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EFFECT OF GINKGO BILOBA ON CLOMIPRAMINE INDUCED SEXUAL DYSFUNCTION IN MALE RATS

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ABSTRACT
The present study aimed to investigate the effect of G*inkgo biloba* leaves extract (40 mg/kg, dose) on sexual behaviour: mount latency and frequency (ML&MF), intromission latency and frequency (IL&IF), ejaculation latency (EL), post ejaculatory pause (PEP) and couplatory frequency and interval (CF&CI) in Clomipramine - induced sexually dysfunction male rats. The results showed in relation to the Clomipramine treated group, the G*inkgo biloba* extract, significantly restored the normal sexual behavior and antioxidant activities.

KEYWORDS: G*inkgo biloba*, Clomipramine, mount latency, sexual dysfunction.

INTRODUCTION
Clomipramine (CLMP) is a tricyclic antidepressant, which has demonstrated efficacy in depression, obsessive compulsive disorder (OCD) and panic disorder. Clomipramine is the imipramine analogue of chlorpromazine. Due to its action against anxiety disorders and panic attacks, it is the only drug with 2 entries in the essential drugs list of the World Health Organization (WHO). With regards to compulsive disorders, it is now the "gold standard" of therapy against which other drugs are measured.6-10 However, chronic use of Clomipramine leads to sexual dysfunction in humans[1,2] and neonatal males.[3]

Ginkgo biloba extract (GBE) facilitates blood flow, influences nitric oxide systems, and has a relaxant effect on smooth muscle tissue. These processes are important to the sexual response in women and, hence, it is feasible that GBE may have a therapeutic effect. The present study was the first to provide an empirical examination of the effects of both short- and long-term GBE administration on subjective and physiological (vaginal photoplethysmography) measures of sexual function in women with Sexual Arousal Disorder.

The *Ginkgo biloba* is an ancient tree that the Chinese have cultivated and held sacred for its health-promoting properties for over two millennia.[4,5] In recent decades, concentrated extracts from *Ginkgo biloba* leaves (GBE) have been sold in Western countries as herbal medicines for treating peripheral vascular disease and for enhancing cerebral blood flow.[6] Indeed, a number of clinical trials have shown that GBE is effective in treating a wide range of problems associated with impaired circulation, including hearing problems, visual disturbances, edema, varicose veins, leg ulcers, stroke, and intermittent claudication[7], as well as symptoms of cerebrovascular insufficiency, such as difficulties of concentration and memory, confusion, lack of energy, depressed mood, dizziness, and tinnitus.[8]

Clinical and pharmacological studies have shown that GBE promotes increased blood flow both in the arteries and capillaries.[9] Clinical reports also indicate GBE is effective in treating a variety of conditions responsive to improved circulation.[7] Given GBE's ability to facilitate peripheral blood flow to various bodily regions (e.g., arms, legs, hands), it is reasonable to speculate that it might also be effective in facilitating blood flow to the genital region, thus enhancing sexual arousal mechanisms.[10]

A second means by which GBE might facilitate sexual function is via the relaxation of the muscular cells of blood vessels. In addition to the above mechanisms, Cohen and Bartlik speculated that GBE could enhance vascular flow to the genitals through inhibition of platelet-activating factor, in a similar manner to the mechanism by which it enhances cerebral perfusion, and by having a direct effect on prostaglandins, which are known to enhance sexual arousal in men. Recent research has also shown GBE to

have nitric oxide-scavenging abilities[11], which point to its potential therapeutic value in treating conditions in which nitric oxide (NO) reactivity is important. In men, sexual stimulation leads to the production of NO and the subsequent release of guanylate cyclase. Guanylate cyclase converts guanosine triphosphate to cyclic guanosine monophosphate (cGMP) and cGMP produces relaxation of the smooth muscles of the penile arteries and corpus cavernosum, resulting in increased blood flow into the penis.[12] Therefore, in the present study, we investigated the effect of *Ginkgo biloba*.

ANIMALS

Healthy adult Wistar albino male rats (150-200 g) were used. The animals were housed individually, maintained under standard conditions (12 h light and 12 h dark cycle, 25-30OC, 35-60% relative humidity), the animals were fed with standard rat pellet diet (Venkateshwara enterprises, Bangalore, India) and water *ad libitum*. The study was approved by the Institutional Ethical Committee prior to commencement.

Induction of sexual dysfunction in male rats[13]

Sexually experienced male rats were divided into two groups, group 1 served as control and received vehicle only (normal saline) and group 2 served as Clomipramine treated group and received oral dose of 27 mg/kg Clomipramine suspension (suspension was prepared daily in Tween 80, suspended in 0.9% saline solution). Dosing was done for 30days once in a day. At the end of 30 days, after 30 min of last dosing, estrus female was introduced into respective cages and observed for mating performance and results were recorded and compared with control group. Mating performance analyses were conducted in the dark phase of the light-dark cycle under dim light condition. Assessment of IL&F, EL, ML&F, CE&I and PEP were monitored for 30 min after pairing (20). Animals which showed minimum 25% reduction in sexual behavior were considered as sexually impaired and they were incorporated for subsequent study.

Effects of Leaf extract of *Ginkgo biloba* on sexual behavior in Clomipramine-induced sexual dysfunction male rats[14]

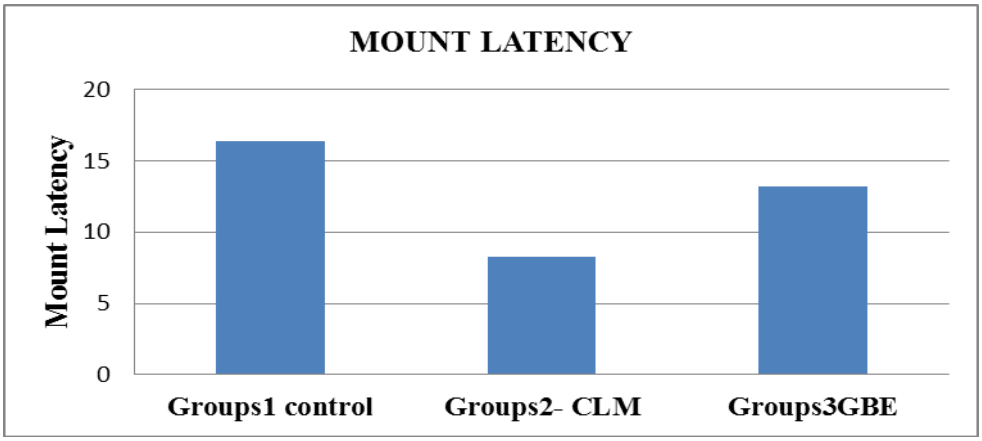
Normal sexually experienced male rats and sexually impaired male rats were employed for this investigation. Three groups of animals were formed, group I served as normal control (sexually experienced normal male rats) and received vehicle only, group II served as negative control (sexually impaired male rats, obtained from above study) and received Clomipramine suspension orally (27 mg/kg), group III (sexually impaired male rats obtained from above study), received 40 mg/kg of GBE suspension orally (suspension was prepared daily in Tween 80, suspended in 0.9% saline solution) besides Clomipramine suspension (27 mg/kg). Dosing frequency was once in a day for 30 days. At the end of 30th day, after 30 min of last dosing, estrus female was introduced into respective cages and observed for mating performance and results were recorded and statistically analyzed.

Statistical analysis

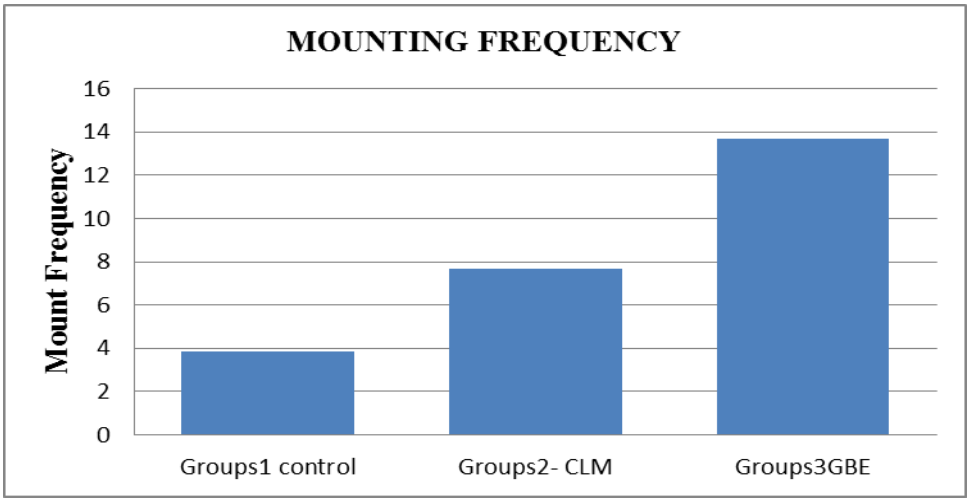
Data were presented as the mean ± SEM. The data were subjected to analysis of variance (ANOVA) followed by Tukey's test. $p < 0.05$ was taken as significant.

RESULTS

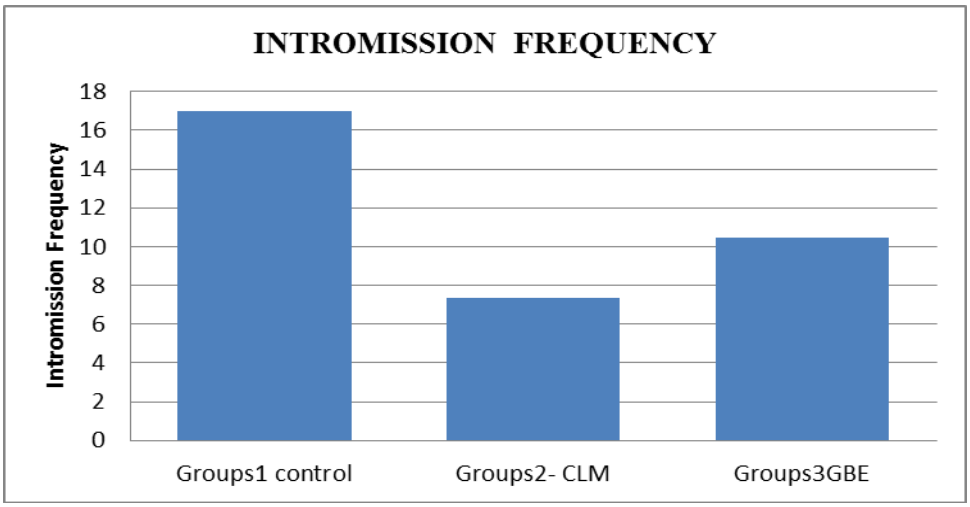
The results of sexual behavior analysis showed that in group I, normal rats exhibited usual sexual behavior, evident by ML (80.1 ± 1.4), CF (0.86 ± 0.32) MF (3.88 ± 1.2), IF (11.6 ± 1.7), EF (3.6 ± 1.2), IL (127.89 ± 1.77), EL (149.2 ± 1.03), and PEP (159.2 ± 1.43). In paroxetine treated group (group II), males demonstrated diminished sexual behavior as reflected by decreased ML (148.2 ± 1.2), CF (7.33± 1.23) MF (7.66 ± 0.42), IF (4.28 ± 1.49), EF (1.8± 1.1) and increased IL (184.16 ± 1.11), EL (194.1 ± 1.04), and PEP (284.16 ± 2.4) in comparison to normal control. On the other hand, animals of *G.biloba* treated group (group III), restored sexual performance significantly compared to Clomipramine treated group (group II). The males of group III showed significant increase in the frequencies ML (121.12 ± 1.4), CF (0.64± 0.13) MF (13.6 ±1.24), IF (9.53 ±1.06), EF (3.2 ±1.17) and decrease in latencies IL (153.77 ±1.88 d), EL (168.19 ±1.1), PEP (214.2 ±1.24) in relation with normal control.



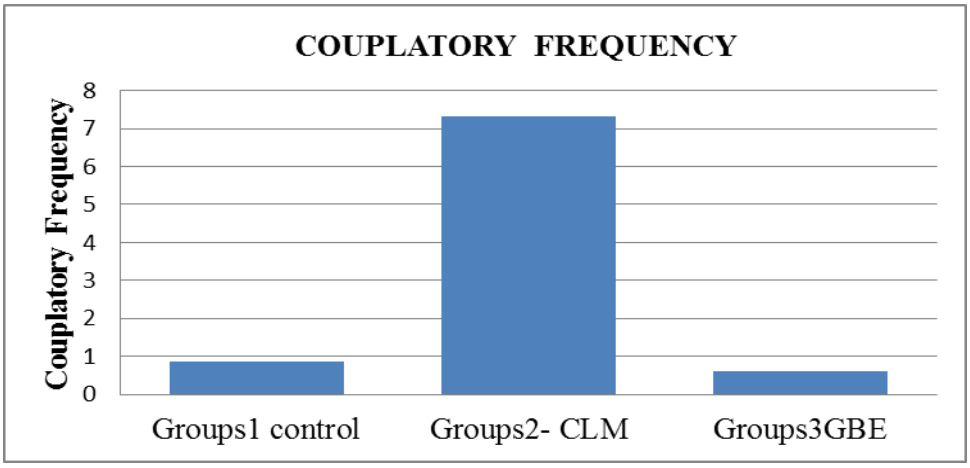
Graph 1: Effect of *G.biloba* extract on Mounting Latency in Clomipramine induced sexual dysfunction male rats. Group-I: vehicle treated; Group-II: Clomipramine treated; Group-III: ethyl acetate fraction treated. Values are the mean ± SEM, n = 6; a p < 0.05 and d p < 0.01, a: compared to normal control; d: compared to negative control. Data were analyzed by ANOVA followed by Tukey's test.



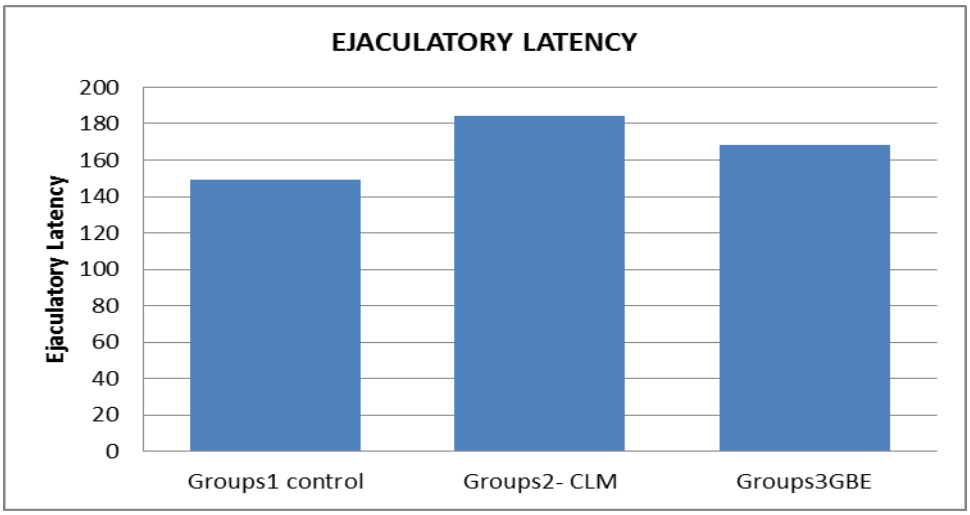
Graph 2: Effect of *Ginkgo biloba* on mounting frequency in Clomipramine induced sexual dysfunction male rats. Group I: vehicle treated; Group-II: Clomipramine treated; Group-III: *Ginkgo biloba* treated. Values are the mean ± SEM, n = 6; a, p < 0.05 and d p < 0.01, a,: compared to normal control; d: compared to negative control. Data were analyzed by ANOVA followed by Tukey's test.



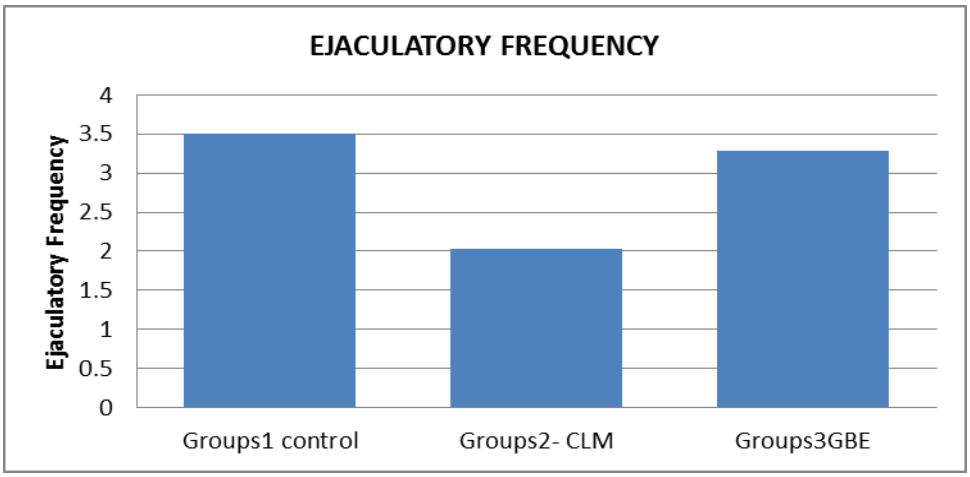
Graph 3: Effect of *Ginkgo biloba* on intromission frequency in Clomipramine induced sexual dysfunction male rats. Group I: vehicle treated; Group-II: Clomipramine treated; Group-III: *Ginkgo biloba* treated. Values are the mean ± SEM, n = 6; a, p < 0.05 and d p < 0.01, a,: compared to normal control; d: compared to negative control. Data were analyzed by ANOVA followed by Tukey's test.



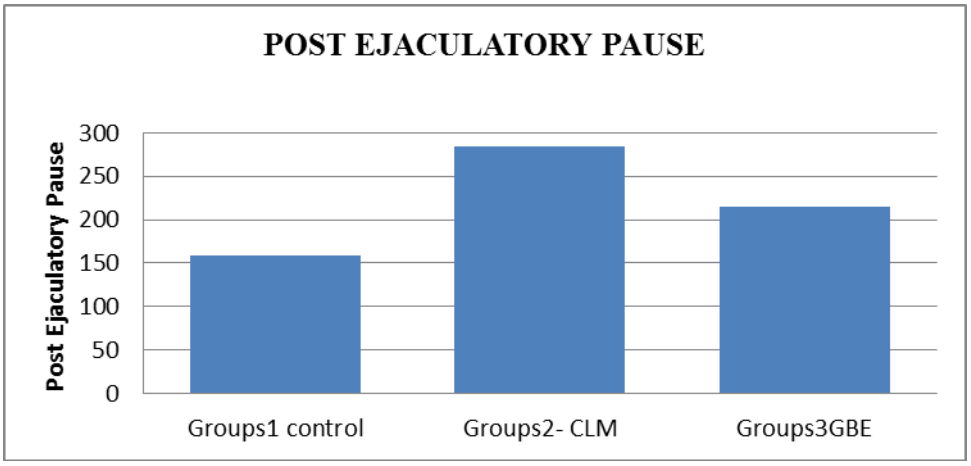
Graph 4: Effect of *Ginkgo biloba* on Couplatory frequency in Clomipramine induced sexual dysfunction male rats. Group I: vehicle treated; Group-II: Clomipramine treated; Group-III: *Ginkgo biloba* treated. Values are the mean ± SEM, n = 6; a, p < 0.05 and d p < 0.01, a,: compared to normal control; d : compared to negative control. Data were analyzed by ANOVA followed by Tukey's test.



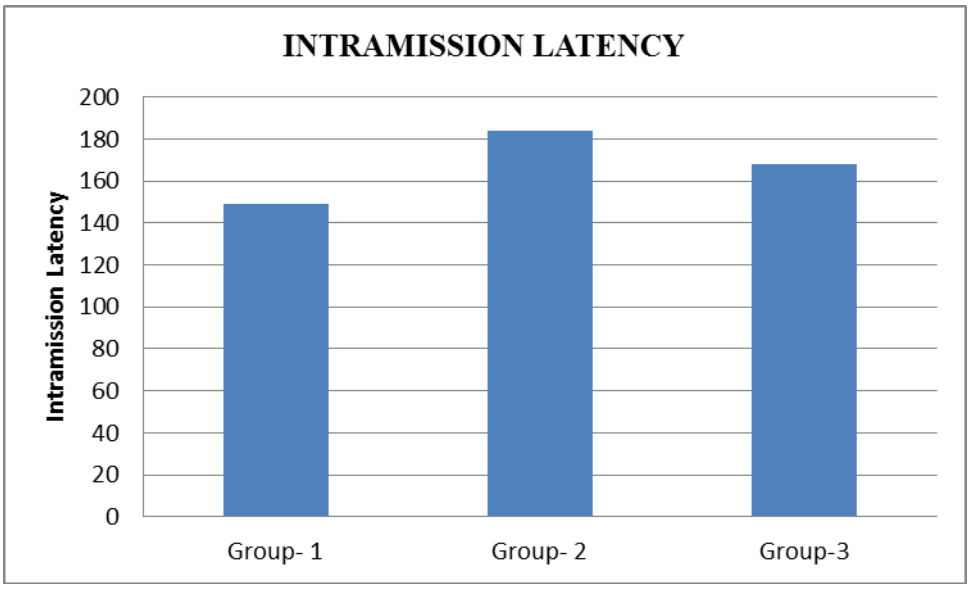
Graph 5: Effect of *Ginkgo biloba* on Ejaculatory Latency in Clomipramine induced sexual dysfunction male rats. Group I: vehicle treated; Group-II: Clomipramine treated; Group-III: *Ginkgo biloba* treated. Values are the mean ± SEM, n = 6; a, p < 0.05 and d p < 0.01, a,: compared to normal control; d : compared to negative control. Data were analyzed by ANOVA followed by Tukey's test.



Graph 6: Effect of *Ginkgo biloba* on Ejaculatory frequency in Clomipramine induced sexual dysfunction male rats. Group I: vehicle treated; Group-II: Clomipramine treated; Group-III: *Ginkgo biloba* treated. Values are the mean ± SEM, n = 6; a, p < 0.05 and d p < 0.01, a,: compared to normal control; compared to negative control. Data were analyzed by ANOVA followed by Tukey's test.



Graph 7: Effect of *Ginkgo biloba* on Post Ejaculatory Pause in Clomipramine induced sexual dysfunction male rats. Group I: vehicle treated; Group-II: Clomipramine treated; Group-III: *Ginkgo biloba* treated. Values are the mean ± SEM, n = 6; a, p < 0.05 and d p < 0.01, compared to normal control; compared to negative control. Data were analyzed by ANOVA followed by Tukey's test.



Graph 8: Effect of *Ginkgo biloba* on Intromission Latency in Clomipramine induced sexual dysfunction male rats. Group I: vehicle treated; Group-II: Clomipramine treated; Group-III: *Ginkgo biloba* treated. Values are the mean ± SEM, n = 6; p < 0.05 and d p < 0.01, compared to normal control: compared to negative control. Data were analyzed by ANOVA followed by Tukey's test.

DISCUSSION

Sexual activity is a multifaceted activity, involving complex interactions between the nervous system, the endocrine system, the vascular system and a variety of structures that are instrumental in sexual excitement, intercourse and satisfaction. Normal male sexual response cycle can be functionally divided into five interrelated sequence that occur in a defined sequence: libido, erection, ejaculation, orgasm, and detumescence. Problem anywhere in the entire sequence may lead to sexual dysfunction. Sexual dysfunction is a common side effect of psychoactive medications as well as a number of other frequently prescribed medications. Considerable attention has been recently focused on antidepressants, perhaps because of their widespread use and because they are often taken for long periods of time (e.g., months or years). Clomipramine is the imipramine analogue of chlorpromazine. Compared to other tricyclic antidepressants (TCA), it has a greater effect upon dopamine blockade and serotonin uptake inhibition. This has implications for prolactin release and orgasmic dysfunction mediated through $5HT_2$ receptors[15,16] (Blackwell, 1984 & Sorcher & Dilsaver, 1986). Moreover, peripheral antimuscarinic and alpha-adrenergic blockade effects have also been implicated in the mechanism of Clomipramine-induced orgasmic dysfunctions. Sexual dysfunctions such as decreased libido, delayed orgasm, difficulties in maintaining an erection, and inhibition of ejaculation are common side effects of Clomipramine.

CF, PEP, ML, MF, IF, IL EF and EL are useful indices of vigor, libido and potency.[10] An increase in ML (Graph 1) MF (Graph 2) reflects sexual motivation and increase in the number of IF (Graph3), IL (Graph-8) CF (Graph 4)EF (Graph 6)and PEP (Graph 7) shows the efficiency of erection, penile orientation and the ease by which ejaculatory reflexes are activated. Therefore, the increase in sexual parameters, enhanced sexual behavior of male rats. Results of sexual performance analysis showed that *G.biloba* extract remarkably increases the CF, PEP, MF, ML, IF, IL and EF, Although GBE has long been used to treat disorders involving restricted vascularity. There are many active agents found in GBE and it is unknown whether the therapeutic benefits of GBE are attributable to single active ingredients or the combined, or perhaps even synergistic, action of many ingredients. *Ginkgo biloba* leaves contain two major groups of substances: flavonoids (e.g., kaempferol, quercetin, isorhamnetin derivatives) and terpenes (e.g., ginkgolides, bilobalide).[17] Ginkgolides, which have not been found in any other living species, can be divided into several types (i.e., A, B, C) which differ only in the number and position of hydroxyl groups.[8]

Whereas in Clomipramine-induced experimental rats CF, PEP, MF, ML, IF,IL and EF decreases remarkably. Mount latency, Mount frequency, intromission frequency & latency and ejaculatory frequency & latency post ejaculatory pause are indicators of sexual motivation are inversely proportional to sexual motivation.[18] According to biological theory, serotonin reuptake inhibitors increase the accumulation of serotonin in the synapse, which lead to delayed or inhibited ejaculation and ejaculatory inhibiting effects of serotonin in the brain are enhanced.[19] Therefore, the decrease in the CF, PEP, MF, ML, IF, IL and EF observed in *G.biloba* extract treated animals might imply stimulation of sexual motivation and arousability as compared to Clomipramine treated group. It may also be an indication of enhanced sexual appetitive behavior in the male rats. All these facts further support the sexual function improving effect of the *G.biloba* extract.

CONCLUSION

In conclusion, the present study has demonstrated that *G.biloba* extract has potential to restore the normal sexual behavior in experimental animal.

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