# Effects of Herbal Formulas on Oxidative Status in the Male Reproductive System and Penile Tissue of Hyperglycemia-and Hypercholesterolemia-Induced Rats

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## Abstract

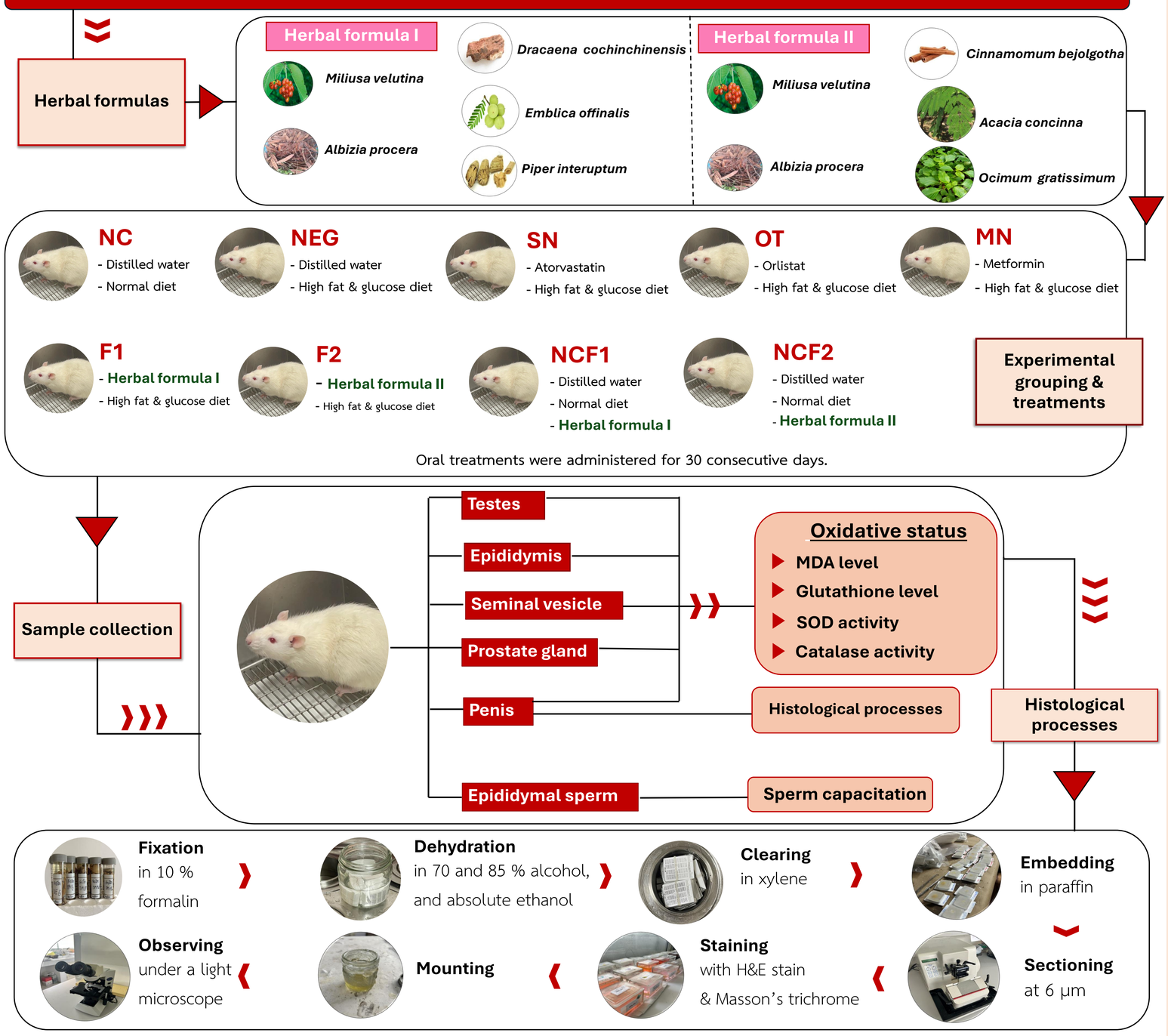
Obesity and diabetes are significant factors contributing to oxidative stress, which results from an imbalance between free radicals and antioxidants. Oxidative stress in male reproductive system leads to erectile dysfunction and reduced sperm fertilization capacity. Currently, herbal medicine has gained popularity for treating various diseases and alleviating male sexual dysfunction caused by obesity and diabetes. Thus, the objective of this study was to evaluate the effectiveness of two novel herbal formulations in high-fat-diet and streptozotocin-induced hyperglycemia and hypercholesterolemia in male rats. The two novel herbal formulas consisted of Formula 1 (*Dracaena cochinchinensis*, *Miliusa velutina*, *Emblica officinalis*, *Piper interruptum*, and *Albizia procera*) and Formula 2 (*Cinnamomum bejolghota*, *Miliusa velutina*, *Acacia concinna*, *Ocimum gratissimum*, and *Albizia procera*). Hyperglycemia-and hypercholesterolemia-induced rats were treated with these herbal formulas for one month, and the effects were compared to those in normal rats. Synthetic drugs commonly used to treat obesity and diabetes, including orlistat, metformin and atorvastatin, were used as positive controls. Oxidative stress indices, including malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) were examined in testes, epididymis, seminal vesicles, seminal fluid, prostate gland, and sperm suspension. Additionally, sperm capacitation and histology of erectile tissue in penis were investigated. The results demonstrated that both herbal formulations effectively reduced oxidative stress in the male reproductive system by lowering MDA levels and increasing antioxidant enzyme activities. Furthermore, Formula 1 significantly ($p<0.05$) promoted penile erection by reducing fibrosis in the corpus cavernosum of hyperglycemia-and hypercholesterolemia-induced rats. However, neither herbal formula had any effect on enhancing sperm fertilization capacity.

## Introduction

Currently, diabetes and obesity are growing health concerns worldwide. According to surveys, the number of diabetes patients has doubled over the past 20 years (Zimmet *et al*., 2014). Diabetes and obesity are associated with oxidative stress, which results from an accumulation of free radicals in the body due to mitochondrial dysfunction and imbalanced cellular metabolism (Giacco *et al*., 2010). Oxidative stress affects various body systems, including the male reproductive system, which plays a crucial role in sperm production and hormone regulation. Studies have shown that patients with diabetes and obesity often experience erectile dysfunction and infertility due to tissue damage in the reproductive organs (Kaltsas *et a*l., 2024) and structural changes in sperm cells (Nada *et al*., 2015).

Recent research suggests that antioxidants found in various herbal plants can help reduce oxidative stress and restore male reproductive function. However, despite numerous studies on the antioxidant properties of individual herbs, research on the the effects of herbal formulations in reducing oxidative stress and improving male reproductive health in diabetes and obesity remains limited. Therefore, this study aimed to investigate the efficacy of an herbal formulation in reducing free radicals, restoring male reproductive function, and preventing oxidative stress-induced damage in hyperglycemia- and hypercholesterolemia-induced experimental rats.

## Materials and Methods




## Results and Discussion

**Table 1** MDA levels in the reproductive organs of hyperglycemia-and hypercholesterolemia-induced rats treated with herbal formulas and synthetic drugs.

| | MDA (nM/mgProtein) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | NC | NEG | SN | MN | OT | F1 | F2 | NCF1 | NCF2 |
| **Testes** | $202.24^{bc}$ | $234.70^{c}$ | $232.14^{c}$ | $221.82^{c}$ | $211.59^{c}$ | $174.87^{a}$ | $232.75^{c}$ | $145.74^{a}$ | $202.41^{bc}$ |
| **Epididymis** | $241.32^{a}$ | $309.91^{cd}$ | $282.96^{bc}$ | $259.92^{ab}$ | $266.11^{ab}$ | $323.32^{de}$ | $277.21^{abc}$ | $265.67^{ab}$ | $349.46^{e}$ |
| **Seminal vesicle** | $187.23^{a}$ | $237.56^{c}$ | $186.68^{a}$ | $181.76^{a}$ | $225.65^{bc}$ | $193.94^{ab}$ | $238.41^{c}$ | $318.93^{d}$ | $227.53^{bc}$ |
| **Seminal fluid** | $423.92^{e}$ | $454.94^{e}$ | $313.90^{ab}$ | $351.83^{cd}$ | $356.33^{d}$ | $327.67^{abcd}$ | $301.46^{a}$ | $347.62^{bcd}$ | $318.67^{abc}$ |
| **Prostate gland** | $247.96^{b}$ | $253.98^{b}$ | $265.68^{bc}$ | $254.6^{b}7$ | $368.91^{d}$ | $249.63^{b}$ | $287.99^{c}$ | $195.84^{a}$ | $214.22^{a}$ |
| **Penis** | $315.33^{abc}$ | $325.06^{bc}$ | $249.54^{a}$ | $677.64^{e}$ | $260.31^{ab}$ | $285.97^{ab}$ | $359.77^{c}$ | $373.55^{cd}$ | $436.31^{d}$ |

The superscript letters indicate statistically significant differences ($p < 0.05$) between the experimental groups (Duncan's test).

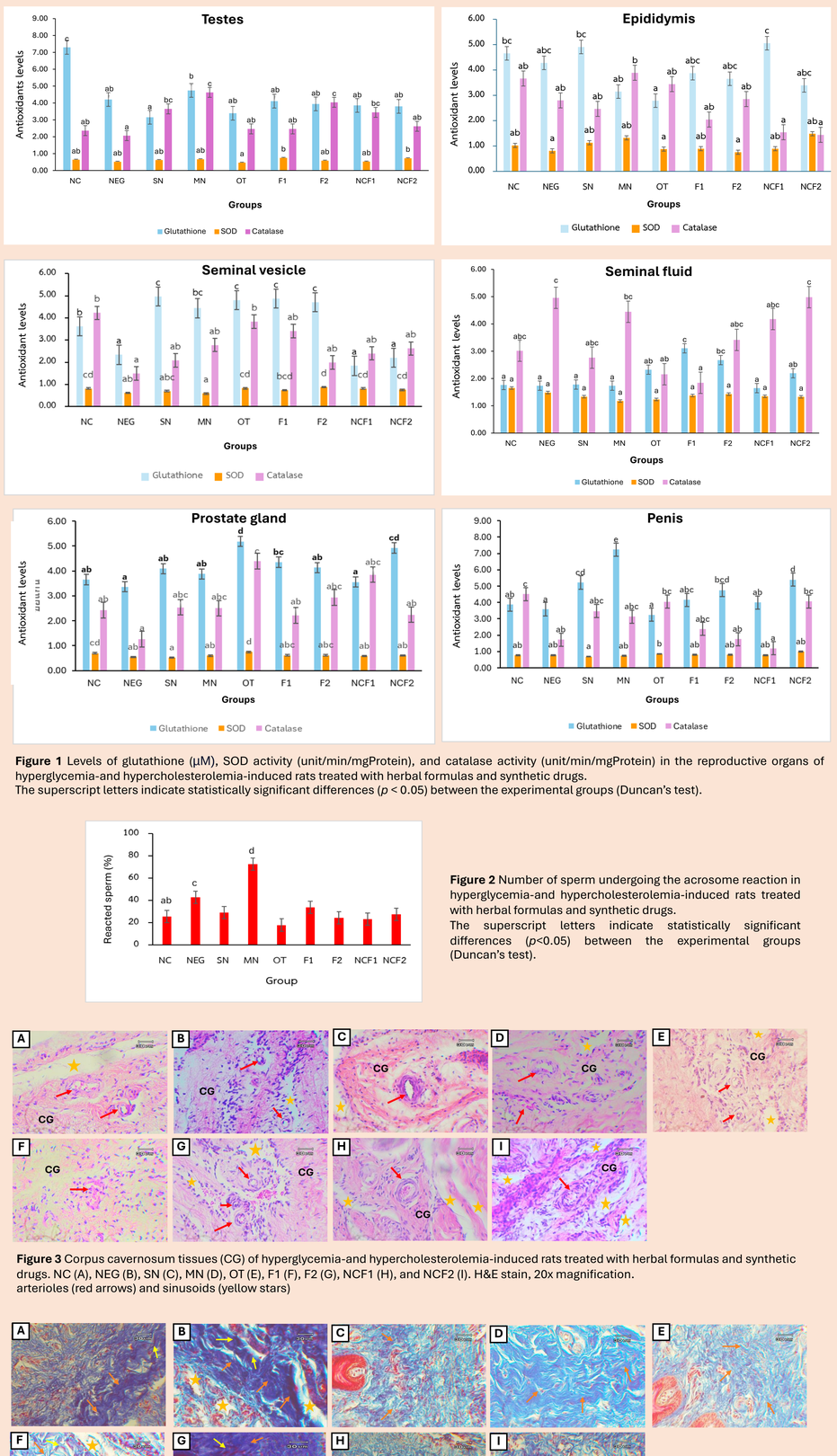



**Figure 1** Levels of glutathione (µM), SOD activity (unit/min/mgProtein), and catalase activity (unit/min/mgProtein) in the reproductive organs of hyperglycemia-and hypercholesterolemia-induced rats treated with herbal formulas and synthetic drugs.
The superscript letters indicate statistically significant differences ($p < 0.05$) between the experimental groups (Duncan's test).

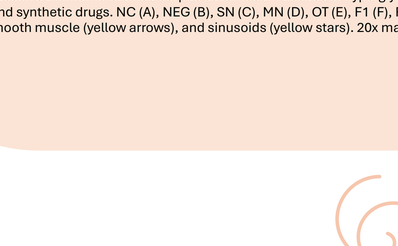



**Figure 2** Number of sperm undergoing the acrosome reaction in hyperglycemia-and hypercholesterolemia-induced rats treated with herbal formulas and synthetic drugs.
The superscript letters indicate statistically significant differences ($p<0.05$) between the experimental groups (Duncan's test).




**Figure 3** Corpus cavernosum tissues (CG) of hyperglycemia-and hypercholesterolemia-induced rats treated with herbal formulas and synthetic drugs. NC (A), NEG (B), SN (C), MN (D), OT (E), F1 (F), F2 (G), NCF1 (H), and NCF2 (I). H&E stain, 20x magnification.
arterioles (red arrows) and sinusoids (yellow stars)




**Figure 4** Masson's trichrome-stained corpus cavernosum tissues of hyperglycemia-and hypercholesterolemia-induced rats treated with herbal formulas and synthetic drugs. NC (A), NEG (B), SN (C), MN (D), OT (E), F1 (F), F2 (G), NCF1 (H), and NCF2 (I) shows fibrous tissue (orange arrows), smooth muscle (yellow arrows), and sinusoids (yellow stars). 20x magnification.

## Conclusion

Herbal formulation 1 exhibited a greater ability to reduce free radicals, increase antioxidant levels in the reproductive system, and enhance erectile function compared to herbal formulation 2. This was demonstrated through TBARS, DTNB, catalase activity, NBT reduction assays, and histological analysis. Formulation 1 effectively reduced free radicals in the testes, seminal vesicle, seminal fluid, and penis. However, neither formulation 1 nor 2 had a significant impact on improving sperm quality.

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