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Dietary Supplementation Of Black Seed Oil And Sunflower Oil Along With Vit-E Improves Sperm Characteristics And Reproductive Hormones In Male Albino Rats.

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ABSTRACT: This study aimed to investigate the potential effects of dietary supplementation with black seed oil (BSO) and sunflower oil (SFO), in conjunction with vitamin E (Vit-E), on sperm characteristics and reproductive hormone levels in male albino rats. 24 Male albino rats were randomly divided into four groups: control (received standard diet), BSO group (received black seed oil supplementation), SFO group (received sunflower oil supplementation), and BSO+SFO+Vit-E group (received a combination of black seed oil, sunflower oil, and vitamin E supplementation). The supplementation period lasted for six weeks.

The results revealed a significant improvement in sperm characteristics, including sperm count, motility, and morphology, in the BSO, SFO, and BSO+SFO+Vit-E groups compared to the control group. Additionally, reproductive hormone levels, such as testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH), showed significant enhancements in the supplemented groups. Dietary supplementation with black seed oil, sunflower oil, and vitamin E demonstrated positive effects on sperm characteristics and reproductive hormones in male albino rats. These findings suggest the potential use of these dietary supplements as a supportive approach to enhance male reproductive health. Further research is warranted to elucidate the underlying mechanisms and to assess the translational relevance of these findings to human reproductive health.

Key words: sunflower oil, black seed oil, reproductive hormones, sperm quality, vitamin E.

INTRODUCTION

Human reproductive health is a continuing focus of scientific research due to the complex interactions between many elements. The effect of food and nutrition on male reproductive capacity is one such aspect[1]. The possible impact of certain nutrients and organic compounds on male fertility has attracted increasing attention in recent years. Among these, black seed oil, sunflower oil and Vit-E have become notable contenders. Alternative medicine has become increasingly popular during the past 10 years[2]. The ineffectiveness of conventional medications, the perception that alternative therapies have fewer side effects, patients' desire to maintain control over their treatment, and a preference for a more "natural" approach are some of the factors behind this trend[3].

Male fertility can be measured by the quantity of the sperm cells generated in the seminiferous tubules of the testis, which is a well-planned and organised process known as sperm production[4]. The testis functions as an endocrine organ, synthesising the necessary amounts of testosterone for healthy sperm generation and the development of physical characteristics[5]. In order for spermatogenesis to occur, Leydig, Sertoli, peritubular myoid cells, and germ cells of the testis must communicate with one another in a paracrine and autocrine manner. Because each of these cell types has a specific purpose, the process is well-coordinated at various phases[6].

Sperm formation is mostly dependent on spermatogonial stem cells, which develop into the process known as spermatocytes, and spermatids. Testosterone, LH, and FSH are only a few of the hormonal systems that work together to control this process. The growth and development of sperm cells through meiosis, spermiogenesis, and the discharge of mature spermatids are only a few of the crucial roles that testosterone plays[7]. It supports the development of spermatocytes, the transformation of spermatids from round to elongated, the gonads' creation, the differentiation of the testicles, and the survival of the sperm cells and spermatids[8]. Mentioned that sunflower oil (SFO; a rich source of PUFAs) and vitamin C improved the reproductive performance in the rams[9]. The other study showed that inclusion of SFO (omega-6) and fish oil (omega-3) improved cryopreservation of rooster semen and positively affected semen parameters post freezing-thawing[10]. The increased sperm count and the decreased DNA fragmentation were observed by supplementing SFO in the human trial[11]. Showed that SFO improved semen parameters in adult male Sprague-Dawley rats. Vitamin E is applied to the poultry diet to prevent from the lipid peroxidation caused by PUFAs[12]. It has been shown that dietary intake of SFO and vitE improved reproductive function of male rats[13].

In addition, DPP consumption in rats showed a significant increase in their serum testosterone levels[14]. Also, increased testosterone levels on the one hand, increased sperm concentration; motility and increased natural morphology on the other hand have been observed in studies with palm pollen[15]. In addition, date pollen contains gonadal stimulating estrogen compounds, and sterols that can contribute to the treatment of male infertility[16]. Linolenic acid and oleic acid in black seed increase sperm parameters such as sperm count and motility,but reduce abnormal sperm[17]. Phenolic and alkaloids black seed compounds stimulate the secretion of testosterone and follicle stimulating hormone (FSH) [18]. Thymoquinone in black seed improves semen quality[19]. Although small amounts of reactive oxygen species (ROS) are essential for sperm activity

such as capacity, acrosomal reaction, and oocyte adhesion[20], high concentrations damage sperm cells, reducing sperm motility and ultimately inhibiting sperm fertility[21]. Extensive research has been conducted on the antioxidant properties of plants as a substance that reduces oxidative stress[22]. The present clinical trial study, investigated the combined effect of sun flower and black seed pollen on male sex hormones and prolactin.

NEED OF THE STUDY.

Infertility is a growing concern worldwide, prompting the need for innovative research aimed at understanding and improving reproductive health. This proposed study aims to investigate the effects of dietary supplementation with black seed oil and sunflower oil, combined with Vitamin E, on sperm characteristics and reproductive hormones in male albino rats. Several factors contribute to male infertility, and exploring natural interventions like these oils along with Vitamin E presents a promising avenue for potential therapeutic strategies.

Limited Research on Natural Supplements:

There is a scarcity of comprehensive studies examining the impact of dietary supplementation, especially involving black seed oil, sunflower oil, and Vitamin E, on male reproductive health.

Existing research has primarily focused on pharmaceutical interventions, and there is a gap in understanding the potential benefits of natural compounds in improving sperm quality and reproductive hormone levels.

Potential Synergistic Effects:

Black seed oil, sunflower oil, and Vitamin E are rich in bioactive compounds known for their antioxidant properties and potential effects on hormonal balance.

Investigating the combined impact of these substances may reveal synergistic effects, enhancing their individual benefits and providing a holistic approach to addressing male infertility.

Albino Rats as a Model:

Albino Wistar rats are widely used in reproductive research due to their physiological and genetic similarities to humans.

Studying the effects of dietary supplementation in this rat model can provide valuable insights into the translatability of findings to human reproductive health.

Sperm Characteristics and Fertility Parameters:

Sperm quality is a critical determinant of male fertility. Assessing parameters such as sperm count, motility, and morphology in response to dietary supplementation can provide a comprehensive understanding of the potential benefits.

Additionally, evaluating fertility parameters, including mating behavior and reproductive success, will enhance the clinical relevance of the study.

Hormonal Regulation and Endocrine Disruption:

Hormonal imbalances can significantly impact male reproductive function. Investigating the effects of black seed oil, sunflower oil, and Vitamin E on reproductive hormones (e.g., testosterone, luteinizing hormone) can elucidate potential endocrine regulatory mechanisms.

Understanding how these dietary supplements influence hormonal pathways is crucial for designing targeted interventions for individuals with hormonal disruptions contributing to infertility.

MATERIALS AND METHODS:

Experimental Animals: Male albino rats were used in this study. A total of 24 rats, aged 20 week were obtained from Animal house, NIMS University, Rajasthan NH-11C, Delhi – Jaipur Highway, Jaipur, and Rajasthan 303121. The animals were housed in standard laboratory conditions with controlled temperature (22-24°C), humidity (40-60%), and a 12-hour light-dark cycle.

Dietary Supplements: Black seed oil (BSO), sunflower oil (SFO) and Vitamin E (Vit-E) were obtained from RATTAN ORGANIC FOODS PVT LTD, Amla Farm Village Badnagar, Near Paota, Tehisil Kothputli, Jaipur, Rajasthan, India - 303106. The dietary supplements were administered as follows: The dietary supplements were administered as follows:

BSO Group: 2% Black seed oil along with 300 mg vit E was orally administered.

SFO Group: 2% Sunflower oil along with 300 mg vit E was orally administered.

BSO+SFO+Vit-E Group: A combination of 2% black seed oil, 2% sunflower oil, and 300mg vitamin E was orally administered.

Experimental Design: Rats were randomly divided into four groups:

Control Group: Received a standard laboratory diet.

BSO Group: Received 2% black seed oil supplementation.

SFO Group: Received 2% sunflower oil supplementation.

BSO+SFO+Vit-E Group: Received a combination of black seed oil, sunflower oil, and 300mg vitamin E supplementation.

Supplementation Period: The supplementation period lasted for six weeks, during which the dietary supplements were administered daily.

Sperm Collection and Analysis: At the end of the supplementation period, rats were sacrificed, and sperm samples were collected from the cauda epididymis. Sperm count, motility, and morphology were assessed using standard procedures.

Reproductive Hormone Assays: Blood samples were collected to analyze reproductive hormone levels. Enzyme-linked immunosorbent assay (ELISA) kits were used to measure testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) levels.

Statistical Analysis: Data were analyzed using SPSS, and results were expressed as mean ± standard deviation. One-way analysis of variance (ANOVA) followed by post-hoc tests were employed to compare the different groups. Statistical significance was set at $p < 0.05$.

RESULTS: The results of the study on the calculation of testosterone levels, sperm count, motility, and contraction in albino wistar rats treated with the combination of group 2) 2% Black seed oil (BSO)+ vit.E 300mg/kg, group 3) 2% sunflower oil (SFO)+ vit.E 300mg/kg and group 4) 2% BSO+2% SFO along with Vitamin E (Vit-E) 300mg mg/kg of the body weight, showed a difference, the group that showed the highest increase in testosterone levels, , sperm count, motility, and volume was in the group treated group 4) 2% BSO+2% SFO along with Vitamin E (Vit-E) 300mg mg/kg, while the control group was the group with the lowest testosterone level then the 2% sunflower oil (SFO)+ vit.E 300mg/kg and 2) 2% Black seed oil (BSO)+ vit.E 300mg/kg.

DISCUSSION: The combination of Black seed oil (BSO), sunflower oil (SFO) and Vitamin E (Vit-E) appears to positively influence the estrous sperm count, Sperm motility in albino Wistar rats. This combination may hold potential for enhancing reproductive potential in this species.

CONCLUSIONS: In this study investigating the impact of dietary supplementation with black seed oil (BSO) and sunflower oil (SFO), along with vitamin E (Vit-E), on sperm characteristics and reproductive hormones in male albino rats, compelling evidence emerged in support of the potential benefits of the combined intervention.

Representing the semen parameter-

(Semen Volume, Concentration, Viability, Morphology, Membrane Integrity).

Parameters	Days	1 (CTR group)	2 (BSO group)	3 (SFO group)	4(BSO+SFO+vit E group)	SEM	*P*-value
Volume (mL)							
	0	0.68	0.9	0.5	0.43	0.07	0.28
	20	0.66	0.8	0.46	0.48	0.07	0.53
	40	0.54	0.88	0.52	0.44	0.06	0.27
	60	0.67	0.8	0.52	0.4	0.06	0.29
Concentration							
	0	3.63	3.46	3.49	3.75	0.06	0.17
	20	3.89 ^{b}	3.88 ^{b}	3.97 ^{b}	3.70 ^{b}	0.07	0.01
	40	3.73 ^{b}	3.74 ^{b}	3.89 ^{b}	3.55 ^{b}	0.1	0.0007
	60	3.07 ^{c}	3.55 ^{b}	3.22 ^{bc}	2.59 ^{d}	0.16	<0.0001
Viability (%)							
	0	82.00 ^{a}	81.75 ^{a}	81.00 ^{a}	84.50 ^{a}	0.64	0.002
	20	82.50 ^{a}	73.25 ^{c}	79.00 ^{ab}	76.25 ^{bc}	0.86	0.02
	40	84.20 ^{a}	81.67 ^{ab}	82.80 ^{a}	78.00 ^{b}	0.63	0.06
	60	73.20 ^{b}	68.00 ^{c}	67.50 ^{c}	69.33 ^{bc}	1.25	<0.0001
Morphology (%)							
	0	13	16	15.33	16.25	0.51	0.25
	20	14.75	14.25	13.75	14.25	0.41	0.66
	40	16.0 ^{ab}	18.0 ^{a}	13.0 ^{bc}	15.33 ^{ab}	0.54	0.002
	60	23.6	18.8	21.8	19.33	0.85	0.31
Membrane Integrity (%)							

0	**82.60 [a]**	**80.75 [ab]**	**81.67 [a]**	**79.25 [ab]**	**0.77**	**0.03**
20	**79.00 [ab]**	**73.00 [d]**	**81.25 [a]**	**77.00 [bc]**	**0.69**	**0.0001**
40	**77.40 [c]**	**74.33 [c]**	**73.40 [cd]**	**68.67 [d]**	**1.6**	**<0.0001**
60	**70.60 [b]**	**74.60 [b]**	**71.25 [b]**	**70.67 [b]**	**1.09**	**<0.0001**

Data are presented as means SEM (n = 4 Rats per each treatment).
Abbreviations: BSO, Black seed oil; SFO, sunflower oil; vitE, vitamin E.
a-b Different letters within the same row show significant differences among the groups .
(P < 0.05). 1Rats received 4 types of treatments: 1) basal diet as control group (Control), 2) basal diet supplemented with 2% Black seed oil (BSO group), 3) basal diet supplemented with 2% sunflower oil as n-6 source of fatty acids (SFO group), 4) basal diet supplemented with 300 mg/kg vitamin E (BSO + SFO+vit E group).

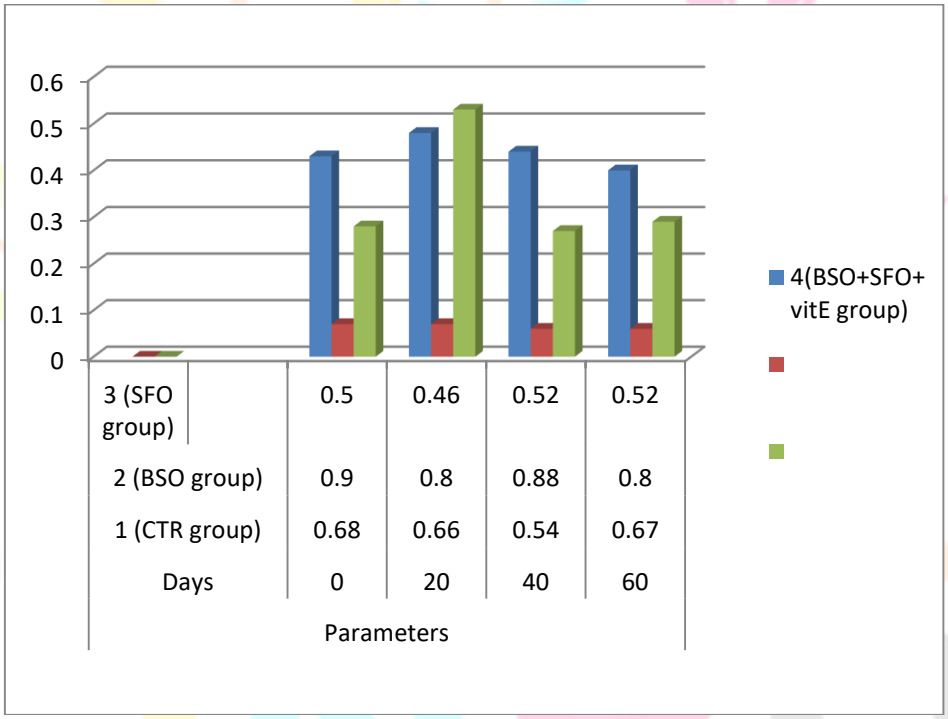

Figure1. Means of semen volume in aged Rats fed diets during 60 days' experiment.Rats received 4 types of treatments: 1) basal diet as control group (Control), 2) basal diet supplemented with 2% Blck seed oil (BSO group), 3) basal diet supplemented with 2% sunflower oil (SFOgroup), 4) basal diet supplemented with 300 mg/kg vitamin E (CTRL + BSO+ SFO+ vitE group), (n = 4 Rats per each treatment). [a-d] Different letters within each day show significant differences among the groups ($P \leq 0.05$). Abbreviations: BSO, pumpkin seed oil; SFO, sunflower oil; vitE, vitamin E.

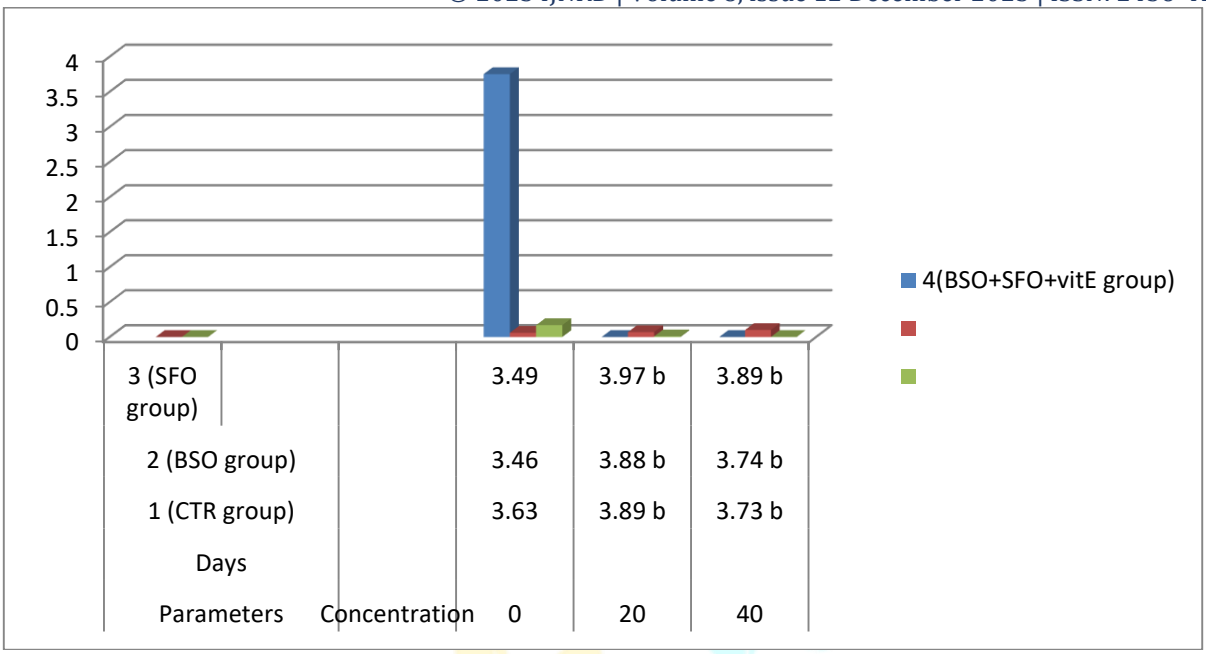

FIGURE 2. MEANS OF SPERM CONCENTRATIONIN AGED RATS FED DIETS DURING 60 DAYS' EXPERIMENT.RATS RECEIVED 4 TYPES OF TREATMENTS: 1) BASAL DIET AS CONTROL GROUP (CONTROL), 2) BASAL DIET SUPPLEMENTED WITH 2% BLCK SEED OIL (BSO GROUP), 3) BASAL DIET SUPPLEMENTED WITH 2% SUNflOWER OIL (SFOGROUP), 4) BASAL DIET SUPPLEMENTED WITH 300 MG/KG VITAMIN E (CTRL + BSO+ SFO+ VITE GROUP), (N = 4 RATS PER EACH TREATMENT). A-D DIFFERENT LETTERS WITHIN EACH DAY SHOW SIGNIfiCANT DIFFERENCES AMONG THE GROUPS (P ⩽ 0.05). ABBREVIATIONS: BSO, PUMPKIN SEED OIL; SFO, SUNflOWER OIL; VITE, VITAMIN E.

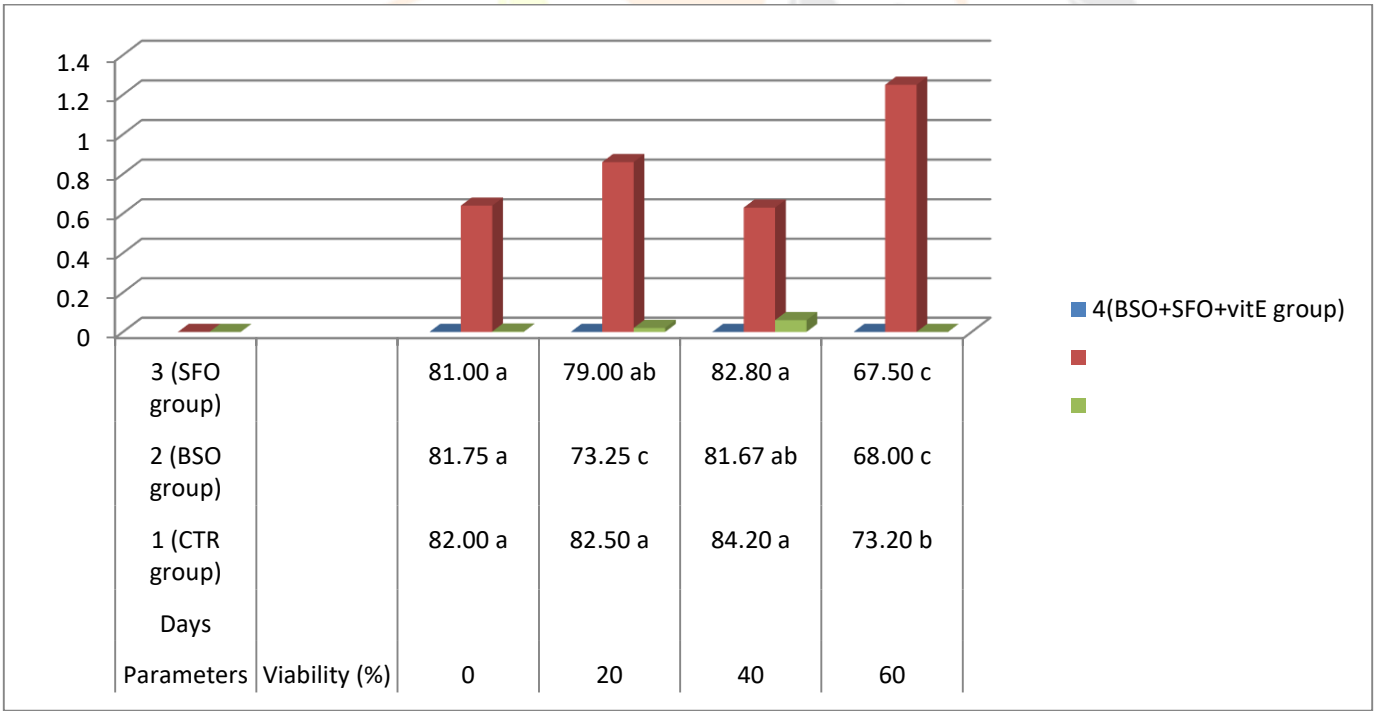

FIGURE 3. MEANS OF SPERM VIABILITY AGED RATS FED DIETS DURING 60 DAYS' EXPERIMENT.RATS RECEIVED 4 TYPES OF TREATMENTS: 1) BASAL DIET AS CONTROL GROUP (CONTROL), 2) BASAL DIET SUPPLEMENTED WITH 2% BLCK SEED OIL (BSO GROUP), 3) BASAL DIET SUPPLEMENTED WITH 2% SUNflOWER OIL (SFOGROUP), 4) BASAL DIET SUPPLEMENTED WITH 300 MG/KG VITAMIN E (CTRL + BSO+ SFO+ VITE GROUP), (N = 4 RATS PER EACH TREATMENT). A-D DIFFERENT LETTERS WITHIN EACH DAY SHOW SIGNIfiCANT DIFFERENCES AMONG

THE GROUPS ($P \leq 0.05$). ABBREVIATIONS: BSO, PUMPKIN SEED OIL; SFO, SUNflOWER OIL; VITE, VITAMIN E.

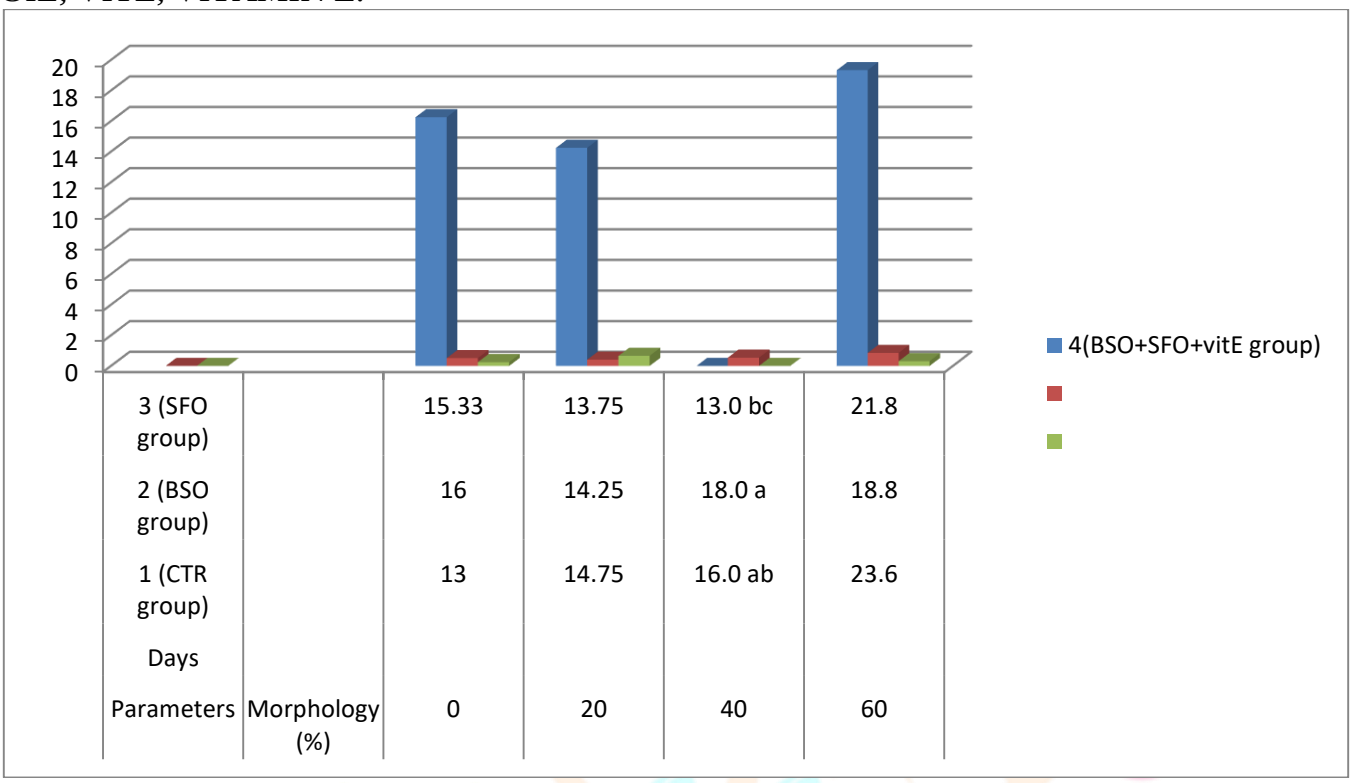

FIGURE 4. MEANS OF SPERM MORPHOLOGY AGED RATS FED DIETS DURING 60 DAYS' EXPERIMENT.RATS RECEIVED 4 TYPES OF TREATMENTS: 1) BASAL DIET AS CONTROL GROUP (CONTROL), 2) BASAL DIET SUPPLEMENTED WITH 2% BLCK SEED OIL (BSO GROUP), 3) BASAL DIET SUPPLEMENTED WITH 2% SUNflOWER OIL (SFOGROUP), 4) BASAL DIET SUPPLEMENTED WITH 300 MG/KG VITAMIN E (CTRL + BSO+ SFO+ VITE GROUP), (N = 4 RATS PER EACH TREATMENT). A-D DIFFERENT LETTERS WITHIN EACH DAY SHOW SIGNIfiCANT DIFFERENCES AMONG THE GROUPS ($P \leq 0.05$). ABBREVIATIONS: BSO, PUMPKIN SEED OIL; SFO, SUNflOWER OIL; VITE, VITAMIN E.

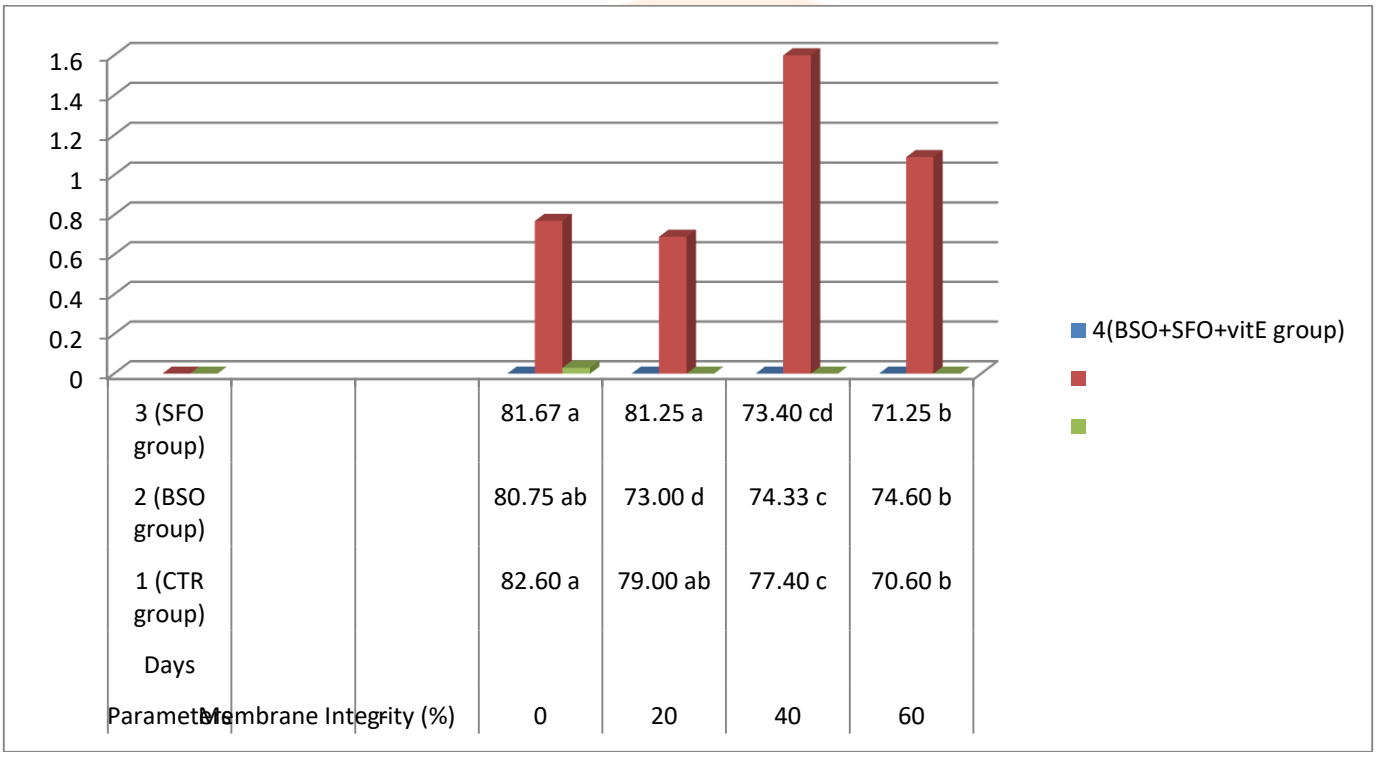

FIGURE 5. MEANS OF SPERM INTEGRITY AGED RATS FED DIETS DURING 60 DAYS' EXPERIMENT.RATS RECEIVED 4 TYPES OF TREATMENTS: 1) BASAL DIET AS CONTROL GROUP (CONTROL), 2) BASAL DIET SUPPLEMENTED WITH 2% BLCK SEED OIL (BSO GROUP), 3) BASAL DIET SUPPLEMENTED WITH 2% SUNFLOWER OIL (SFOGROUP), 4) BASAL DIET SUPPLEMENTED WITH 300 MG/KG VITAMIN E (CTRL + BSO+ SFO+ VITE GROUP), (N = 4 RATS PER EACH TREATMENT). $^{A-D}$ DIFFERENT LETTERS WITHIN EACH DAY SHOW SIGNIFICANT DIFFERENCES AMONG THE GROUPS ($P \leq 0.05$). ABBREVIATIONS: BSO,PUMPKIN SEED OIL; SFO, SUNFLOWER OIL; VITE, VITAMIN E.

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