Experimental Assessment of Relative Efficacy of Drugs of Herbal Origin on Sexual Performance and Hormone Levels in Alcohol Exposed and Normal Rats

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ABSTRACT
Alcohol can affect the hypothalamo-pituitary-gonadal axis leading to impotence and sterility by various mechanisms of actions. In this study evaluation of the effect of three herbomineral formulations, alone and as combinations was done in alcohol exposed and normal rats. The preparations used showed beneficial effects of various degrees with respect to the sexual behavior of animals. There was also improvement in the number of LH-FSH producing basophil cells in the pituitary and raised levels of circulating testosterone. The mean sperm count was also higher in the drug treated animals.

Keywords: alcohol; sexual dysfunction; sperm count; TF (Tentex forte); TR (Tentex Royal); SP (Speman); testosterone; LH-FSH.

INTRODUCTION
Until recently, alcohol induced liver disease was considered to be primarily responsible for sexual dysfunction in alcoholic men. However, recent findings clearly show that sexual changes observed in chronic alcoholic men are the result of alcohol abuse per se (Van Thiel and Gavaler, 1985). Ethanol impairs testosterone production by being a direct testicular toxin and by interfering with pituitary luteinizing hormone secretion (Van Thiel and Gavaler, 1982).

Naloxone-induced release of luteinizing hormone releasing hormone (LHRH) as evaluated with LH levels were blocked by alcohol in rats. In this same species, the castration-induced rise in LH was blocked by alcohol (Cicero 1982) but restored by simultaneous administration of LHRH, further suggesting a hypothalamic site of action of alcohol (Cicero et al., 1978). A direct effect of alcohol on the pituitary has not been substantiated (Noth and Walter, 1984).

On the other hand alcohol induces testicular damage also, probably due to the toxic effects of its intermediate metabolite acetaldehyde (Badr et al., 1977). In chronic alcohol fed rats, testicular atrophy is associated with reduced plasma testosterone levels. Alcohol interferes with testosterone synthesis by reducing the number of LH binding testicular receptors (Bhalla et al., 1979) and also by interfering with the enzymes involved in testosterone biosynthesis (Cicero and bell, 1980; Gordon et al., 1980; Johnston et al., 1981).

SP is an Ayurvedic formulation used in male infertility due to oligospermia (Rajasekharan, 1979). TF is another Ayurvedic preparation useful in impotent men (Khandare et al., 1982). In this study the comparative efficacy of these two preparations along with a new and improved formulation of TF called TR was evaluated for reversing the deleterious effects of alcohol on the male reproductive system in rats. SP in combination with TF and TR was also tried to see if there was any potentiation of the efficacy of one drug by the other.
MATERIALS AND METHODS
Male Wistar rats bred in our laboratory for 45 generations were divided into two groups from birth. One group received in addition to standard laboratory diet, 6% alcohol in feeding bottles. Experimental trials were carried out when the animals were 2.5-3 months old and weighing between 175-200 g. The rats were housed in colony cages at an ambient temperature of 25°C±2°C and 45%-55% relative humidity with a 12 h light-dark cycle. All experiments were conducted between 0900 and 1400 h.

Test drugs: All test drugs were polyherbal formulations and herbs in each drug were procured from authentic sources and were identified by botanists. SP consists of medicinal plants, namely, Orchiis mascula Linn. (Orchidaceae; root) 65 mg, Astercantha longifolia Nees. (Acanthaceae; seed) 32 mg, Argyreia speciosa sweet. (Convolvulaceae; root) 32 mg, Tribulus terrestris Lonn. (Zygophyllaceae; fruit) 32 mg, Leptadenia reticulata W&A (Asclepiadaceae; whole plant) 32 mg. TF contains the following medicinal plants and mineral, namely, Hibiscus abelmoschus Linn. (Malvaceae: seed) 10 mg, Withania somnifera Dunal. (Solanaceae: root) 65 mg, Argyreia speciosa sweet. (Convolvulaceae: root) 32 mg, Mucuna pruriens Bak. (Papilionaceae: seed) 32 mg and Shilajeet (purified rock ooze) 32 mg. TR contains medicinal plants namely, Anacyclus pyrethrum DC. (Compositae: root) 20 mg, Asparagus adscendent Roxb. (Liliaceae: root) 20 mg, Aconitum napellus Linn. (Ranunculaceae: root) 5 mg and Piper betle Linn. (Piperaceae: leaf) 10 mg. The animals in both normal and alcohol treated groups received TF, TR, SP, SP + TR and SP + TF respectively in a dose of 500 mg/kg body weight as an aqueous suspension orally daily for 21 days. The control animals in both groups received tap water for the same duration of time.

The second set of rats (on normal conditions) after 21 days of drug treatment was put on a washout period of 30 days. Testosterone levels before and after the washout period were analyzed. Blood was collected for testosterone assay between 1200 and 1400 h. At the end of the trial period the following parameters were measured in the rest of the animals.

Mounting index: This was done in a specially designed box. Two female rats at proestrus were kept in the box and one male rat was introduced into the box. The rats were identified by picric acid markings. After a 15 min acclimatization period mounting was observed for 45 min and the number of mounts counted.

Total sexual behavior (TSB): This was also assessed after the following 15 min for acclimatization in the mounting box with two female rats per male rat. Male sexual behavior such as genital grooming and sniffing at females was visually monitored and recorded (Pednekar et al., 1993). Both mount test and TSB were done at the beginning and at the end of the trial and percent rise was calculated.

Sperm count: Single cauda of the epididymis was excised and punctured and the fluid was collected in RBC pipette and diluted with phosphate buffered saline (pH 7.1). The sperm count was done on a Neubauer’s chamber (Mortimer, 1985).

Serum testosterone levels: These were assessed at the end of the trial period in all animals by RIA methods (Kirschcher and Jacobs, 1971; Anletta et al., 1974).
**Histology:** Histological sections of anterior pituitary gland were taken for all animals and stained by BR (bromine water), AB (alcian blue), OFG (organe g-acid fuchsingreen) method (Bancroft and Cook, 1984) for basophil cells producing LH, FSH and ACTH. In addition sections of testes were stained with haemotoxylin and eosin and examined. All the results were subjected to statistical analysis by an unpaired Student’s t-test. The minimum level of statistical significance was set at \( p<0.05 \).

**RESULTS**

**Alcohol exposed rats**

Total sexual behavior and mounting index were significantly higher in the TR treated group compared with the alcohol placebo group (Table 1). A significant improvement of these parameters was also seen in the other drug treated groups although to a lesser degree than that with TR.

The sperm count of the drug treated rats showed a significant rise compared with the alcohol placebo (i.e. TR, TF, SP + TF and SP + TR \( p<0.0001 \), SP \( p<0.001 \)) (Table 1).

<table>
<thead>
<tr>
<th>Table 1: Effect of herbal drugs on sexual performance and hormone levels in alcohol exposed rats (values are mean ± SEM)</th>
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<tbody>
<tr>
<td>Parameter</td>
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<tr>
<td>----------------------------------</td>
</tr>
<tr>
<td>Total sexual behaviour</td>
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<tr>
<td>Mounting index</td>
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<tr>
<td>Sperm count (10(^6) mL(^-1))</td>
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<tr>
<td>Serum testosterone (ng/mL)</td>
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</table>

\( n=6 \) rats in each experimental group; statistical significance (\( p \)): \(^a\)<0.01, \(^b\)<0.005, \(^c\)<0.001, \(^d\)<0.0001 compared with alcohol placebo.

The hormone levels in the treated group of animals showed a significant increase in testosterone production compared with the control rats (Tables 1 and 2).

<table>
<thead>
<tr>
<th>Table 2: Effect of herbal drugs on sexual performance and hormone levels in normal rats (values are mean ± SEM)</th>
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<tr>
<td>Parameter</td>
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\( n=6 \) rats in each experimental group; statistical significance (\( p \)): \(^a\)<0.01, \(^b\)<0.005, \(^c\)<0.001, \(^d\)<0.0001 compared with alcohol placebo.
Table 3: Effect of herbal drugs on % cell type counts in pituitaries of alcohol exposed rats (values are mean ± SEM)

<table>
<thead>
<tr>
<th>Cell types</th>
<th>Normal control</th>
<th>Alcohol placebo</th>
<th>TF</th>
<th>SP</th>
<th>TR</th>
<th>SP + TF</th>
<th>SP + TR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basophils (S) (ACTH)</td>
<td>0.495 ± 3.90</td>
<td>0.715 ± 2.01</td>
<td>0.252 ± 1.52</td>
<td>0.325 ± 3.69</td>
<td>0.375 ± 3.62</td>
<td>0.294 ± 1.75</td>
<td>0.315 ± 3.24</td>
</tr>
<tr>
<td>Basophils (R) (FSH, LH)</td>
<td>0.445 ± 4.55</td>
<td>0.275 ± 3.75</td>
<td>0.415 ± 2.74</td>
<td>0.485 ± 3.70</td>
<td>0.545 ± 4.50</td>
<td>0.445 ± 2.60</td>
<td>0.70 ± 5.39</td>
</tr>
</tbody>
</table>

n=6 rats in each experimental group; statistical significance (p): a<0.01, b<0.005, c<0.001, d<0.0001 compared with alcohol placebo.

Histopathological evaluation of the anterior pituitary revealed an improvement in the cellular morphology which was in the order of TR>SP+TR>SP+TF>TF>SP. The cell count of the LH-FSH producing basophils was higher, while there was a reduction in the population of ACTH producing cells (Table 3) in the drug treated group when compared with the control group.

Histological examination of the testicular tissue showed atrophic changes in the alcohol treated rats. These changes were effectively reversed by all five trial drug combinations.

Normal rats
Total sexual behaviour and mounting index were significantly higher in the TR and SP+TR treated groups compared with the placebo. There was a two-fold increase in the sperm count in the TR and SP+TR treated groups. The testosterone levels in the treated group of animals showed a significant rise compared with the placebo rats (p<0.005) Table 2.

After 30 days of washout period the testosterone levels had decreased in all the drug treated groups to near normal levels.

DISCUSSION
This study clearly indicates that the ill effects of alcohol on the hypothalamo-pituitary-gonadal axis can be successfully reversed by using these herbomineral preparations, namely TF, TR and SP. All these formulations contain herbs renowned for their beneficial effects in male impotence and sterility.

Male sexual behavior depends on the circulating levels of testosterone in the blood. Improvement in mounting index and total sexual behavior in this study after treatment with the various drug combinations indicate that the drugs probably act by raising the testosterone levels. Testosterone levels were also found to be raised.

The maximal testosterone levels were seen with TR in the alcohol fed rats and TR with SP in the normal rats. The testosterone level in the TR treated group in alcohol fed rats was comparable to that of normal control rats.

Alcohol has direct toxic effects on testicular tissue as well as an inhibitory effect on the hypothalamic release of LHRH and FSHRH. These herbal combinations probably act at both levels to bring about a rise in the hormone levels. The histological picture of the anterior pituitary reveals abundance in the number of LH and FSH producing cells, which would not be the case if the drugs were acting on the testosterone producing Leydig cells alone.
In addition the improvement in sperm count indicates the increased activity of FSH producing cells. A reduction in basophil(S) cells in the anterior pituitary after drug treatment was also seen, indicating the reversal of alcohol induced hyperstimulation of the adrenal cortex, thus leading to normalization of corticosteroid hormones in blood.

Microscopic examination of the testicular tissue also showed that the atrophic changes due to alcohol were reversed by treatment with the herbal formulations.

The same effects were also seen in normal rats, indicating that the drugs can act even in normal animals. While the improvement in the mean sperm count was about three fold in alcohol fed rats, it was less than double in normal rats indicating that the drugs act optimally in a deficient state and the effect is not all that pronounced in normal animals.

The hormonal effects of these herbal preparations were totally reversible on cessation of therapy, thus ruling out a placebo effect.

Hence it can be concluded that these preparations, particularly TR and a combination of SP and TR can reverse the effects of alcohol in alcohol induced hypogonadism and reverse the hypersecretion of ACTH in rats. Further clinical trails are under progress to study the effects in human beings.

REFERENCES


