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EVALUATION OF THE EFFECTS OF A POLYHERBAL EXTRACT ON SPERM CONCENTRATION, MOTILITY, AND ABNORMALITIES IN THE EPIDIDYMIS OF MALE ALBINO RATS

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ABSTRACT

The present study aims to evaluate the effects of a polyherbal extract on sperm concentration, motility, and abnormalities in male albino rats. Polyherbal formulations, combining multiple medicinal plants, are traditionally used to address various health issues, including reproductive health. The study utilized a blend of clove buds (*Syzygium aromaticum*) and Triphala powder (a combination of *Phyllanthus emblica, Terminalia belerica*, and *Terminalia chebula*), administered at different dose levels of 200 mg/kg, 400 mg/kg, and 600 mg/kg body weight, to assess its impact on male reproductive parameters. Male albino rats were divided into four groups: a control group with normal saline, and remaining three treatment groups with the polyherbal extract at different doses for 30 days. Sperm concentration, motility, and abnormalities were evaluated in the epididymis after treatment. The polyherbal extract significantly reduced sperm concentration in a dose-dependent manner, with the highest dose (600 mg/kg) showing the most substantial decrease. Sperm motility also decreased with increasing doses, with the highest reduction observed at 600 mg/kg. Additionally, the extract led to increased sperm abnormalities, particularly in the head and tail regions, with the most pronounced effects at the highest dose. These findings indicate that the polyherbal extract exerts a dose-dependent impact on sperm parameters, leading to significant reductions in sperm concentration and motility, and an increase in sperm abnormalities. While these effects highlight the potent action of the extract, they also suggest potential adverse impacts on male reproductive health. Further studies are needed to explore the underlying mechanisms and safety of such polyherbal formulations in reproductive health.

Keywords: Polyherbal Extract, Sperm Concentration, Sperm Motility, Sperm Abnormalities, Albino Rats

1. INTRODUCTION

The use of herbal medicine has been a cornerstone of traditional healthcare systems across the world, particularly in the treatment of various reproductive health issues. Polyherbal formulations, which combine multiple medicinal plants, are believed to enhance therapeutic efficacy and minimize side effects compared to single-plant remedies. Recent research has highlighted the potential of such extracts in managing male reproductive health, specifically in improving sperm quality and addressing infertility concerns. For instance, studies have indicated that polyherbal extracts can influence oxidative stress and hormonal balance, both critical factors in male fertility (Goribone et al., 2022; Sharma et al., 2023; Prasad et al., 1993). The complexity of these herbal formulations often leads to a synergistic effect of multiple bioactive compounds, which may contribute to their efficacy in enhancing reproductive parameters.

Sperm concentration, motility, and morphology are crucial indicators of male fertility, and their impairment can significantly affect reproductive success.

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Sperm concentration reflects the number of sperm per milliliter of semen, while motility pertains to the sperm's ability to move on effectively, and morphology assesses the sperm's structural integrity. Recent studies have shown that various herbal extracts can positively or negatively influence these parameters. For example, while some herbs have been reported to improve sperm quality through antioxidant properties and hormonal modulation (Choudhury et al., 2021; Raji et al., 1997), others may cause adverse effects on sperm parameters (Lee et al., 2022). Understanding the effects of polyherbal extracts on these critical aspects of sperm health is essential for developing effective treatments for male infertility.

The present study aims to evaluate the effects of a specific polyherbal extract on sperm concentration, motility, and abnormalities in the epididymis of male albino rats. By administering varying doses of the extract and analyzing its impact on these key reproductive parameters, this research seeks to provide insights into its potential therapeutic or adverse effects. The study will contribute to the understanding of how polyherbal formulations affect on male reproductive health and offer evidence-based guidance for their use in fertility treatments. The primary objective of this research is to assess how different doses of the polyherbal extract influence sperm concentration, motility, and abnormalities, thereby evaluating its overall efficacy and safety in male reproductive health management.

2. MATERIALS AND METHODS

2.1 Preparation of Polyherbal Extract:

The preparation of the polyherbal extract began with sourcing clove buds (Syzygium aromaticum) and fruits of *Phyllanthus emblica, Terminalia belerica*, and *Terminalia chebula* from local vendors in Warangal. Clove buds were used in their dried form, while the fruits of Phyllanthus emblica, Terminalia belerica, and Terminalia chebula were shade-dried for a week before grinding. All plant materials were finely ground into powders to enhance extraction efficiency. A blend was created by combining 250 gr of clove bud powder with 250 gr of Triphala powder, which itself comprised 100 gr each of *Phyllanthus emblica* and *Terminalia belerica*, and 50 gr of *Terminalia chebula*. This homogeneous powder mixture was subjected to cold maceration in 1000 ml of methanol for seven days. After filtering and evaporating the methanol using a rotary evaporator, a semisolid polyherbal extract was obtained for further use.

2.2 Animals:

The study employed Male Albino Wistar Rats weighing between 200 to 250 grams, sourced from the National Institute of Nutrition (NIN) in Hyderabad. These rats were maintained under controlled conditions to ensure consistency in results. They were kept in an environment with a temperature of 22 ± 2 °C, relative humidity of 50 ± 5%, and a 12-hour light-dark cycle. Before starting the experiment, the rats underwent a seven-day acclimatization in sanitized cages with sterile paddy husk bedding and were fed with standard diet with water provided ad libitum.

2.3 Acute Toxicity Study of the Extract:

An acute toxicity study was performed following OECD guidelines to assess the safety of the polyherbal extract (OECD, 2022). Swiss albino mice were used, and the extract was administered at a maximum dose of 2000 mg/kg body weight to evaluate potential adverse effects. The mice were monitored for 14 days for changes in behavior, activity, and health. This extended observation period aimed to detect any immediate or delayed toxic effects, establishing the safety profile of the extract.

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2.4 Experimental:

In this study, the animals were allocated into four groups, each group comprising six rats. This setup was designed to evaluate the effects of the polyherbal extract at different dose levels. Group I acted as the control and induced oral administration of normal saline for 30 days, establishing a baseline for comparison. Groups II, III, and IV were given the different dose levels of polyherbal extract i.e., 200 mg/kg, 400 mg/kg, and 600 mg/kg body weight, respectively, administered orally daily for 30 days. This consistent dosing schedule allowed for the assessment of the extract's effects across different concentrations and ensured reliable measurement of dose-dependent responses. Throughout the study, the animals were monitored for any adverse effects or behavioral changes, ensuring the validity of the results.

2.5 Sperm Count:

The sperm count procedure aimed to quantify the sperm concentration in semen samples to assess male fertility. Prior to collection, rats were acclimatized to their environment for a week under controlled conditions, including a consistent light/dark cycle and stable temperature (22-24°C). After anesthetizing the rats with ketamine and xylazine, a scrotal incision exposed the testes and epididymides, which were then placed in phosphate-buffered saline (PBS) to preserve viability. The epididymides were minced and gently squeezed in PBS to release sperm, with the mixture incubated at 37°C for 10-15 minutes to aid dispersion. A small volume of the sperm suspension was transferred to a hemocytometer for counting. Using a microscope with a 10x or 20x objective lens, sperm in several grid squares were counted to calculate concentration using the formula:

Sperm Concentration (million/mL) =
Number of sperm counted × Dilution factor × Volume of the chamber (mL) / Number of squares counted.

2.6 Sperm Motility and Abnormality:

The goal of evaluating sperm motility and abnormalities was to assess the functional performance and structural integrity of sperm, crucial for determining male fertility potential. To analyze motility, the semen sample was warmed to body temperature, and a slide was prepared with a drop of the sample covered by a coverslip. The slide was then examined under a microscope to measure the percentage of motile sperm and classify their movement patterns, such as progressive or non-progressive. For examining abnormalities, a smear of the semen was prepared, air-dried, and stained if needed. The stained smear was observed under a microscope to evaluate sperm morphology, recording the percentage of sperm with normal versus abnormal shapes. These assessments provided a comprehensive evaluation of sperm quality by identifying potential issues impacting fertility.

2.7 Statistical Analysis:

Sperm count was reported in terms of sperm per milliliter of suspension. The sperm counts among different experimental groups were compared to assess the impact of the treatments or conditions being studied. Statistical methods, such as t-tests or ANOVA, were used to analyze the data and determine if there were significant differences between groups.

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3. RESULTS

3.1 Yield of Polyherbal Extract:

The polyherbal extract was prepared by mixing 250 grams of clove buds powder with 250 grams of Triphala powder, which included 100 grams each of *Phyllanthus emblica* and *Terminalia belerica*, and 50 grams of *Terminalia chebula*. This 500-gram mixture was extracted using 1000 ml of methanol through cold maceration at room temperature for seven days. After maceration, the mixture was filtered through Whatman filter paper #41, and the filtrate was evaporated using a rotary evaporator to obtain a semisolid extract. The final yield of the extract was 62.5 grams, representing a 12.5% yield relative to the initial mixture weight, indicating the efficiency of the extraction process and the solubility of the active compounds.

3.2 Acute Toxicity Studies:

The acute toxicity studies of the polyherbal extract revealed that there was no toxic effects at an oral dosage of up to 2000 mg/kg body weight. This lack of adverse effects suggests that the extract is relatively safe at this high dosage. Based on these results, three lower doses were selected for further fertility testing: 200 mg/kg, 400 mg/kg, and 600 mg/kg body weight. These dosage levels were chosen to assess the extract's therapeutic potential and ensure safety across various concentrations.

3.3 Effect of Polyherbal Extract on Sperm Concentration :

The polyherbal extract markedly decreased sperm concentration in the caput and cauda regions of the epididymis in adult male albino rats, as shown in Figure-1 and Table-1. In Group II, with a 200 mg/kg.bw dose, sperm concentrations fell to 212.30 ± 4.44 million/mL in the caput and 348.70 ± 7.50 million/mL in the cauda, with significant reductions ($p < 0.001$ and $p < 0.05$, respectively).

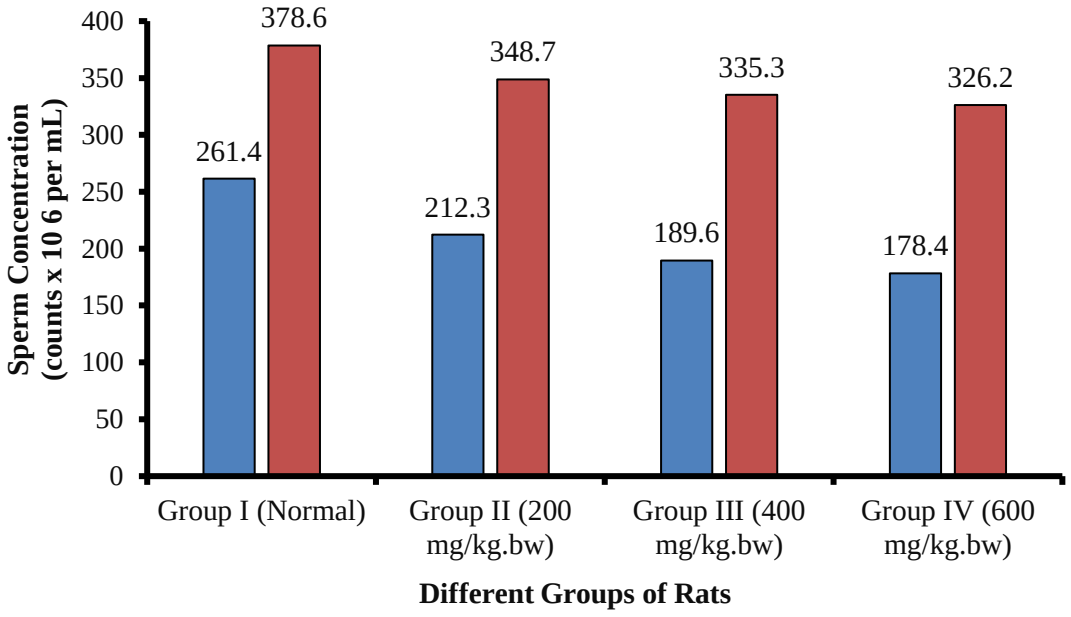

Figure-1. Effect of polyherbal extract on sperm concentration in experimental treated rats (The values are expressed in mean)

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Group III (400 mg/kg.bw) saw further decreases to 189.60 ± 2.44 million/mL ($p < 0.001$) in the caput and 335.30 ± 5.52 million/mL ($p < 0.01$) in the cauda. Group IV (600 mg/kg.bw) had the lowest concentrations, 178.40 ± 7.50 million/mL ($p < 0.001$) and 326.20 ± 3.43 million/mL ($p < 0.001$), respectively. These results reveal a dose-dependent reduction was observed in sperm concentration, with higher doses of the polyherbal extract leading to more significant decreases compared to the control group.

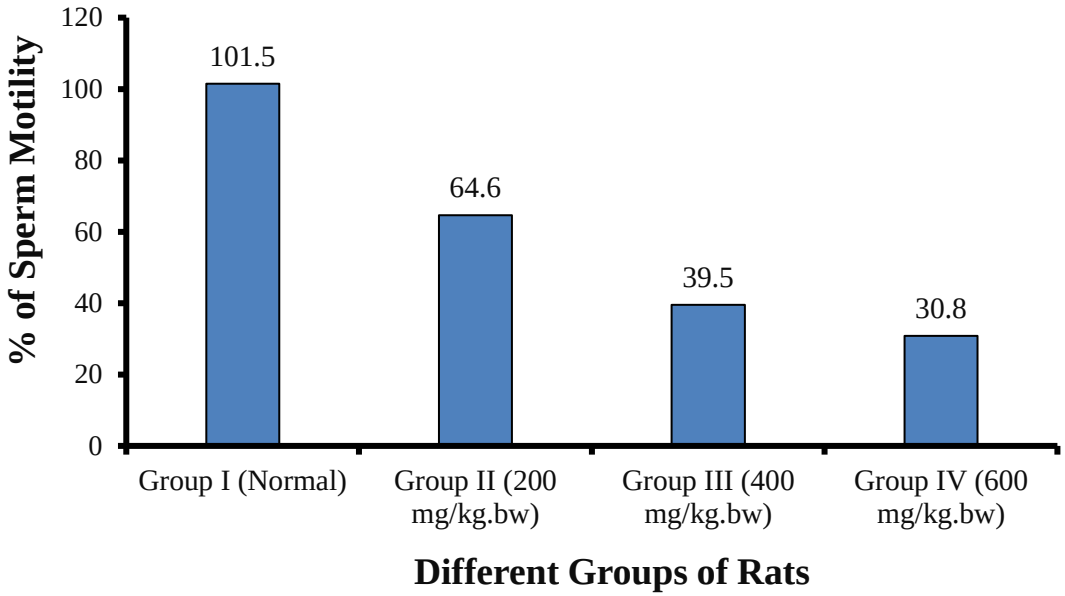

Figure-2. Effect of polyherbal extract on percentage of sperm motility in experimental treated rats (The values are expressed in mean percentage)

3.4 Effect on Sperm Motility:

The polyherbal extract notably impacted sperm motility in the cauda region of the epididymis, as shown in Figure-2. In Group II, treated with 200 mg/kg.bw, sperm motility dropped to 64.60 ± 2.50%, a significant reduction ($p < 0.01$). Group III, receiving 400 mg/kg.bw, experienced a further decline to 39.50 ± 3.67% ($p < 0.001$). Group IV, with the highest dose of 600 mg/kg.bw, showed the most substantial decrease to 30.80 ± 5.20% ($p < 0.001$). Compared to the control group with a sperm motility of 101.50 ± 4.90% in the cauda, the polyherbal extract significantly reduced motility across all treated groups. These findings indicate a dose-dependent impairment in sperm motility, with higher doses causing greater reductions compared to the control group, which had a motility of 101.50 ± 4.90%.

3.4 Effect on Sperm Abnormality:

The polyherbal extract significantly affected sperm abnormalities, increasing both head and tail defects. In Group II (200 mg/kg.bw), the percentage of head abnormalities rose to 8.25 ± 2.12% and tail abnormalities to 8.50 ± 0.73%, though these changes were not statistically significant, suggesting a mild effect (Figure-3).

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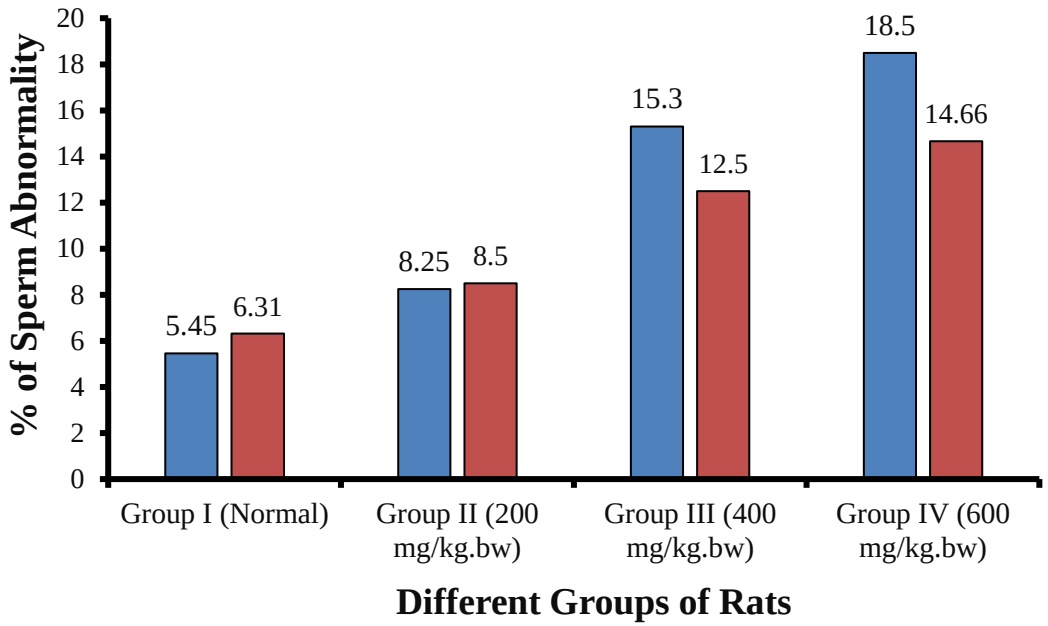

Figure-3. Effect of polyherbal extract on sperm abnormality in experimental treated rats (The values are expressed in mean percentage)

Table-1. Effect of polyherbal extract on sperm concentration, motility and abnormality in the epididymis of adult male albino rats

Group	Sperm concentration (counts x 10^6 per mL)		Sperm motility @ (Cauda)	Sperm abnormality (%)	
	Caput	Cauda		Head	Tail
Group (Normal)	261.40 ± 7.52	378.60 ± 8.04	101.50 ± 4.90	5.45 ± 1.30	6.31 ± 1.80
Group II (200 mg/kg.bw)	212.30 ± 4.44***	348.70 ± 7.50*	64.60 ± 2.50**	8.25 ± 2.12^{NS}	8.50 ± 0.73^{NS}
Group III (400 mg/kg.bw)	189.60 ± 2.44***	335.30 ± 5.52**	39.50 ± 3.67***	15.30 ± 2.25*	12.50 ± 0.93*
Group IV (600 mg/kg.bw)	178.40± 7.50***	326.20 ± 3.43***	30.80 ± 5.20***	18.50 ± 3.80**	14.66 ± 2.50**

- *NS = Non-significant; *p < 0.05; **p < 0.01; ***p < 0.001*
- *All values are expressed as mean ± SD.*

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Group III (400 mg/kg.bw) showed significant increases, with head abnormalities at 15.30 ± 2.25% ($p < 0.05$) and tail abnormalities at 12.50 ± 0.93% ($p < 0.05$). Group IV (600 mg/kg.bw) exhibited the highest levels, with head abnormalities at 18.50 ± 3.80% ($p < 0.01$) and tail abnormalities at 14.66 ± 2.50% ($p < 0.01$) (Figure-3). Compared to the control group, which had 5.45 ± 1.30% head and 6.31 ± 1.80% tail abnormalities, all treated groups displayed higher abnormalities, with Group IV showing the most pronounced increase, indicating a dose-dependent effect of the polyherbal extract.

4. DISCUSSION

The present study assessed the impact of a polyherbal extract on sperm parameters in male albino rats. The extract, comprising clove buds and Triphala, demonstrated a dose-dependent effect on sperm concentration, motility, and abnormalities, highlighting its potential impact on male fertility. Our results reveals that a significant reduction in sperm concentration across all treated groups. Group II (200 mg/kg.bw) showed a decrease in sperm concentration in the caput and cauda regions, with further reductions observed in Groups III (400 mg/kg.bw) and IV (600 mg/kg.bw). These findings were consistent with previous studies demonstrating that herbal extracts can significantly alter sperm concentration. For instance, Agarwal et al. (2012) reported reduced sperm counts following the administration of certain herbal extracts, attributing it to alterations in endocrine function or direct toxicity.

The polyherbal extract also led to a marked decline in sperm motility. Group II exhibited a significant reduction, with Groups III and IV showing even more pronounced impairments. This decrease in motility corroborates findings by Kaur et al. (2015), who observed similar effects with other herbal formulations, suggesting that the active compounds in these extracts might interfere with sperm functionality. The reduction in motility aligns with the work of Madaan et al. (2010) and Buchipal et al., (2023), who linked reduced motility to oxidative stress and altered metabolic pathways.

An increase in sperm abnormalities was noted, especially in Groups III and IV, where both head and tail abnormalities were significantly higher. This result supports the observations by Prasad et al. (2018) and Dwivedi et al., (1990), who found that herbal extracts can induce morphological changes in sperm, possibly due to toxic effects on spermatogenesis or structural components of spermatozoa.

The polyherbal extract's impact on sperm parameters demonstrates its potential to affect on male reproductive health. The observed dose-dependent effects align with existing literature on herbal extracts' influence on sperm quality, suggesting a need for further research to elucidate the underlying mechanisms.

5. CONCLUSION

The present study concludes that the polyherbal extract significantly affects on male reproductive parameters in a dose-dependent manner. Administration of the extract led to a notable reduction in sperm concentration and motility, along with an increase in sperm abnormalities. These effects were most pronounced at higher doses, suggesting potential adverse impacts on male fertility. The findings underscore the need for cautious use of polyherbal extracts and further investigation into their long-term effects on reproductive health. Overall, while the polyherbal extract exhibits promising therapeutic properties, its implications for fertility warrant comprehensive evaluation.

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Conflicts of Interest

Authors declare that there is no conflict of interests regarding the publication of this paper.

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