

# **Tribulus terrestris L. extract improves spermatozoa motility and increases the efficiency of acrosome reaction in subjects diagnosed with oligoastheno-teratozoospermia**

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## **SUMMARY**

Here we investigated the effects of *Tribulus terrestris* L. on sperm morphology and acrosome reaction in subjects with oligoastheno-teratozoospermia. A double-blind placebo-controlled trial was performed with 30 primary and secondary infertile men. Half received oral *Tribulus* (Libilov, 500mg dose) three times per day for 60 days, and the rest were given sugar pills. The ejaculate volume, sperm concentration, morphology, acrosome reactions, and other parameters were evaluated before and after treatment.

A significant increase in the percentage of slow or sluggish progressive motile sperm in the treated group was observed when compared to the control group. The percentage of the sperm with normal acrosome reaction was also increased, whereas the percentage of immotile sperm decreased significantly.

The frequency of sexual intercourse was also increased significantly in the *Tribulus* group. No side effect was observed. We concluded that *Tribulus terrestris* L., given at 500mg three times a day for 60 days proved to be effective in restoring some of the sperm functions, such as motility and acrosome reactions.

## **ABSTRACT**

The increasingly popular use of the herbal extract of *Tribulus terrestris* L, reflective of the increased use of traditional medications in the recent years, has resulted in scientific research efforts to identify the active components. The identification of protodioscin, a furostanol saponin as the active ingredient followed the findings of its beneficial effects on the improvement of spermatogenesis, and on the increase in sperm mobility and viability. In this study, we investigated the effects of *Tribulus terrestris* L. treatment on sperm morphology and acrosome reactions in subjects with oligoastheno-teratozoospermia. Here, a double-blind and placebo controlled clinical trial was performed at Academic Hospital Dr. Soetomo in Surabaya, Indonesia. This study consisted of 30 primary and secondary infertile men (age 25-40 years), half of which received 500 mg tablets of *Tribulus terrestris* L. extracts three times a day for sixty days. The other half were treated with placebo. Ejaculate volume, sperm concentration, fitness and morphology, acrosome reaction efficiency, as well as other parameters were evaluated before and after treatment.

We found a significant increase in the motility of sperm in the treated group ( $1.666 \pm 12.344$ ) as compared to the control placebo group ( $-9.000 \pm 10.889$ ,  $p < 0.05$ ). We also found the proportion of sperms with normal acrosome reactions was increased in the treated group ( $6.633 \pm 6.282$ ) as compared to the control group ( $-0.333 \pm 3.406$ ,  $p < 0.05$ ). Furthermore, the proportion of immotile sperm in the treated group ( $-10.333 \pm 16.198$ ) was significantly decreased as compared to the control group ( $12.666 \pm 21.865$ ,  $p < 0.05$ ). The ejaculate volume, sperm concentration, morphology and motility of rapid, progressive and non-progressive sperms did not differ significantly. The frequency of sexual intercourse of these subjects with their partners was also increased significantly in the *Tribulus terrestris* L. treated group ( $p < 0.05$ ). No harmful or other side effects were observed.

In conclusion, *Tribulus terrestris* L., when given at a dose of a 500 mg tablet three times daily for sixty days proved to be effective in restoring some sperm functions in subjects diagnosed with oligoastheno-teratozoospermia, by improving sperm motility and acrosome reaction.

In the United States, approximately 10 - 20% of married couples are infertile. This number increased from 15% in the past decade alone. In a 1992 clinical study conducted at Academic Hospital Dr. Soetomo in Surabaya, Indonesia, involving 598 infertile couples showed that 522 (87.2%) of these couples suffered from primary infertility, whereas the remaining 76 (12.8%) couples suffered from secondary infertility.

Many factors are involved in the process of conception and the ensuing pregnancy, including psychological, anatomical and immunological factors, which affect both men and women. According to

Jaffe and Jewelewicz (1991) as well as Bayer and co-workers (1993), 40-50% of infertility cases are the results of male infertility. This is most commonly caused by varicoceles, infections of the testes, immunological disorders, and sperm abnormalities. Proper spermatogenesis, including good sperm morphology and motility, as well as normal sexual functions or libido are important for successful conception.

The importance of sperm viability on conception has given rise to the importance of laboratory tests in the diagnosis of male infertility, as only these tests can give objective and accurate analyses of spermatogenesis as well as anatomical inspections. Most common of these tests are the analyses of sperm morphology, motility and concentration. In addition to their relative ease, these tests are also practical, inexpensive and readily repeatable. Another useful analysis of sperm viability is the efficiency of acrosome reaction, which is extremely important for fertilization in vivo and in vitro.

The use of hormone-based medications to treat infertility and other sexual dysfunctions have not been very successful. In addition to their transitory benefits, these treatments are often ineffective, and in some cases even result in decreased hypothalamic hormone productions or functions. Therefore, the development of a non-hormonal treatment, in the form of *Tribulus terrestris* L. extract that is effective in treating infertility, but without the harmful or dangerous side-effects, contributes greatly to its medical popularity.

*Tribulus terrestris* L. extract, a non-hormonal plant-derived extract, has been successfully used in Europe and Asia to treat sexual dysfunctions. Active components of this extract were determined to be a furostanol saponin, named protodioscin. Oral administration of this extract to laboratory animals resulted in the stimulation of spermatogenesis and the proliferation of the spermatogonia, which involved cell divisions of the spermatocytes and spermatids. These increases in cellular divisions were not accompanied by the increase of the diameter of the seminiferous tubules. In addition to the increased mitotic activity of the spermatozoa and the increased number of Sertoli cells, sperm viability and survival were also significantly increased. Detailed clinical trials found that protodioscin was not toxic and had no undesirable side-effects.

In Indonesia, a multi-center clinical trial of the effect of *Tribulus terrestris* L. on oligozoospermia was conducted in Jakarta, Surabaya and Denpasar. Spermatozoa analyses of the group treated with *Tribulus terrestris* L. showed increased sperm concentration and motility as compared to the placebo control group. In addition to these analyses, subjects involved in the trials were also inspected for changes in their liver and kidney functions, as well as for changes to the sex hormone levels in the bloodstream. In these aspects, no significant differences were observed between the treated and the control groups.

This clinical trial is designed to answer the following questions:

1. Does *Tribulus terrestris* L. treatment improve the morphology and motility of spermatozoa of subjects suffering from oligoasthenoteratozoospermia?
2. Does *Tribulus terrestris* L. treatment improve the acrosome reaction of spermatozoa in these subjects?

The general goal of this study is to evaluate the use of *Tribulus terrestris* L. as a medical treatment of oligoasthenoteratozoospermia, with specific goals of the following:

1. To determine the morphology and mobility of spermatozoa of oligoasthenoteratozoospermia subjects before and after treatment with *Tribulus terrestris* L.
2. To determine the proportion of spermatozoa with normal acrosome reaction in these subjects before and after *Tribulus* treatment.

The result of this study is summarized as follows:

1. Confirms significant improvements in the motility of the spermatozoa as well as improved acrosome reaction in these subjects after *Tribulus* treatment. No substantial improvement in general spermatozoa morphology.
2. Affirms the effectiveness of *Tribulus terrestris* L. as treatment for oligoasthenoteratozoospermia.

3. Provides suggestion as to the mechanism of Tribulus-induced improvement in spermatozoa functions.

## MATERIALS AND METHODS

This nine-month clinical study was performed at Academic Hospital Dr. Soetomo in Surabaya, Indonesia. Laboratory analyses were performed on spermatozoa collected from infertile male subjects suffering from primary and secondary infertility that conformed to the following criteria:

- Married for more than two years and of the ages from 25 to 40 years
- Did not use contraceptives
- Had semen with normal pH
- Had semen with normal viscosity
- Had spermatozoa concentration of 5 - 20 million/ml
- Had spermatozoa mobility Category A < 25% or Category A+B < 50%
- Had normal spermatozoa morphology < 30%
- Otherwise physically and mentally healthy

This study involved thirty male subjects diagnosed with oligoasthenoteratozoospermia, composed of 28 subjects suffering from primary infertility and 2 subjects suffering from secondary infertility. Division of these subjects to the treated and untreated control groups were performed randomly and in a double-blind manner. Materials were supplied by PT TeguhSindo LestariTama in form of purified Tribulus terrestris L. extract 250 mg tablets (under name of Libilov or Libilon) and placebo tablets. Patients from both groups were administered two tablets three times daily for sixty days.

Spermatozoa evaluation involved ejaculate volume, sperm concentration, mobility and morphology using strict-criteria. Here, saffranin/cresyl violet staining assay was performed to analyze spermatozoa morphologies. This assay used glass slides, methanol, 0.1% saffranin solution, buffer I and II, 0.25% cresyl violet stain solution, aquadest and light microscope. Acrosome reaction was performed by a triple-stain assay. Here, trypan blue stained the spermatozoa blue, Bismarck brown stained the post-acrosome region light brown and rose Bengal stained the acrosome light red. This assay was performed in TMPA culture medium, 2% trypan blue, bovine serum albumin, 3% glutaraldehyde in 0.1 M cacodylate buffer pH 7.4, 0.8% Bismarck brown Y pH 1.8, 0.8% Rose Bengal in 0.1M Tris-Cl buffer pH 5.3. Efficiency of acrosome reactions were scored by the following criteria:

- Samples containing post-acrosome regions that were stained dark blue and acrosome regions that were stained light red were scored as dead spermatozoa with fully or partially inactive acrosome.
- Samples containing post-acrosome regions that were stained dark blue and acrosome regions that were stained blue or white were scored as dead spermatozoa with missing acrosome, or had degenerated acrosome.
- Samples containing post-acrosome regions that were stained light brown and acrosome regions that were stained light red were scored as live spermatozoa without active acrosome.
- Samples containing post-acrosome regions that were stained light brown and acrosome region that were stained white were scored as live spermatozoa with active or normal acrosome.

Samples were performed in a completely randomized block. Patients were also asked to report any other effects felt during the course of the treatment.

## RESULTS

### ***Effects of Tribulus terrestris L. Treatment on Ejaculate Volume, Sperm Concentration and Motility as Compared to Placebo***

Based on statistical analyses, the effects of Tribulus treatment on ejaculate volume, sperm concentration and motility were not significant as compared to placebo ( $p > 0.05$ ). There were no considerable differences in the rapid progressive and stationary motilities between the treated and the control groups ( $p > 0.05$ ), whereas there was a statistically significant difference in the slow progressive and non-progressive spermatozoa ( $p < 0.05$ ).

Parameter	Tribulus	Placebo	p	Note
Ejaculate volume (ml)	0.373 ± 1.102	0.240 ± 0.783	>0.05	NS
Concentration (million/ml)	-1.104 ± 8.157	-0.346 ± 7.183	>0.05	NS
Motility (%)				
fast and linear	1.666 ± 5.563	1.000 ± 5.732	>0.05	NS
slow and nonlinear	1.666 ± 12.344	-9.000 ± 10.889	<0.05	S
stationary motility	6.000 ± 11.052	-4.666 ± 20.482	>0.05	NS
no movement	-10.333 ± 16.198	12.666 ± 21.865	<0.05	S

**Table I.** Differences in ejaculate volume, spermatozoa concentration and motility after treatment with Tribulus terrestris L extract (Libilov, 250mg) or placebo. NS: not statistically significant; S: statistically significant.

#### ***Effects of Tribulus terrestris L. Treatment on Spermatozoa Morphology as Compared to Placebo***

Based on statistical analyses, the effect of Tribulus treatment on the spermatozoa morphology, including acrosome, mid-piece and tail shapes and sizes were mostly not significant as compared to placebo.

Sperm Morphology Parameter	Tribulus	Placebo	p	Note
<b>Normal (%)</b>	2.866 ± 5.222	2.533 ± 6.058	>0.05	NS
<b>Head (%)</b>				
oval/normal acrosome	-0.200 ± 10.818	2.466 ± 11.057	>0.05	NS
oval/no acrosome	0.933 ± 5.338	-0.733 ± 4.636	>0.05	NS
oval/small acrosome	-1.733 ± 2.815	-0.066 ± 3.105	>0.05	NS
lepto	3.200 ± 5.321	-2.400 ± 6.045	<0.05	S
elongated	0.466 ± 7.549	2.466 ± 8.202	>0.05	NS
roughened	-0.333 ± 1.589	0.266 ± 2.738	>0.05	NS
pin	0.600 ± 2.640	-0.733 ± 2.120	>0.05	NS
double	-0.133 ± 0.352	0.066 ± 0.961	>0.05	NS
megalosperm	0.200 ± 1.699	0.733 ± 2.815	>0.05	NS
microsperm	-0.666 ± 4.655	-2.200 ± 5.281	>0.05	NS
amorphous	-2.333 ± 6.161	0.133 ± 8.043	>0.05	NS
<b>Mid-piece</b>				
normal	2.600 ± 3.135	1.466 ± 3.021	>0.05	NS
abnormal	-2.266 ± 2.738	-5.333 ± 2.475	>0.05	NS
cytoplasmic droplet	-0.333 ± 1.175	-0.933 ± 1.486	>0.05	NS
<b>Tail</b>				
normal	2.133 ± 1.995	1.666 ± 2.193	>0.05	NS
abnormal	-2.133 ± 1.995	-1.666 ± 2.193	>0.05	NS

**Table II.** Differences in spermatozoa morphology after treatment with Tribulus (Libilov, 250mg) or placebo. NS: statistically not significant; S: statistically significant.

#### ***Effects of Tribulus terrestris L. Treatment on Acrosome Reaction as Compared to Placebo***

Based on statistical analyses, the effects of Tribulus treatment on dead spermatozoa and spermatozoa with degenerated acrosomes were not significant as compared to placebo control. There was, however, a considerable difference in the proportion of live spermatozoa with normal acrosome in the treated group, as compared to the control.

Acrosome Parameters (%)	Tribulus	Placebo	p	Note
Dead sperm with whole acrosome	-4.600 ± 22.814	2.400 ± 17.837	>0.05	NS
Dead sperm with deformed acrosome	5.416 ± 10.637	4.833 ± 10.276	>0.05	NS
Live sperm with whole acrosome	-7.450 ± 26.986	-6.900 ± 22.914	>0.05	NS

Live sperm with normal acrosome reaction	6.633 ± 6.282	-0.333 ± 3.406	<0.05	S
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**Table III.** Differences in acrosome reaction after treatment with Tribulus (Libilov, 250mg) or placebo. NS: statistically not significant; S: statistically significant.

#### ***Effects of Tribulus terrestris L. Treatment on the Frequency of Sexual Intercourse***

Ten out of the fifteen people who received Tribulus terrestris L. extract experienced substantially increased frequency of sexual intercourse after treatment. The remaining five people experienced the same frequency of intercourse before and after treatment. No subjects experienced less frequency of intercourse after treatment. This effect was statistically significant ( $p < 0.05$ ).

Final Frequency (per week)	Starting Frequency (per week)					Total
	1	2	3	4	5	
2	-	1	-	-	-	1
3	1	2	3	-	-	6
4	-	1	5	1	-	7
7	-	-	-	-	1	1
Total	1	4	8	1	1	15

**Table IV.** Effect of Tribulus (Libilov, 250mg) treatment on the frequency of sexual intercourse. Starting frequency is the frequency of sexual intercourse before treatment; Final frequency is the frequency of sexual intercourse after treatment.

#### ***Effects of Placebo on the Frequency of Sexual Intercourse***

From the fifteen subjects in the control group, six subjects experienced more frequent sexual intercourse after treatment with placebo, one patient reported less frequent intercourse, and eight subjects reported the same frequencies of sexual intercourse. This effect was not statistically significant ( $p > 0.05$ ).

Final Frequency (per week)	Starting Frequency (per week)			Total
	2	3	4	
2	1	-	-	1
3	3	7	1	11
4	-	3	-	3
Total	4	10	1	15

**Table IV.** Effect of placebo (sugar pill) treatment on the frequency of sexual intercourse. Starting frequency is the frequency of sexual intercourse before treatment; Final frequency is the frequency of sexual intercourse after treatment.

## **DISCUSSION**

#### ***Effects of Tribulus terrestris L. Treatment on Ejaculate Volume, Sperm Concentration and Motility as Compared to Placebo***

There were no significant effects of Tribulus treatment on ejaculate volume, sperm concentration and mobility, specifically progressive or stationary mobility, as compared to placebo ( $p > 0.05$ ). In comparison, statistically significant improvements on slow and non-progressive sperm mobility were observed in the treated group ( $1.67 \pm 12.34$  % after completion of Tribulus treatment) compared to the placebo control group ( $-9.00 \pm 10.89$  % after treatment). Significant improvement on the decreased proportion of immotile sperm was also observed. Here, the Tribulus treated group reported a decreased proportion of immotile sperm of  $-10.33 \pm 16.20$  %, whereas the placebo control reported an increased proportion of  $12.67 \pm 21.9$ % ( $p < 0.05$ ). Based on these results, we concluded that Tribulus terrestris L.

treatment substantially improved the slow non-progressive and the proportion of immotile spermatozoa as compared to placebo treatment.

It was previously reported that Tribulus supplement given to normal men improved their luteinizing hormone (LH) and testosterone levels, without changing their follicle stimulating hormone (FSH). This increase in LH and testosterone levels resulted in the improvements of the male reproductive functions, specifically in the improvements of spermatozoa mobility and viability. Furthermore, Dysson and Orgebin-Crist reported that sperm mobility and fertilization capability also depended on the androgen level. It was reported that a significant fraction of spermatozoa that originated from the caput epididymis were either immotile or showed uncoordinated tail movements. The motility of these spermatozoa improved during its transit through the epididymis duct, as a result of increased exposure to testosterone in the testes secretion that entered the duct through the ductus efferent. These conclusions were further supported by our data, which showed that the male reproductive capacity in subjects with oligoasthenozoospermia was further improved by the increased slow non-progressive spermatozoa motility and decreased proportion of defectively immotile sperms.

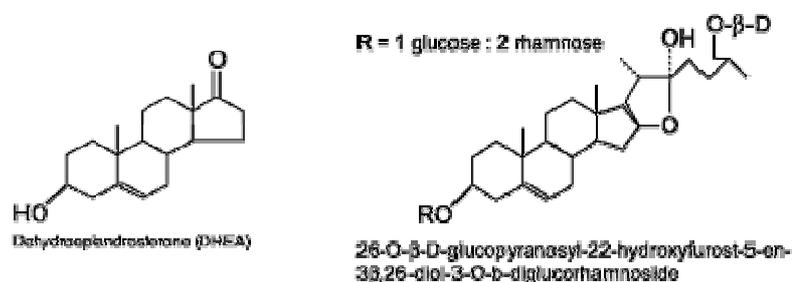
#### ***Effects of Tribulus terrestris L. Treatment on Spermatozoa Morphology as Compared to Placebo***

Tribulus treatment resulted in the increased proportion of lepto shaped head region ( $3.20 \pm 5.31$  %) as compared to placebo control ( $-2.4 \pm 6.05$  %) ( $p < 0.05$ ). Previous research on Tribulus administration in laboratory animal models suggested increased proliferation of germinativum, spermatogonia and Sertoli cells, without the proliferation of the Leydig cells. Sertoli cells were involved in the male reproductive function by producing Androgen Binding Protein (ABP) which was necessary for spermatogenesis in the seminiferous tubules. Furthermore, Sertoli cells also provided nutrients to spermatogonium cells, the cellular precursor to mature spermatozoa.

The slight increase in the proportion of spermatozoa with lepto head in the Tribulus treated group did not constitute an improvement in sperm morphology, as this did not lead to increased fertility capability of the cells. Therefore, we concluded that Tribulus terrestris L. treatment did not improve general spermatozoa morphology.

#### ***Effects of Tribulus terrestris L. Treatment on Acrosome Reaction as Compared to Placebo***

A significant improvement was observed in the proportion of live spermatozoa with normal acrosome reaction in the Tribulus treated group ( $6.63 \pm 6.28$  %), as compared to placebo control ( $-0.33 \pm 3.41$  %) ( $p < 0.05$ ). Presumably, this was the largest contribution of Tribulus treatment on the improvement of spermatozoa function. The mechanism of this action was presumed to be due to the active ingredient in Tribulus terrestris L. extract, which was identified to be protodioscin. The structure of protodioscin was very similar to dihydroepiandrosterone (DHEA), which was intimately involved in the male reproductive function through the production of testosterone. Here, in Leydig cells, cholesterol was chemically modified to DHEA through intermediates of pregnenolone and 17- $\alpha$ -hydroxypregnenolone. DHEA was further modified to create androstenedione, then to testosterone and dihydrotestosterone in the epididymis tissue. DHEA also played a crucial role in the maturation of spermatozoa in the epididymis: increased DHEA levels increased sperm functions, as observed in our study by the increase in the efficiency of spermatozoa acrosome reaction.



**Figure 1.** Chemical structures of DHEA and protodioscin (the active ingredient in Tribulus terrestris L. extract)

#### ***Effects of Tribulus terrestris L. Treatment on the Frequency of Sexual Intercourse***

The beneficial effects of *Tribulus terrestris* L. extract on male sexual behavior and sex drive were observed by calculating the frequency of sexual intercourse pre- and post-treatment. From the fifteen subjects in the treated group, ten reported an increase, whereas five reported no change in the frequency of sexual intercourse ( $p < 0.05$ ). In contrast, from the fifteen subjects in the placebo control group, six reported an increase, one reported a decrease, and eight reported no change in the frequency of intercourse ( $p > 0.05$ ). Therefore, our data agreed with previously reported observations that *Tribulus terrestris* L. treatment was significantly beneficial to the male sexual drive or libido.

## CONCLUSION

1. *Tribulus terrestris* L. treatment did not cause any improvement in spermatozoa morphology in subjects with oligoasthenoteratozoospermia.
2. *Tribulus* treatment increased spermatozoa acrosome reaction in these subjects.
3. *Tribulus* treatment increased the motility of slow and non-progressive spermatozoa, and decreased the proportion of immotile spermatozoa.
4. *Tribulus* treatment increased the frequency of sexual coitus.
5. No side effects were observed during nine months of treatment.

## RECOMMENDATION

1. Due to the cost of treatment, laboratory analyses of spermatozoa morphology is recommended. Patients with sperm of normal oval head and intact acrosome will receive the maximal benefit of *Tribulus terrestris* L. treatment.
2. Future research on the effect of DHEA on spermatozoa functions is warranted.

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