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Antifertility proficiency of aqueous stem extract of *Tinospora cordifolia* (willd.) on seminal parameters of male albino mice

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Abstract:-

In this research the effect of aqueous stem extract of *Tinospora cordifolia* on epididymal sperm count, seminal pH, sperm motility, sperm mortality and abnormality of spermatozoa in male albino mice were studied. Mice were treated with 0.1 ml (200 mg /Kg bd.Wt.) of aqueous stem extract of *T. cordifolia* daily up to 60 days and sacrificed mice after12, 24, 36, 48 and 60 days of exposure. Mice after 60 days of treatment showed highly significant decrease ($P<0.001$) in sperm count, seminal pH and sperm motility, while highly significant ($P<0.001$) increase in sperm mortality and abnormality of spermatozoa were observed as compared to control group of mice. The finding of this research work is Suggest that, the aqueous Stem extract *T. cordifolia* cause antifertility among male albino mice.

Key word- Antifertility, Sperm Count, Sperm motility, Sperm mortality, Seminal pH, *Tinospora cordifolia*.

Introduction:-

India is the second most populous country in the world, next to China. India comprises more than 17.7 % of world's total population. The ever increasing human population is threatening not only the survival of many other species, but also its own survival. It is therefore the prime responsibility of each one of us to help educate people and take necessary steps to check our fast growing population. Control of population growth is very important in populated countries. Current methods of Contraceptation result in an unacceptable rate of unwanted side effects.

The objective of this research work is to search an ideal, specific, cheaper safe, effective, indigenous & reversible contraceptive agent of plant origin for male without any side effects. Various studies showed that many herbal plants have antifertility property. These plants have been used as antifertility agent for population control. Since

ancient time *T. cordifolia*is one of them has various medicinal properties and antitesticular activities. There are very less informations are available regarding antifertility property of *T. cordifolia.*

The stems are pungent bitter & show medicinal properties. The aqueous extract of *T. cordifolia* (stem) possess activities like antioxidant (Premanath et. al., 2010), antihyperglycemic (Puranik et. al., 2010), antineoplastic (Jagetia et. al., 2005), antistress activity (Singh et. al., 2003), antiasthmatic & chronic cough treatment (Spelman et. al., 2001), antileprosy (Asthana et. al., 2001), antihela cell activity (Jagetia et. al., 2006), antioxidant and antiinflammatory activity (Shwetha et. al., 2016), antidiabetic activity (Kammar et. al., 2010), antibacterial activity (Jeyachandran et. al., 2003), antimicrobial activity (Kommidi et. al., 2020), anti-HIV activity (Estari et. al., 2012), anti-osteoporotic activity (Tiwari et. al., 2018), antifertility effect (Gupta ea.al., 2003) etc . Therefore it is decided to study the antifertility proficiency of aqueous stem extract of *T. cordifolia* on seminal profile of male albino mice.

Material and Methods:-

Adult (age 12-14 weeks) Swiss albino mice weighing 25g to 30g were selected for the investigation. Mice were maintained under hygienic condition in well ventilated room. All animal were fed twice with bread, dalia, green vegetable, milk, and supplemented with germinated seed, nut, white bread, corn bread, along with tap water *ad libitum*. Male mice were kept in a polypropylene cage.

Fresh mature *T. cordifolia* stem were taken. These stem were washed in tap water and dried in shade. For making aqueous extract of Giloe stem, 50 gm of stem powder were dissolved in 1000ml of distilled water for dose 200mg/kg bd. wt. Doses were provided to male albino mice 0.1ml to the Group- B (Treated) animals (Singh et. al., 2011). In this investigation 60 male albino mice were selected and all mice were divided in two groups Group-A (control), Group- B (treated). Control and treated groups further divided into exposure days 12, 24, 36, 48 and 60 days. Each exposure days were contain six mice. Treated groups of mice were fed 0.1 ml of giloe stem extract orally by gastric catheter. While Group- A (control) group of mice were fed same amount of distilled water daily up to 60 days. Each group of mice were sacrificed after the exposure of aqueous stem extract of *T. cordifolia* (giloe) for 12, 24, 36, 48 and 60 days, while control group of mice were sacrificed after feeding with same amount of distilled water in similar exposure days as treated group. Mice were sacrificed by cervical dislocation method. After sacrifice each mice were operated and both the cauda epididymis of each male mice were exposed and kept in watch glass and tinged with 2ml of normal saline. Both cauda epididymis were crushed with the help of forcep for sperm count, seminal pH, sperm mortality, sperm motility and abnormality of Spermatozoa counts were done after the method of Eliasson (1975), while motility of spermatozoa were observed after the method of Tijee and Oentoeng (1968). pH of seminal fluid was measurd with the help of pH indicator, which was procured from Merck Limited Worly, Mumbai (Kumar et al., 2017).

Statistical Analysis: -

Student t-test was applied for the test of significance.

Results:-

In this research the effect of aqueous stem extract of *Tinospora cordifolia* on seminal parameters like sperm count, sperm motility, mortality, seminal pH, and abnormality of spermatozoa have been studied and presented in the Table -1.

In the present study, administration of aqueous stem extract of *T. cordifolia* up to 12 days the sperm count decreased significantly ($p<0.05$) up to ($224.16\pm3.62 \times 10^4$ Sperms/ml) as compared to control group of mice. During 24 days of treatment the sperm count decreased significantly ($p<0.001$) up to ($200.33\pm5.83 \times 10^4$ Sperms/ml) as compared to control group of mice. During 36 days of treatment the sperm count decreased significantly ($p<0.001$) up to ($178.83\pm4.8 \times 10^4$ Sperms/ml) as compared to control group of mice. During 48 days of treatment the sperm count decreased highly significantly ($p<0.001$) up to ($152.66\pm4.39 \times 10^4$ Sperms/ml) as compared to control group of mice. During 60 days of treatment of aqueous stem extract of *T. cordofolia* the sperm count decreased highly significantly ($p<0.001$) up to ($115.34\pm3.07 \times 10^4$ Sperms/ml) as compared to control group of mice.

In the present study, administration of aqueous stem extract of *T. cordifolia* up to 12 days of exposure the motility decreased significantly ($p<0.05$) up to (75.60 ± 1.64) as compared to control group of mice. During 24 days of treatment of aqueous stem extract of *T. cordofolia* the motility of spermatozoa decreased significantly ($p<0.001$) up to (62 ± 1.77) as compared to control group of mice. During 36 days of treatment of aqueous stem extract of *T. cordofolia* the motility of spermatozoa decreased significantly ($p<0.001$) up to (56.83 ± 1.06) as compared to control group of mice. During 48 days of treatment the motility of spermatozoa decreased highly significantly ($p<0.001$) up to (47 ± 2.56) as compared to control group of mice. During 60 days of treatment the motility of spermatozoa decreased highly significantly ($p<0.001$) up to (38.66 ± 1.8) as compared to control group of mice.

In the present study, administration of aqueous stem extract of *T. cordifolia* up to 12 days the mortality increased significantly ($p<0.05$) up to (24.4 ± 1.46) as compared to control group of mice. During 24 days of treatment the mortality increased significantly ($p<0.001$) up to (38 ± 1.32) as compared to control group of mice. During 36 days of treatment the mortality increased significantly ($p<0.001$) up to (43.16 ± 1.48) as compared to control group of mice. During 48 days of treatment the mortality increased significantly ($p<0.001$) up to (53 ± 1.24) as compared to control group of mice. During 60 days of treatment of aqueous stem extract of *T. cordofolia* the mortality increased highly significantly ($p<0.001$) up to (61.33 ± 1.55) as compared to control group of mice.

In the present study, administration of aqueous stem extract of *T. cordifolia* up to 12 days the seminal pH decreased significantly ($p<0.05$) up to (6.7 ± 0.14) as compared to control group of mice. During 24 days of treatment the seminal pH decreased significantly ($p<0.01$) up to (6.3 ± 0.15) as compared to control group of mice. During 36 days of treatment the seminal pH decreased significantly ($p<0.001$) up to (5.8 ± 0.21) as compared to control group of mice. During 48 days of treatment the seminal pH decreased significantly ($p<0.001$) up to (5.2 ± 0.10) as compared to control group of mice. During 60 days of treatment the seminal pH decreased highly significantly ($p<0.001$) up to (4.5 ± 0.19) as compared to control group of mice.

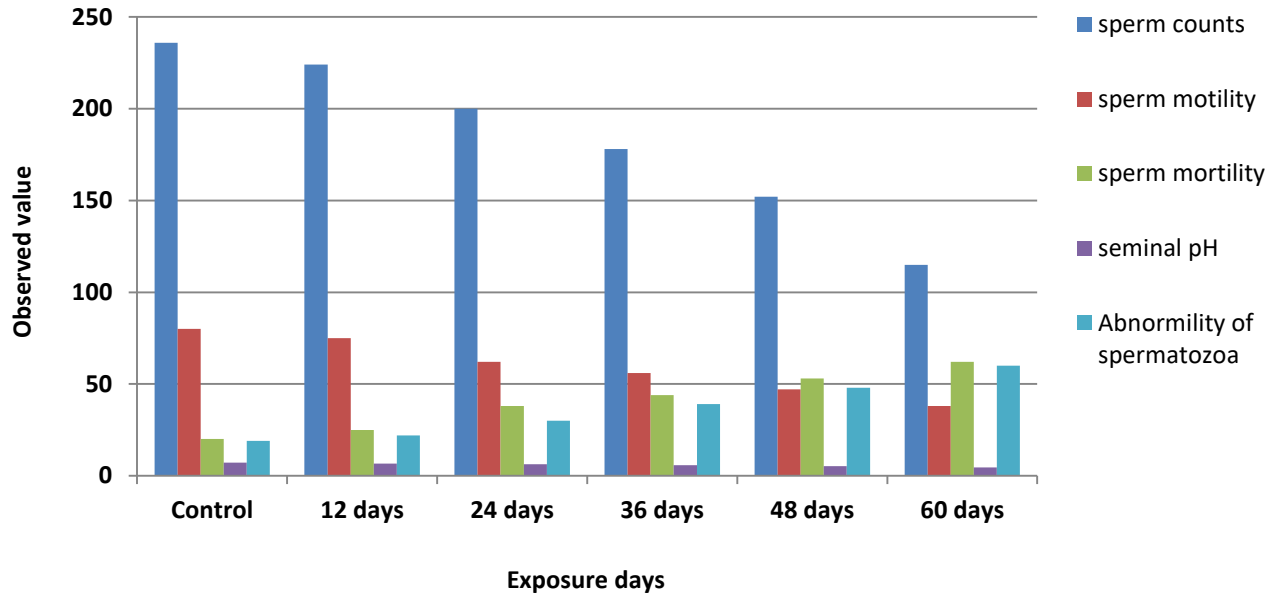
In the present study, administration of aqueous stem extract of *T. cordifolia* up to 12 days the abnormality of spermatozoa increased significantly ($p<0.05$) up to (22.83±1.12) as compared to control group of mice. During 24 days of treatment of aqueous stem extract of *T. cordofolia* the abnormality of spermatozoa increased significantly ($p<0.001$) up to (30.50±1.63) as compared to control group of mice. During 36 days of treatment the abnormality of spermatozoa increased significantly ($p<0.001$) up to (39.16±1.32) as compared to control group of mice. During 48 days of treatment the abnormality of spermatozoa increased significantly ($p<0.001$) up to 48±1.86) as compared to control group of mice. During 60 days of treatment of aqueous stem extract of *T. cordofolia* the abnormality of spermatozoa increased highly significantly ($p<0.001$) up to (60±1.55) as compared to control group of mice.

Table-1 showing the effects of aqueous stem extract of *Tinospora cordifolia* (Willd.) on Sperm Counts (x10^4 Sperms/ml), Motility Percent, Mortality Percent, Seminal pH, Abnormality of Spermatozoa percent of male albino mice.

Group	Sperm Counts (x10^4 Sperms/ml)	Motility (in Percent)	Mortality (in Percent)	Seminal pH	Abnormality of Spermatozoa (in percent)
Control	236.50±4.76	80.50±1.3	19.50±1.64	7.2±0.17	19.50±0.96
12 days	224.16±3.62*	75.6±1.64*	24.4±1.46*	6.7±0.14*	22.83±1.12*
24 days	200.33±5.83***	62±1.77***	38±1.32***	6.3±0.15**	30.50±1.63***
36 days	178.83±4.80***	56.83±1.06***	43.16±1.48***	5.8±0.21***	39.16±1.32***
48 days	152.66±4.39***	47±2.56***	53±1.24***	5.2±0.10***	48±1.86***
60 days	115.34±3.07***	38.66±1.80***	61.33±1.55***	4.5±0.19***	60±1.55***

Data presented as Mean± SEM; *, **, *** shows significance at 0.05, 0.01 and 0.001 levels with value in control. Number within parenthesis denotes number of samples.

Histogram showing the changes in Sperm Counts (x10^4 Sperms/ml), Motility, Mortality, Seminal pH, Abnormality of Spermatozoa during treatment of aqueous stem extract of *Tinospora cordifolia* in Swiss albino mice.



Discussion:-

In the present research, it was observed that sperm count decreased highly significantly ($p<0.001$) from 12 to 60 days of exposure in *Tinospora cordifolia* treated mice as compared to control group of mice. Reduction in sperm count indicates the interference in testicular spermatogenesis and the process is under surveillance of androgen; hence *Aegle marmelos* extract causes significant decrease in androgen level and it's also reduce in sperm counts, which lead antifertility proficiency among the treated group of mice(Kumar et al., 2017).

As indicated in Table-1 sperm motility and seminal pH also decreased significantly ($p<0.001$) in *T. cordifolia* stem extract treated group as compared to control group of mice. Decrease in sperm motility, is one of the main reason for antifertility proficiency.The decrease in sperm count associated with *T. cordifolia* can be attributed to hormonal modulation, potential oxidative stress, histological changes in testicular tissue, and its anti-inflammatory properties. While it has beneficial effects, caution is warranted due to its antifertility potential, especially for those concerned about reproductive health. In a similar study Kumari et al., (2017) reported that oral administration of aqueous extract of *Carica papaya* showed significant ($p<0.001$) decrease in sperm counts in treated group of mice than the control group of mice. In a similar study kumar et al. (2017) reported that oral feeding of aqueous extract of *Aegle marmelos* shows significantly ($p<0.001$) decrease in sperm counts in treated group of mice than the control group of mice. This suggest that *T. Cordofolia* causes decreased in sperm counts may be present that type of chemical which present in *Carica papaya* and *Aegle marmelos,* That leads to decreased in sperm counts. It might be possible due to the same reason the sperm count decreased in *T. cordifolia* treated group of mice.

In the present research, it was observed that, the administration of aqueous stem extract of *T. cordofolia* from 12 to 60 days of exposure cause highly significant ($p<0.001$) decreased in motility of spermatozoa as compared to control group of mice. Decrease in sperm motility suggests sperm maturation in epididymis and it also decreases the rate of fusion of female gamete (Lohiya& Goyal, 1992), which depend on androgen level (Satyaraj et al., 2010).

In this research, it was also observed that, the mortality of spermatozoa increased highly significant ($p<0.001$) from 12 to 60 days of exposure of aqueous stem extract of *T. cordifolia* as compared to control group of mice.. The increased percentage of mortality of spermatozoa in seminal fluid of treated group of mice may be due to androgen deficiency (Ahmed et al., 2002b). The increase in mortality may be due to low level of seminal pH; at low pH sperms are fragile (Pragya et al., 2012; Hembrom et al., 2011; Das et al., 2018). It might be possible that *T. cordifolia* decreased the pH level in semen of mice. Which increased the mortality of spermatozoa.

In the present investigation, it was observed that the pH of seminal fluid decreasedhighly significantly ($p<0.001$) after 12, 24, 36, 48 and 60 days of treatment as compared to control group of mice. Decrease in pH of seminal fluid also decrease sperm motility and increase sperm mortality of spermatozoa, then finally decreased in sperm count of treated group of mice. The mortality and abnormality of spermatozoa increased highly significantly ($p<0.001$) in the *T. cordifolia* treated group of mice. While direct studies specifically examining the effect of *Tinospora cordifolia* on seminal pH in mice may be sparse, its influence on reproductive health, antioxidant properties, and hormonal modulation suggests that it could have an indirect effect on the pH of semen. More focused research is needed to elucidate these relationships fully. If the pH of seminal plasma decreases, it become acidic and in acidic pH sperms became highly fragile that resulted into high rate of mortality of spermatozoa (Turner and Reich, 1985). If pH range is decreased the medium of seminal plasma become acidic and in acidic pH sperms are highly fragile that causes highly rate of mortality (Kumari et al. 2017). In another study Kumar et al., (2017) found that *Aegle marmelos* decreased seminal pH in treated group of mice. Which is one of the reason of increase sperm mortality and abnormality.

In this research, it was observed that, the abnormality of spermatozoa increased highly significantly ($p<0.001$) from 12 to 60 days of exposure of aqueous stem extract of *T. cordifolia* as compared to control group of mice. The mortality and abnormality of spermatozoa increased highly significantly after *Aegle marmelos* treatment (Kumar et al., 2017). Abnormal sperms are not able to fertilize the female ovum in the *Aegle marmelos* treatment (Lohiya & Goyal., 1992). In the similar study Pragya et al., (2016) proved that *Ocimum sanctum* increase sperm abnormality. In this study double headed, banana shaped, club shaped, tailless spermatozoa observed.

Conclusion:-

India is the second most populous country in the world, next to China. Therefore it is highly necessary to control over growing population in our country. Apart from the major taken by the government towards the growing population *T. cordifolia* could be a good herbal male contraceptive agent which is reversible, cheaper, easily available indigenous and without any side effects.

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Conflicts of interest

There are no conflicts of interest.

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