PR type B percentage increased from 7.08 to 12.84% and progressive motility combined PR (A+B) percentage has increased from 16.26 to 38.38% after DPP Administration for three months in a statistically significant value (P < 0.001).

As is shown in (Table 3), non progressive motility (NP) type C percentage after DPP Administration for three months has increased (from 8.84% to 10.10%) but in non-significant value. This increment in the (NP) type C percentage strengthens the percentages of motility partitions are in a positive way towards improving the motility parameters [43-45].The immotile spermatozoa percentage (IM% Class D) has dropped significantly from 70.13% in the pretreatment measure to 51.52% in the post treatment (Table 3), which can be regarded as a good sign of general sperm motility improvement.

The improvement in sperm motility function in the current study can be attributed to DPP through the antiapoptic ability of DPP in the urogenital system especially in testis and prostate [46], DPP reduces the sperm DNA denaturation which triggers apoptosis, and therefore, seems to improve its DNA quality, improve sperm quality, and enhance fertility in the male adult rat. Therefore, it may be useful in solving male sub-fertility problems in humans. Several studies of apoptosis on male infertility establish an association between increased cell death and fertility problems [47]. Signs of apoptosis in mature spermatozoa are found primarily associated with the mitochondrial pathway:

activation of initiator caspase-9, disruption of mitochondrial membrane potential, activation of the main executioner caspase-3 and subsequent cellular collapse [48,49]. Increases of reactive oxygen species in tubules and in seminal plasma and of apoptosis are reputed to affect sperm motility [50,51]. Actually, an increase in apoptotic germ cells in testis of patients suffering severe oligozoospermia has been reported [47]. DPP treatment has ameliorated the histopathological and immunohistochemical changes in atypical prostatic hyperplasia (APH) induced in rats [52]. In these prostate diseases, there is an imbalance between prostate cell growth and apoptosis. Moreover, histopathological examination after DPP treatment revealed increased cellular proliferation and reduced apoptosis in ventral prostate. In the current study, motility sperm parameters have been improved probably through the antioxidant activity of DPP, because antioxidants play an important role in protecting semen from ROS and can improve basic sperm parameters in case of idiopathic oligoasthenozoospermia [53]. DPP contain Vitamins E and C [54,38]. Studies have shown that these vitamins possess antioxidant functions, and their inhibitory effects on ROS accumulation vary from one vitamin to the other [55]. As shown in the current study, one of the causes that lie beyond the improvement in sperm motility with DPP therapy could be the control of presence sperm DNA fragmentation as it contain vitamin E which is beneficial in subfertile men and improve sperm DNA fragmentation index and chromatin integrity by reducing sperm DNA damage [56-59]. Vitamins C and E contents of DPPcan significantly increase glutathione content, superoxide dismutase and catalase enzyme activity, while also decreasing lipid peroxidation products, total protein and nitric oxide contents and this can explain the improvement in sperm motility parameters after DPP administration in oligoasthenozoospermic patients [60-62]. The significant improvement in sperm motility parameters by DPP administration in the current research can be explained by the effect of DPP on sperm tail abnormalities, where DPP causes a decrease in sperms with tail abnormalitiesnumbers which interfere with sperm motility [63]. It is a fact that, the tail of the sperm (the flagellum) - confers motility upon the sperm by having the principal components of sperm motility controllers [64].

DPP treatment Effects on sperm tracks and velocities measured in oligoasthenozoospermic patients stated by CASA dynamic parameter analysis report II:VCL (Track velocity) in pre-treatment measures increased significantly from 29.59 to 46.92 μm/s, while (VSL) Progressive velocity increased significantly from 14.94 to 29.09 μm/s. Finally, average path velocity (VAP) increased significantly from 18.30 to 33.45 μm/s in the post treatment values (Table 4).

The three commonly reported CASA parameters include curvilinear velocity (VCL), average path velocity (VAP) and straight line velocity (VSL). These CASA motility parameters have been modelled and refined mathematically to describe the best motion parameters of each spermatozoon as it travels through a microscopic field [65]. The two important movements of spermatozoa, the vigour movement (circular or whiplash) that could be represented by the VCL and the progression movement can be represented by VSL. VAP represents the general trajectory of the spermatozoa [66,67]. A research found that there is significant positive correlations between sperm velocities and sperm mobility [68] and as the motility parameters improved in our current research DPP administration (Table 2 and 3) in oligoasthenozoospermic patients, so that we can conclude that sperm velocities improvement is a reflection for sperm motility improvement. Both the progressive linear motility (VSL) and the vigorous non-linear non- progressive motion (VCL) are of high importance [43], in our current study both increased and improved by the administration of DPP (Table 4) in oligoasthenozoospermic patients.

Table 6. The pre-post treatment values of general semen parameters (Sperm count, total motility percentage and progressive motility percentage) measured in azoospermic patients after DPP treatment

Group	Measurement group	Mean ±SD		
		Conc. (Mill\ml)	Total motility (%)	Progressive motility(%)
1	Pre-treatment measurement	0.000± 0.000	0.000± 0.0000	0.000± 0.000
2	Post-treatment measurement	.000±0.0000	0.000±0.000	0.000± 0.000

n=19

Table 7. The pre-post values of serum levels of alkaline phosphate and creatinine in oligo asthenozoospermic patients after DPP treatment

Group	Measurement group	Mean ±SD	
		Serum Conc. of Alk. Phoph. (IU\L)N.R(43-119)	Serum Conc. of Creatinine (mg\dl)N.R(0.6-1.4)
1	Pre-treatment measurement	80.67±21.19	.876± .2180
2	Post-treatment measurement	91.67±17.18 $^{N.S}$	0.852± .081$^{N.S}$

n=21 N.S = not significant

Table 8. The pre-post values of hemoglobin concentration, RBC, WBC and platelets count measured in oligoasthenozoospermic patients after DPP treatment

Group	Measurement group	Mean ±SD			
		Hemoglobin Conc.(gm\dl) N.R(14±1)	RBC Count ×10^6/Ul N.R (5Mill.-6 Mill.\dl)	WBC Count× 10^3/Ul N.R(4000-11000)	Platelets Count× 10^3/Ul)N.R(150000-400000)
1	Pre-treatment measurement	14.03± 1.187	5.37± .56	5.86±1.280	220.67± 38.351
2	Post-treatment measurement	14.31± 1.352$^{N.S}$	5.39± .521$^{N.S}$	6.22±1.342$^{N.S}$	231.57± 52.467$^{N.S}$

n=21 N.S = not significant

Table 9. The pre-post values of serum levels of alkaline phosphate and creatinine in azoospermic patients after DPP treatment

Group	Measurement group	Mean ±SD	
		Serum Conc. of Alk. Phoph. (IU\L)	Serum Conc. of Creatinine (mg\dl)
1	Pre-treatment measurement	91.89± 12.35	.826± .1790
2	Post-treatment measurement	91.89± 12.35 $^{N.S}$	0.805±(.112)$^{N.S}$

n=19 N.S = not significant

Table 10. The pre-post values of hemoglobin concentration, RBC, WBC and platelets count measured in azoospermic patients after DPP treatment

Group	Measurement group	Mean ±SD			
		Hemoglobin Conc.(gm\dl) N.R(14±1)	RBC Count×10^6/UIN.R (5Mill.-6 Mill.\dl)	WBC Count ×10^3/UIN.R(4000-11000)	Platelets Count×10^3/Ul) N.R(150000-400000)
1	Pre-treatment measurement	13.35± 0.947	5.38±0.400	5.73±1.9	224.58± 62.58
2	Post-treatment measurement	13.69± 1.054$^{N.S}$	5.12±.351$^{N.S}$	5.54±1.46$^{N.S}$	226.00± 63.06$^{N.S}$

n=19 N.S = not significant

Azoospermic patients were shown no changes in general semen parameters after the treatment (Tables 6).Normal serum level of ALP is [N.R:43-119 IU\L], in the current study, mean value of ALP in the oligoasthenozoospermic patients in the pre-treatment level changed from 80.67 to 91.67 IU\L in the post-treatment values as shown in Table 7, while in azoospermic patients mean value in the pretreatment level changed from 91.89 IU\L to 91.63 IU\L in the post treatment values as shown in Table 9, in both of the cases, DPP induced a non - significant differences in serum levels of ALP.

The above results in the current research show the safe use of DPP on liver function and this was in agreement with other studies that showed that, DPP significantly reduced CCl_4-induced elevation in plasma activities of aspartate aminotransferase (AST), alanine

aminotransferase (ALT), alkaline phosphatase (ALP) enzymes and billirubin concentration and ameliorated morphological and histological liver damage, suggesting that CCl_4-induced liver damage in rats can be reversed by treatment with DPP and moreover it can also be used prophylactically as a dynamic liver support [69]. DPP also contains safe and effective novel herbal composition for the prevention of liver disorders in acute and chronic alcoholics [70]. However, it is possible that the recorded DPP content of vitamin C may also play a role in hepatoprotection [71,72].

Oligoasthenozoospermic patients showed a normal mean serum creatinine level in the pre-treatment measures 0.876 mg\dl which was changed to 0.852 mg\dl in the post-treatment as shown in Table 7, while azoospermic patients was showed 0.826 mg\dl in pre-treatment and which was changed to 0.805 mg\dl in the post treatment values as shown in Table 9 (Creatinine NR: 0.6-1.4 mg\dl). In both of the cases, DPP induced non - significant differences in serum levels of creatinine. The above results in the current research show the safe use of DPP on renal function and this was in agreement with other studies that show DPP significantly reduces the increase in plasma creatinine and urea concentrations induced by gentamycin nephrotoxic doses and ameliorated the proximal tubular damage. Antioxidant components in the DPP (e.g. melatonin, vitamin E and ascorbic acid) were suggested to be the basis of the nephroprotection [69], while in another recent study they found that, DPP causes significant decrease in serum creatinine due to the ability of DPP to promote the filtration process and increase the efficacy of the two kidneys [73].

In the oligoasthenozoospermic patients the pre-treatmentlevelof hemoglobin concentration changed from 14.03 to 14.31 g\dl in the post-treatment values as shown in Table 8, while in azoospermic patients the mean value in the pretreatment level changed from 13.35 to 13.69 g\dl in the post treatment values as shown in Table 10 (Normal hemoglobin concentration is 14±1 g\dl).

In this study, all the patients have a normal hemoglobin concentration and DPP induced a non-significant changes in both oligoasthenozoo-spermic and azoospermic patients in hemoglobin concentration. This result is in contrary to the finding of a recent study which claims that, DPP causes significant increase in hemoglobin concentration [73].

The mean value of RBC countin the oligoasthenozoospermic patients included in the current study changed insignificantly, in the pre-treatmentlevel changed from 5.37 to 5.39×10^6 cell\dl in the post-treatment values as shown in Table 8, while in azoospermic patients the mean value in the pretreatment level changed from 5.38 to 5.12 $\times10^6$ cell\dl in the post treatment values as shown in table 10 (Normal RBCs count NR:5-6 $\times10^6$ cell\dl). In the current study, all the patients have a normal RBC count and DPP induced a non-significant change in both oligoasthenozoospermic and azoospermic patients in RBC count. This result is in agreement to the finding of a recent study which claims that, DPP causes no changes in RBCs count [73].

The mean value of WBC countin the oligoasthenozoospermic patients in the pretreatmentlevel changed from 5.86 to 6.22 $\times10^3$ cell\dl in the post-treatment values as shown in Table 8, while in azoospermic patients the mean value in the pretreatment level changed from 5.73 to 5.54 $\times10^3$ cell\dl in the post treatment values as shown in Table 10 (WBC count NR: 4-11$\times10^3$ cell\dl). In the current study, all the patients have a normal WBC count and DPP induced a non-significant changes in both oligoasthenozoospermic and azoospermic patients in WBCs count, this result in agreement to the finding of a recent study which claim that, DPP causes no changes in WBCs count.

The mean level of platelet count in the oligoasthenozoospermic patients in the pre-treatmentlevel was changed from 220.67×10^3 cell\dl to 231.57×10^3 cell\dl in the post-treatment as shown in Table 8, while in azoospermic patients mean value in the pretreatment level changed from 224.58 to 226.00 $\times10^3$ cell\dl in the post treatment values as shown in Table 10 (platelet count NR: 150-400$\times10^3$cell\dl), in the current study, mean value of platelet countin both of the cases, DPP induce a non - significant differences in platelet count.

5. CONCLUSION

Date palm pollen grains administration induced significant increase in testosterone level (in oligoasthenozoospermic patients) and FSH level (in azoospermic patients). It also induced significant changes towards improvement in the total and progressive sperm motility percentages measured in oligoasthenozoospermic patients by CASA dynamic analysis report I and II. The

toxicological studies for DPP approved their safety use in human.

CONSENT

Written agreement and consent had been taken from the participated patients.

ETHICAL APPROVAL

The experiment was done in accordance with the Ethical Clearance, approved by the Committee for Ethics at National medicines and poisons board, Republic of Sudan.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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APPENDIX

Quality assurance approval (Appendix 1)

AMIPHARMA Laboratories Ltd.
Quality Control Department
Sheet for Uniformity of Weight of Capsules.

Product Name:_____

Batch No.:_____ **Q.C. No.:**_____

Manufacturing Date:_____ **Expiry Date:**_____

Pharmacopoeia Requirements:
Not more than two of the individual weights deviate from the average weight by more than 1
by more than 20% when the average of the capsule weight is less than 300mg.
Not more than two of the individual weights deviate from the average weight by more than 7
by more than 15% when the average of the capsule weight is 300mg or more.

Capsule No.	Filled Capsule Weight g	Empty Capsule Weight g	Quantity of powder (x) g	$(x-\bar{x})$	% Deviat average weig
1	0.6534	0.1091	0.5443	0.0054	1.00
2	0.6461	0.1085	0.5376	-0.0013	-0.2
3	0.6613	0.1118	0.5495	0.0106	1.96
4	0.6479	0.1058	0.5421	0.0032	0.59
5	0.6470	0.1073	0.5397	0.0008	0.14
6	0.6415	0.1051	0.5364	-0.0025	-0.4
7	0.6512	0.1122	0.5390	0.0001	0.01
8	0.6358	0.1089	0.5269	-0.0120	-2.2
9	0.6464	0.1038	0.5426	0.0037	0.68
10	0.6410	0.1029	0.5381	-0.0008	-0.1
11	0.6546	0.1106	0.5440	0.0051	0.94
12	0.6432	0.1094	0.5338	-0.0051	-0.9
13	0.6470	0.1086	0.5384	-0.0005	-0.1
14	0.6506	0.1099	0.5407	0.0018	0.33
15	0.6481	0.1154	0.5327	-0.0062	-1.1
16	0.6494	0.1078	0.5416	0.0027	0.50
17	0.6510	0.1082	0.5428	0.0039	0.72
18	0.6582	0.1113	0.5469	0.0080	1.48
19	0.6425	0.1073	0.5352	-0.0037	-0.6
20	0.6273	0.1011	0.5262	-0.0127	-2.3
SUM	12.9435	2.1650			

Total Weight of filled powder (Σ x) = 10.7785 g
Mean filled weight ($\bar{x}$) = 0.5389 g
Standard Deviation (SD) = 0.006
Relative Standard Deviation (RSD) = 1.105

Applies if average weight is less than 300mg:
Number of individual weights deviating by : more than 10% Not Applicable more than
Applies if average weight is 300mg or more:
Number of individual weights deviating by : more than 7.5% 0 more than

Test Results Passes / Fails Pass

Date: 13/1/21 Analysts M. Elmlo Q.C. Manager_____

QC calculatio

001:N + 0.6534 g
002:N + 0.6273 g
003:N + 0.6461 g
004:N + 0.6613 g
005:N + 0.6479 g
006:N + 0.6470 g
007:N + 0.6415 g
008:N + 0.6512 g
009:N + 0.6358 g
010:N + 0.6464 g
011:N + 0.6410 g
012:N + 0.6546 g
013:N + 0.6432 g
014:N + 0.6470 g
015:N + 0.6506 g
016:N + 0.6481 g
017:N + 0.6494 g
018:N + 0.6510 g
019:N + 0.6582 g
020:N + 0.6425 g

n 20
$\bar{x}$ 0.64718 g
s 0.00760 g
srel 1.17 %
Σx 12.9435 g
min 0.6273 g
max 0.6613 g
Diff 0.0340 g

001:N + 0.1091 g
002:N + 0.1011 g
003:N + 0.1085 g
004:N + 0.1118 g
005:N + 0.1058 g
006:N + 0.1073 g
007:N + 0.1051 g
008:N + 0.1122 g
009:N + 0.1089 g
010:N + 0.1038 g
011:N + 0.1029 g
012:N + 0.1106 g
013:N + 0.1094 g
014:N + 0.1086 g
015:N + 0.1099 g
016:N + 0.1154 g
017:N + 0.1078 g
018:N + 0.1082 g
019:N + 0.1113 g
020:N + 0.1073 g

n 20
$\bar{x}$ 0.10825 g
s 0.00338 g
srel 3.12 %
Σx 2.1650 g
min 0.1011 g
max 0.1154 g
Diff 0.0143 g

Agreement and consent from the participated patients (Appendix 2)

In the name of God, The merciful, the compassionate

Declaration of Agreement
For Participation in the Research

I, ... Declare of my own accord, willingly and voluntarily, and in my legally accepted state, to participate in the research

.....
.....
.....

After it was explained to me; which will be carried out by the researcher ..... Address of the participant/evidence of identity.....
Telephone:.....
...

And, in my full mental faculties I do agree to:

1. My entry into the abovementioned research.
2. And that I shall take the medicine ..... Dosage ..... number of times ..... And I do agree to take a number of ..... sample(s) of my blood or other requested samples in the appropriate times to the research, and to inform the medical team with any complications or side-effects that might occur to me, and I have no objection for the carryout of the necessary medical and diagnostical examination.

And, may God bear witness of what I say.

Signature of volunteer/or his Guardian:.....
Date:.....

Ethical Clearance (Appendix 3)

Republic of Sudan
National Medicines & Poisons Board
Secretariat General

المجلس القومي للأدوية والسموم
الأمانة العامة

Date:/04/2017

Ethical Clearance Certificate

This is to certify that the proposal entitled (Usage of some natural& pharmaceutical preparations in management of male sub fertility: aclinical trial.) Introduced by Dr. Hosam Aldeen Salim Mohammed Saeed, Faculty of pharmacy- University of Khartoum. The research is to be conducted at Reproductive Health Care Center in Khartoum state. Has been approved by clinical trials and drug research on human and animal committee, National medicines& poisons board, to be carried out in the Sudan.

Yours sincerel.....

Excitation: The principal investigator is requested to submit a copy of the final report to clinical trials and drug research on human and animal committee.

Dr/Sakina Babikir Elamin

F /Secretary General of National Medicines& Poisons Board

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