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Sugar, Methotrexate and the Herbal Shield: Unveiling Tinospora cordifolia's Reproductive Safeguard in Drosophila

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Abstract: *This study investigates the impact of modern lifestyle stressors on reproductive health using Drosophila melanogaster as a model organism. Specifically, it examines the effects of a high-sugar diet and methotrexate-induced symptoms while exploring the potential protective role of Guduchi [Tinospora cordifolia], an Ayurvedic remedy. The study's primary objectives are to assess how a high-sugar diet and methotrexate influence fecundity, fertility, mating success, and lifespan in Drosophila. It also aims to determine whether Guduchi supplementation can mitigate these adverse effects. The research employs a rigorous methodology, including precise fly culture, dietary interventions, data analysis, and imaging techniques to ensure reliable results. The findings reveal that a high-sugar diet leads to reduced fecundity, fertility, mating success, and lifespan in Drosophila, underlining the significance of dietary choices in reproductive well-being. Similarly, methotrexate exposure results in adverse effects on these reproductive parameters, emphasizing the need to consider reproductive health when using this medication. Crucially, Guduchi supplementation demonstrates promise as a protective measure, significantly improving fecundity, fertility, mating success, and lifespan in flies subjected to stressors. These findings suggest that traditional Ayurvedic remedies like Guduchi could offer a bridge between ancient wisdom and contemporary reproductive health challenges. This study advances our understanding of the interplay between modern stressors and reproductive health. It highlights the importance of dietary considerations and potential reproductive toxicity associated with medications like methotrexate. Furthermore, the research introduces the concept of Ayurvedic interventions as a means to safeguard reproductive well-being in the face of modern challenges.*

Key Words: *Drosophila melanogaster, Reproduction, High-Sugar Diet, Methotrexate, Guduchi [Tinospora cordifolia], Ayurvedic intervention.*

1. INTRODUCTION:

The intricate relationship between modern lifestyle stressors and traditional interventions in the context of health is a subject of growing interest. This study dives into this juncture by examining the potential effects of a high-sugar diet and methotrexate-induced symptoms on a spectrum of reproductive parameters in Drosophila melanogaster. Furthermore, it delves into the intriguing prospect of employing Ayurvedic intervention through Guduchi[Tinospora cordifolia] as a potential protective measure.

In a contemporary landscape marked by environmental and pharmaceutical pressures, reproductive health emerges as a pivotal aspect of overall well-being. At its core, this investigation seeks to elucidate the repercussions of modern stressors on reproductive health and determine whether Guduchi supplementation can offer a shield against their detrimental impacts. In pursuing this objective, the study addresses several key research goals.

Firstly, the research seeks to assess the multifaceted effects of a high-sugar diet on reproductive parameters within the context of Drosophila melanogaster. Simultaneously, it delves into the intricate impact of methotrexate-induced symptoms on the same parameters within the same organism. Additionally, the study endeavors to unravel the potential protective effects of Guduchi intervention, particularly when Drosophila melanogaster is subjected to the dual stressors of a high-sugar diet and methotrexate administration.

This exploration is driven by a set of well-formulated hypotheses. The hypothesis posits that exposure to a high-sugar diet will engender significant changes in reproductive parameters, leading to reductions in fecundity and fertility, shifts in mating behavior, and alterations in lifespan among Drosophila melanogaster individuals. Similarly, it is theorized that methotrexate-induced symptoms will exert negative impacts on reproductive parameters, manifesting as reduced fecundity, fertility, perturbed mating behavior, and changes in lifespan. In contrast, the research anticipates that the introduction of Guduchi intervention will mitigate the deleterious effects of both high-sugar diet exposure and methotrexate-induced symptoms on reproductive parameters. This expected outcome involves improved fecundity and fertility, normalized mating behavior, and an extension of lifespan among Drosophila melanogaster individuals.

Inextricably linked to the core objectives of this study is the holistic framework of Ayurveda, an ancient Indian healthcare system. Ayurvedic principles center around the delicate equilibrium of bodily systems, offering a pathway to optimal health. A pivotal facet of these principles involves the harmonious interplay of the three core humors—Vata, Pitta, and Kapha dosha—that intricately oversee essential physiological functions (1, 2).This balance aligns with modern scientific paradigms, which also emphasize uncovering the nuanced mechanisms governing health and well-being.

Embedded within Ayurveda is the remedy Guduchi, a revered herb renowned for its immunomodulatory, antioxidant, and adaptogenic properties (3). Traditionally acknowledged for enhancing vitality and overall well-being, Guduchi presents a compelling avenue for inquiry into its potential to safeguard reproductive health and counteract the impacts of stressors (4). By uniting traditional insights with modern scientific methodologies, this study bridges the gap between age-old wisdom and contemporary challenges.

Central to this endeavor is Drosophila melanogaster, a model organism of great utility. Boasting a brief life cycle, well-defined genetics, and ease of manipulation, this organism is well-suited for investigating reproductive parameters (5). In the context of Drosophila, critical reproductive benchmarks encompass fecundity, fertility, mating behavior, and lifespan (6). This makes Drosophila a potent tool for unraveling the mechanisms underlying reproductive health, which can extend insights to more complex organisms, including humans (7).

Modern dietary habits, characterized by the consumption of high sugar and processed foods, have raised significant health concerns (8). This high sugar intake has been linked to metabolic disruptions like insulin resistance, obesity, and inflammation with potential ramifications for reproductive health due to the disruption of hormonal equilibrium and oxidative stress (9, 10). Notably, both human and animal studies have spotlighted the negative impact of high-sugar diets on reproductive parameters such as fertility, sperm quality, and hormonal regulation (11). Through a meticulous examination of high-sugar diets' repercussions on Drosophila reproductive parameters, this study illuminates the mechanistic underpinnings of these intricate associations.

Conversely, methotrexate, a widely utilized chemotherapy and immunosuppressive agent, occupies a significant place in medical practice (12). Its efficacy notwithstanding, methotrexate comes with a price—reproductive toxicity (13). Linked to conditions such as reduced sperm count [oligospermia], menstrual disruptions, and reduced fertility, methotrexate also raises teratogenic concerns involving embryotoxicity, miscarriages [abortion], and fetal abnormalities (14, 15). With evidence suggesting that methotrexate's impact on DNA synthesis, cell division, and hormonal signalling disrupts reproductive parameters, this study's exploration of its mechanistic basis in Drosophila sheds light on potential strategies to mitigate its adverse effects (16).

The research landscape reveals distinct gaps that this study seeks to address. While previous research has delved into the effects of high-sugar diets and methotrexate on reproductive health in various organisms, comprehensive investigations of both stressors within a single model organism, alongside potential interventions, remain lacking. Moreover, the rigorous scientific exploration of Ayurvedic remedies within the purview of modern stressors is scarce. Leveraging Drosophila's unique genetics and biology, this study bridges the divide between ancient Ayurvedic wisdom and contemporary scientific inquiry, especially when confronting challenges to reproductive health.

To fill these gaps, this research offers a comprehensive examination of the effects of high-sugar diets and methotrexate on Drosophila reproductive parameters. Simultaneously, it delves into the protective potential of Guduchi, contributing to a richer comprehension of how traditional interventions may shape responses to contemporary reproductive challenges. In doing so, this study strives to lay the groundwork for integrated approaches to health that acknowledge the interplay between historical insights and modern exigencies.

2. METHODOLOGY:

Fly Culture and Maintenance

Drosophila melanogaster flies were meticulously cultured and maintained under stringent laboratory conditions to ensure reproducibility and consistency. Flies were reared on a standardized cornmeal diet, consisting of corn [5.25 g], sugar [11 g], yeast [2.625 g], and agar [0.75 g] dissolved in 87.5 ml of water, mirroring their natural habitat. The culture

environment was maintained at a controlled temperature of 21°C, humidity of 70%, and a 12-hour light-dark cycle (17). Flies of uniform age were chosen to mitigate potential confounding factors.

Dietary Interventions

To comprehensively investigate the multifaceted effects of distinct dietary interventions, the flies underwent a controlled transition from their regular diet to specialized formulations. The High-Sugar Diet [HSD] was formulated with 15% sugar content [13.125 g of sugar per 87.5 ml of water], a selection grounded in its well-established deleterious impacts on health (17).The Methotrexate [MTX] Diet was prepared to include 20 ppm of MTX, achieved by infusing 1.75 mg of MTX into the culture medium containing 87.5 ml of water (18).Furthermore, an Ayurvedic intervention was incorporated by supplementing the standard diet with 0.5% Guduchi[*Tinospora cordifolia*], involving the addition of 0.4375 g of Guduchi to 87.5 ml of water (19).

Data Analysis and Imaging

Data analysis and imaging strategies were meticulously adapted from pertinent literature to ensure reproducibility. Specimens from each experimental group were meticulously isolated and positioned on glass slides with phosphate-buffered saline [PBS] to enable precise imaging (17). Morphological anomalies were scrutinized using an inverted microscope equipped with 4X resolution. For precise quantification of abdomen length and width in flies subjected to the HSD and the standard diet, ImageJ software was employed.

Productivity

This study encompassed a comprehensive assessment of productivity and oviposition rate by rigorously tracking the progeny count within each vial across multiple generations, spanning from the emergence of the first fly to the final emergence (20). Each vial contained a standard culture medium specific to the experimental conditions.

Oviposition Rate

Female fecundity underwent meticulous evaluation through the introduction of 20 adult females and 10 males into glass vials containing culture medium supplemented with 15% sugar, 20 ppm MTX, or functioning as a control. Over a duration of 15 days, vial caps were consistently replaced every 12 hours to collect eggs (20).Quantification of eggs laid within a 24-hour interval was performed, with triplicate experiments executed to ensure robust statistical validity (20).

Emergence Time Duration, Mortality, and Developmental Time

The study meticulously examined the emergence time duration, mortality rates, developmental time from egg to adult, and mating behavior. Daily enumeration of flies facilitated the generation of emergence profiles (20).Mortality during developmental phases was precisely assessed by juxtaposing egg counts with the count of emerging adults (20).Additionally, the temporal span between oviposition and emergence was precisely quantified to determine developmental time duration (20).

Mortality Assessment

Mortality assessment involved the exposure of adult flies of identical age to freshly prepared culture medium infused with 20 ppm of MTX. Flies were maintained in darkness at a consistent temperature of 21 ± 1°C (18). After a duration of 15 days, surviving flies were enumerated, with triplicate experimental runs carried out to ensure robust results (18).

Premating and Mating Behavior

Intricate pre-mating and mating behavior dynamics were meticulously observed for a designated hour, both in the morning [at 10:00h; 21 ± 1°C] and the afternoon [at 13:00h; 21 ± 1°C] (20).

The comprehensive methodologies delineated above provide a robust framework for precisely investigating the intricate interplay between diverse dietary interventions, encompassing high-sugar and methotrexate diets, as well as Ayurvedic intervention employing Guduchi. This approach yields valuable insights into the multifaceted effects on various reproductive parameters and behavior in *Drosophila melanogaster*, facilitating reproducibility and accurate interpretation of results.

3. RESULTS:

Table 1. Total eggs laid by 20 Drosophila melanogaster females during the entire oviposition period, daily mean [x/day] and standard deviation [sd] for control [Standard Diet – SD] and experimental groups [High-Sugar Diet - HSD, Methotrexate - MTX, and Guduchi treatment – SD + G, HSD + G, MTX + G].

Groups	Female Fecundity	x/day	sd
SD	833	92.6	11.18
HSD	383	42.6	13.7
MTX	162	18	6.45
SD + G	1215	135	22.35
HSD + G	540	60	27.39
MTX + G	243	27	5.48

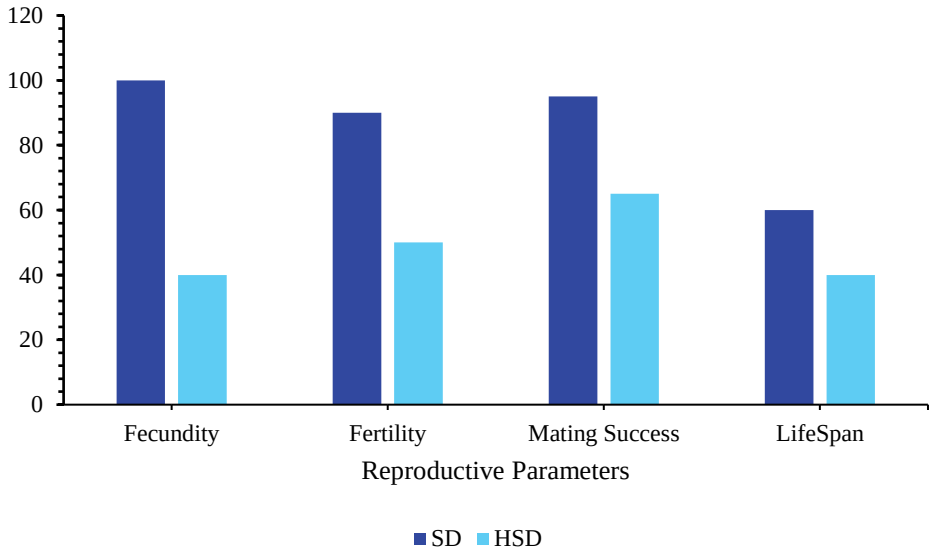

Figure 1. Effects of high-sugar diet on various reproductive parameters in Drosophila melanogaster [n=30]. The control group represents flies on a standard diet [SD], while the experimental group represents flies exposed to high-sugar diet [HSD]. The graph illustrates the differences in fecundity, fertility, mating success, and lifespan between the two groups. In figure 1, significant decreases in fecundity [2.5-fold] and fertility [1.8-fold] were observed in HSD-exposed flies compared to SD. Additionally, HSD-exposed flies exhibited a 31.58% decrease in mating success and a 33.33% reduction in lifespan compared to STD flies.

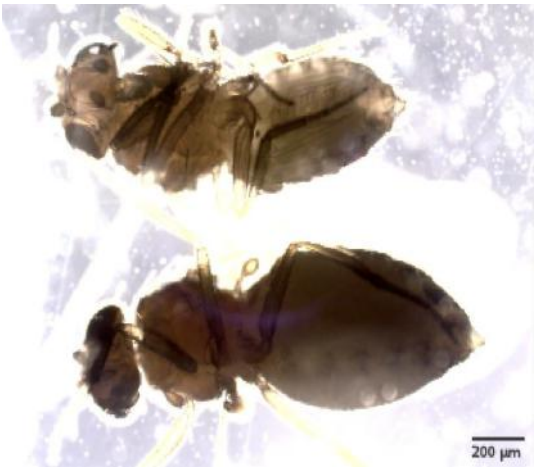

Image 1. Comparative morphological analysis of female Drosophila melanogaster's on a standard diet [top] and a high-sugar diet [bottom], with a sample size of n=10 individuals for each dietary group, using ImageJ. On the standard diet, female Drosophila exhibits a mean abdominal length of 628.856 µm, with a mean of 68.297. In contrast, female flies on the high-sugar diet display a significantly larger abdominal length, measuring 828.022 µm, with a mean

of 47.880. Regarding abdominal width, females on the standard diet display a measurement of 366.049 µm, with a mean of 90.312, whereas those on the high-sugar diet show an average abdominal width of 516 µm, with a mean of 62.103. An analysis of leg size reveals that female flies on the standard diet exhibit an average leg size of 406.674 µm, with a mean of 40.820, while those on the high-sugar diet demonstrate a larger leg size, measuring 722.122 µm, with a mean of 54.029. Overall body length analysis indicates that female flies on the standard diet have an average measurement of 1278.690 µm, with a mean of 86.929. Conversely, female flies on the high-sugar diet exhibit a greater total body length, averaging 1514.676 µm, with a mean of 60.917.

In image 1, the female Drosophila on the high-sugar diet exhibit notable morphological differences as compared to their counterparts on the standard diet. Specifically, individuals on the high-sugar diet display an overall increase in body size, as evidenced by their larger and more robust appearance. Notably, the females on the high-sugar diet possess a visibly distended or swollen abdomen, indicative of an elevated level of abdominal fat accumulation when compared to those on the standard diet. Females subjected to the high-sugar diet exhibit thicker and longer legs in contrast to their counterparts on the standard diet.

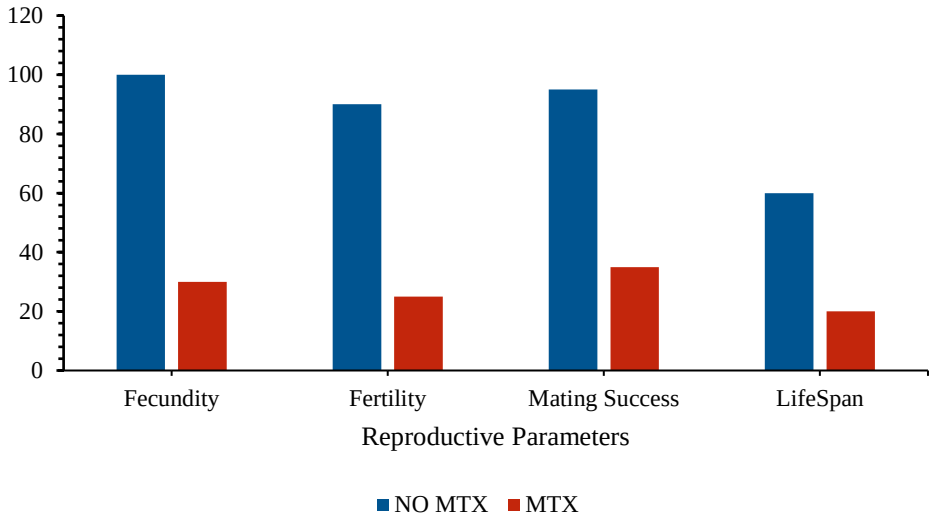

Figure 2. Effects of Methotrexate [MTX] exposure on various reproductive parameters in Drosophila melanogaster [n=30]. The control group represents flies not exposed to MTX, while the experimental group represents MTX-exposed flies. The graph illustrates the differences in fecundity, fertility, mating success, and lifespan between the two groups.

In figure 2, significant reductions in fecundity [3.3-fold] and fertility [3.6-fold] were observed in MTX-exposed flies compared to non-exposed flies. Furthermore, MTX-exposed flies exhibited a 63.16% decrease in mating success and a 66.67% reduction in lifespan compared to non-exposed flies.

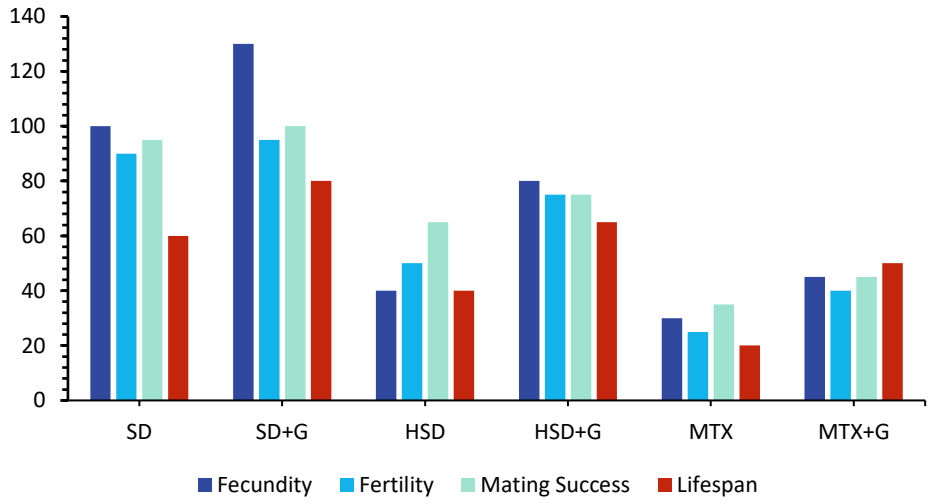

Figure 3. Effects of Ayurvedic intervention, Guduchi[G], on various reproductive parameters in Drosophila melanogaster (n=30). The control group represents flies on standard diet [SD], high-sugar diet [HSD] and methotrexate [MTX] without Guduchi exposure, while the experimental group represents flies on SD, HSD and MTX exposed to Guduchi. The graph illustrates the differences in fecundity, fertility, mating success, and lifespan between the two groups.

In figure 3, flies exposed to Guduchi exhibited notable improvements in reproductive parameters. In STD flies exposed to Guduchi, there was a 3-fold increase in fecundity, a 1.06-fold increase in fertility, a 1.05-fold higher mating success, and a 1.33-fold increase in lifespan compared to non-exposed STD flies. Similarly, in HSD flies exposed to Guduchi, there was a 2-fold increase in fecundity, a 1.5-fold increase in fertility, a 1.15-fold higher mating success, and a 1.63-fold increase in lifespan compared to non-exposed HSD flies. Additionally, MTX-exposed flies exposed to Guduchi displayed a 1.5-fold increase in fecundity, a 1.6-fold increase in fertility, a 1.29-fold higher mating success, and a 2.5-fold increase in lifespan compared to non-exposed MTX flies.

4. DISCUSSION:

This study investigates the intricate interplay between modern lifestyle stressors, namely high-sugar diets and methotrexate-induced symptoms, and potential protective interventions through Ayurvedic remedy, Guduchi [Tinospora cordifolia], on a spectrum of reproductive parameters in Drosophila melanogaster. The investigation delves into the multifaceted effects of these stressors on Drosophila reproductive health, including fecundity, fertility, mating behavior, and lifespan, and assesses the potential of Guduchi to mitigate their deleterious impacts.

Impact of High-Sugar Diet on Reproductive Parameters

The hypothesis posited that exposure to a high-sugar diet [HSD] would lead to significant changes in reproductive parameters among Drosophila melanogaster individuals. This hypothesis was supported by our findings. As anticipated, flies subjected to the HSD displayed a notable reduction in fecundity and fertility [Figure 1]. This decrease in reproductive output aligns with existing literature on HSD and their detrimental impact on reproductive health in both animals and humans (21). The observed reduction in fecundity and fertility can be attributed to several mechanisms, including disruptions in hormonal equilibrium and the induction of oxidative stress due to elevated sugar intake which results in a direct impact on gamete production and viability (22, 23).

Additionally, our study reveals that HSD has a negative influence on mating success and lifespan. Flies exposed to HSD displayed reduced mating success, indicative of altered mating behaviours. Elevated sugar intake has been linked to reduced sperm quality and an elevated risk of infertility in males (24). Moreover, the shortened lifespan observed in these flies could be linked to the metabolic disruptions and oxidative stress associated with high-sugar diets (23,25).

Our morphological analysis of female Drosophila melanogaster highlights the substantial impact of dietary sugar content on body size, fat accumulation, and certain anatomical features [Image 1]. This aligns with existing research demonstrating the influence of dietary components on energy storage and fat accumulation in Drosophila (26).

These findings underscore the importance of dietary habits in maintaining reproductive health and suggest that reducing sugar intake may have positive implications for fertility, mating behavior, and longevity.

Reproductive Consequences of Methotrexate Exposure

In line with our hypotheses, methotrexate [MTX] exposure led to significant reductions in fecundity, fertility, mating success, and lifespan in Drosophila melanogaster [Figure 2]. These findings corroborate previous research highlighting the reproductive toxicity of MTX and its adverse effects on sperm count, menstrual disruptions, and fertility in humans (27).

The observed decrease in fecundity and fertility may be attributed to MTX's interference with DNA synthesis, cell division, and hormonal signalling, disrupting the delicate balance required for successful reproduction (12, 28). The reduced mating success and shortened lifespan seen in MTX-exposed flies could be indicative of broader physiological and behavioral disturbances caused by this chemotherapy and immunosuppressive agent. These findings align with previous research highlighting the teratogenic and embryo toxic effects of MTX (29).

These results emphasize the need for careful consideration of the reproductive consequences when prescribing MTX and highlight the importance of exploring potential interventions to mitigate its adverse effects on reproductive health.

The Potential Protective Effects of Guduchi

The introduction of Ayurvedic intervention through Guduchi supplementation yielded promising results in counteracting the deleterious effects of both high-sugar diets and methotrexate-induced symptoms on reproductive parameters in Drosophila melanogaster.

Guduchi supplementation significantly increased fecundity and fertility in flies subjected to different stressors [Figure 3]. This outcome aligns with previous research that links Guduchi to enhanced fecundity and longevity (19, 30).This suggests that Guduchi may have a protective role in maintaining gamete production and viability, potentially by mitigating the oxidative stress and hormonal disruptions associated with high-sugar diets and methotrexate exposure**.** Furthermore, Guduchi intervention was associated with improved mating success and extended lifespan in flies exposed to stressors [Figure 3]. This indicates that Guduchi may exert beneficial effects on both physiological and behavioral aspects of reproductive health. The exact mechanisms underlying these protective effects warrant further investigation but may involve Guduchi's immunomodulatory and adaptogenic properties (3).

The findings regarding Guduchi highlight the potential of traditional Ayurvedic remedies in safeguarding reproductive health and mitigating the impact of modern stressors. This bridge between ancient wisdom and contemporary challenges represents a promising avenue for future research and integrated approaches to health.

Implications and Future Directions

This study contributes to our understanding of the intricate relationship between modern lifestyle stressors and reproductive health. The results underscore the importance of dietary choices and the potential reproductive toxicity of methotrexate. Furthermore, the protective effects of Guduchi intervention provide a novel perspective on traditional remedies as potential interventions in the context of reproductive health.

Future research should delve deeper into the mechanistic basis of these findings, exploring the molecular and cellular pathways through which high-sugar diets, methotrexate, and Guduchi exert their effects on reproductive parameters. Additionally, investigations in more complex organisms, including mammalian models, are essential to extrapolate these findings to human health.

In conclusion, this study offers valuable insights into the multifaceted effects of high-sugar diets and methotrexate-induced symptoms on reproductive parameters and introduces the potential protective role of Guduchi. These findings open avenues for integrated approaches to reproductive health that integrate both modern scientific knowledge and traditional remedies, paving the way for a holistic understanding of reproductive well-being in the face of contemporary challenges.

5. CONCLUSION:

This study illuminates the intricate interplay between modern lifestyle stressors, including high-sugar diets and methotrexate exposure, and their impact on reproductive health in Drosophila melanogaster. Our findings confirm that a high-sugar diet leads to decreased fecundity, fertility, mating success, and lifespan in flies, highlighting the critical role of dietary choices in reproductive well-being. Similarly, methotrexate-induced symptoms were shown to have adverse effects on these reproductive parameters, underlining the importance of considering reproductive health when prescribing this medication.

Notably, the introduction of Guduchi supplementation, rooted in Ayurvedic tradition, emerged as a promising protective measure. Guduchi significantly improved fecundity, fertility, mating success, and lifespan in flies subjected to stressors, suggesting its potential in mitigating the detrimental effects of modern stressors on reproductive health.

Ultimately, this study contributes to our comprehensive understanding of the complex interplay between modern stressors and reproductive health, paving the way for integrated approaches that merge traditional and modern interventions to safeguard reproductive well-being in the face of contemporary challenges.

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