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ANTIOXIDANT AND ANTI-INFERTILITY EFFICACY OF N-MIRACLE (POLYHERBAL FORMULATION) IN ETHANOL INDUCED INFERTILITY IN MALE ALBINO RATS

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ABSTRACT

The aim of this research was to investigate In-vivo enzymatic antioxidant and anti-infertility efficacy of N-Miracle (Polyherbal formulation) in ethanol induced infertility in male albino rats. A total of 30 male albino rats were selected, divided in to five groups. Ethanol induced testicular damage was done on Group III, IV, and V. The present study exhibited the enzymatic antioxidant impact of N-Miracle (Polyherbal formulation) against ethanol induced infertility in rats by increasing the levels of enzymatic antioxidants such as superoxide dismutase, catalase, glutathione peroxidase and decreasing the activity of Glutathione-S-transferase in the liver. In the same time the polyherbal formulation increases the levels of enzymatic antioxidants such as superoxide dismutase, catalase, glutathione peroxidase and decreasing the activity of Glutathione-S-transferase in the kidney. The present study showed androgenic effect of N-Miracle (Polyherbal formulation) by increasing testosterone and decreasing estrogen, LH, and FSH in ethanol induced infertile rats.

KEYWORDS: Antioxidant, N-Miracle, Polyherbal formulation, Ethanol, Infertility, Testosterone.

INTRODUCTION

Infertility is “a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse (The

International Committee for Monitoring Assisted Reproductive Technology - World Health Organization, 2009). Male infertility is a world-wide medical and social problem. The fact that more than 15 percent couples worldwide are affected by infertility speaks the volume about worsening reproductive health worldwide (World Health Organisation, Biennial Report, 1992-93).[1]

Recently, the substances in the environment that can bother male fertility have been increased. Ethanol is among the most widely abused drug, which can destroy reproductive function and sexual behaviour.[2] Alcohol abuse is considered as one of the problems associated with poor semen production and sperm quality.[3] The lack of sexual craving in long-term alcohol users has been reported from 31 to 58%.[4] [5] [6] Whalley (1978) reported that about 54% of hospitalized alcoholic men and 24% of healthy controls had erectile dysfunction. Alcohol abuse is well known to damage reproductive performance in experimental animals and also in human beings. Alcoholics are found to have fertility anomalies, with low sperm count, impaired testosterone production, and testicular atrophy.[7]

Alcohol is toxic for testes and causes fertility disorders through low sperm count and motility in men.[8] Chronic ethanol exposure decreases plasma testosterone level and cause testicular atrophy.[9], [10] Ethanol significantly enhances lipid peroxidation in the testis and inhibits the conversion of both dehydroepiandrosterone and androstenedione to testosterone by reducing the activities of 3-beta hydroxyl steroid dehydrogenase.[11]

Long-term effects of chronic alcohol abuse come along with gynecomastia, impotence, testicular atrophy, and loss of libido, as it had been reported before.[12], [13], [6] Ethanol consumption also produces a considerable decrease in the percentage of motility, concentration[8], and normal morphology in human and animal spermatozoa.[14] Martinez et al.,[15] reported histological abnormalities in testicular tissue of alcoholic animals. These included severe intercellular spaces, improper diameter of the seminiferous tubules, and high number of necrotic cells in the lumen compared with controls. In addition, they showed that the epididymal sperm motility is declined in ethanol-treated rats. Brzek[16] reported a reduction of semen volume, density, and motility was caused by alcohol consumption. In addition, it was stated that daily alcohol consumption decreases normal sperm morphology.[17] Ethanol users showed a substantial decrease in mean sperm count, ranging from 113 to 66 million/mL.[18]

The medicinal plants, which contain high number of polyphenols, are deemed to be a good supply of natural antioxidant compounds and more often possess higher antioxidant potential than that of dietary fruits and vegetables. Consumption of these plant products certainly inhibits the free radical mediated damage of cell and therefore protects the body from several health problems.[19] Research during the past twenty years has stretched focus on impotence (erectile failure), premature ejaculation and male infertility. There are a number of prescription drugs which may act as sex stimulant and increasing the sexual desire and activity in both men and women. Although the use of allopathic medicines has shown considerable improvement in treating sexual disorders, but at the same time there are large number of contraindications noted. These include irregularities of the rhythm of the heart, suicidal tendencies, mental disorders and tremors.[20] Thus, there is mounting need to look for aphrodisiacs more of herbal origin as opposed to synthetic compounds. In Ayurveda, single or multiple herbs (polyherbal) are used for the treatment. The concept of polyherbalism is to attain greater therapeutic effectiveness. The active phytochemical constituents of individual plants are not sufficient to achieve the desirable therapeutic effects.

When combining the multiple herbs in a particular ratio, it will give a better therapeutic efficacy and reduce the toxicity.[21]

N-Miracle, a polyherbal formulation, was prepared by the combination of the following medicinal plants.

A. *Conium maculatum* L.

B. *Lycopodium Clavatum.*

C. Selenium.

D. Vitex agnus castus L.

E. *Pausinystalia yohimbe.*

F. *Caladium seguinum.*

The above mentioned medicinal plants have diversified pharmacological effect, however, the anti-infertility effect of the combination of the above plants are not yet carried out. Hence, the present study has been designed with the following results.

MATERIALS AND METHODS

Combination of N-Miracle (Polyherbal Formulation)

N-Miracle, a polyherbal formulation, was prepared by the combination of the following medicinal plants in Sai Brindavan Clinic, Omalur Main Road, Salem, Tamil Nadu, India. Each 100 g contains the following composition:

A. *Conium maculatum* L.

B. *Lycopodium Clavatum.*

C. Selenium.

D. Vitex agnus castus L.

E. *Pausinystalia yohimbe.*

F. *Caladium seguinum.*

Drugs and Chemicals

N-Miracle (Polyherbal formulation) was provided by Dr. Ramesh Shankar, Sai Brindavan Clinic, Omalur Main Road, Salem, Tamil Nadu, India as a gift sample and it was used to carry out the research work. All other chemicals and reagents used in the present study were obtained commercially and were of analytical grade.

Experimental Animals

Male Wistar albino rats weighing 150-200 g were used for the study. The animals were fed with commercial pellet feed and water was given ad libitum. The animals were subjected to a 12:12 h light: dark cycle under standard laboratory conditions at a temperature of 24-28 °C with a relative humidity of 60%-70%. The animal experiments were carried out as per the guidelines of Institutional Animal Ethical Committee (CPS/IAEC/AH/P/19/20).

Experimental Design

After one week of acclimatization period, male albino rats were divided randomly into five groups of six animals each.

Group I: Normal control rats were treated with oral dose of distilled water for 30 days.

Group II: Rats were treated with N-Miracle (Polyherbal formulation) 20 mg/kg body weight/day for 30 days.

Group III**:** Rats were treated with ethanol 20%v/v, 1.6 g/ kg body weight/day for 30 days.

Group IV**:** Rats were treated with ethanol as given in Group III for 15 days. After ethanol induction for 15 days, N-Miracle (Polyherbal formulation) was given as given in Group II for next 15 days.

Group: V: Animals were treated with ethanol as given in Group III and N-Miracle (Polyherbal formulation) as given in Group II simultaneously for 30 days.

Blood Samples

After the treatment period, the rats were anaesthetized by light ether anaesthesia in lethal chamber. Blood was collected in a dry test tube and allowed to clot at ambient temperature for 30 min. Serum was separated by centrifugation at 3500 rpm for 10 min for fertility hormone analysis.

Tissue Homogenate Preparation

The collected liver and kidney tissues of different experimental groups were homogenized in Tris-HCl buffer (0.1M, pH 7.4) using a Teflon homogenizer at 4°C, the crude tissue homogenate was then centrifuged at a speed of 9000 rpm for 15 minutes in cold centrifuge, the supernatant was kept at -20°C for assay of enzymatic antioxidant analysis.[22]

Enzymatic Antioxidant Analysis

Superoxide dismutase in the tissues homogenate was assayed by the method of Kakkar et al., 1984.[23] The activity of catalase in the tissues homogenate was determined by the method of Sinha in the year of 1972.[24] The activity of GPx in the tissues homogenate was measured by the method of Rotruck et al., 1973.[25] Glutathione-S-transferase activity was measured by the method of Habig et al., 1974.[26]

Fertility Hormone Analysis

The serum Testosterone, Estradiol, Luteinizing Hormone and Follicle Stimulating Hormone concentration was quantitatively determined using the direct human serum testosterone enzyme immunoassay kit as outlined in manufacturer's protocol. The determination was based on the principle of direct assay of limited (competitive) type following the general Antibody-Antigen reaction based on ELISA as described by Tietz, 1995 Span Diagnostics, Surat, India.[27]

Statistical Analysis

The results were expressed as the mean value ± SD. Group comparisons were performed by using one-way analysis of variance (ANOVA) test. Significant difference between normal control and experimental groups were assessed by student′s t-test. A probability level of less than 5% ($P<0.05$) was considered as significant.[28]

RESULTS

Enzymatic Antioxidant in Liver Tissues in Different Experimental Groups of Rats

In rats treated with ethanol (Group III), there was significant ($p<0.05$) decrease in SOD, CAT, GPx levels as compared to control animals (Group I). There was a significant ($p<0.05$) increase in SOD, CAT, and GPx levels in N-Miracle treated animals (Group IV). During combined treatment of ethanol and N-Miracle (Polyherbal formulation) (Group V), there was a significant ($p<0.05$) increase in SOD, CAT, and GPx levels as compared to ethanol treated rats, which indicated the combined effect of N-Miracle (Polyherbal formulation). We found that there was significant ($p<0.05$) increase in GST levels in Group III as compared to Group I. We also found that there was significant ($p<0.05$) decrease in GST levels in Group IV. In Group V, there was a significant ($p<0.05$) decrease in GST levels as compared to ethanol treated rats, which indicated the combined effect of N-Miracle (Polyherbal formulation).

Table 1: Enzymatic Antioxidant in Liver Tissues in Different Experimental Groups of Rats.

Groups	SOD (U/min/mg of protein)	CAT (µmol /min/ mg of protein)	GPx (µmol /min/ mg of protein)	GST (µmol/min/mg protein)
I	9.45 ± 0.75^{a}	75.75 ± 4.89^{a}	11.28 ± 1.10^{a}	3.13 ± 0.43^{a}
II	9.25 ± 0.60^{a}	76.86 ± 5.25^{a}	10.80 ± 0.90^{a}	3.28 ± 0.43^{a}
III	4.30 ± 0.44^{b}	45.10 ± 3.90^{b}	4.29 ± 0.19^{b}	6.36 ± 0.20^{b}
IV	8.90 ± 0.45^{a}	73.90 ± 4.15^{a}	10.78 ± 0.80^{a}	3.90 ± 0.35^{a}
V	9.18 ± 0.86^{a}	72.49 ± 6.35^{a}	11.10 ± 0.12^{a}	4.10 ± 0.2^{a}

Values were mean ± SD of six rats

Values not sharing a common superscript differ significantly at $P < 0.05$

Enzymatic Antioxidant in Kidney Tissues in Different Experimental Groups of Rats

There was significant ($p<0.05$) decrease in SOD, GPx levels in ethanol induced rats (Group III) as compared to normal control animals (Group I). There was significant ($p<0.05$) increase in SOD and GPx levels in rats treated with N-Miracle after ethanol induction for 15 days (Group IV) as compared to ethanol induced animals (Group III). During combined induction of ethanol and N-Miracle (Polyherbal formulation) (Group V), there was a significant ($p<0.05$) increase in SOD and GPx levels as compared to only ethanol induced animals (Group III), which indicated the combined effect of N- Miracle (Polyherbal formulation). There was significant ($p<0.05$) increase in catalase and GST levels in ethanol induced rats (Group III) as compared to control animals (Group I). There was a significant ($p<0.05$) decrease in GST levels in N-Miracle (Polyherbal formulation) treated animals (Group IV)

compared to ethanol alone treated animals (Group III). There was a significant ($p<0.05$) decrease in GST levels in co-treatment group (Group V) as compared to ethanol treated rats (Group III), which indicated the combined effect of N-Miracle (Polyherbal formulation).

Table 2: Enzymatic Antioxidant in Kidney Tissues In Different Experimental Groups of Rats.

Groups	SOD (U/min/mg of protein)	CAT (µmol /min/ mg of protein)	GPx (µmol /min/ mg of protein)	GST (µmol/min/mg of protein).
I	15.55±0.95[a]	38.19±2.75[a]	8.25±0.65[a]	6.35±0.30[a]
II	15.65±0.75[a]	38.78±2.25[a]	8.15±0.45[a]	6.76±0.58[a]
III	12.10±0.50[b]	46.54±1.95[b]	5.23±0.20[b]	7.18±0.58[b]
IV	14.85±0.45[a]	37.19±2.20[a]	7.97±0.50[a]	6.32±0.32[a]
V	14.96±0.28[a]	35.69±2.85[a]	8.12±0.28[a]	6.90±0.59[a]

Values were mean ± SD of six rats

Values not sharing a common superscript differ significantly at $P < 0.05$

Testosterone, Estrogen, LH, and FSH in Different Experimental Groups of Rats

The results showed there was a significant ($p<0.05$) decrease in testosterone in rats treated with ethanol (Group III) as compared to control group (Group I). There was a significant ($p<0.05$) increase in testosterone in rats treated with N-Miracle (Polyherbal formulation) (Group IV) as compared to ethanol induced rats (Group III). In rats treated with ethanol and N-Miracle (Polyherbal formulation) simultaneously (Group V), there was a significant ($p<0.05$) increase in testosterone as compared to ethanol treated groups (Group III), which indicated the combined effect of N- Miracle (Polyherbal formulation). On the other hand, our results showed that there was a significant ($p<0.05$) increase in estrogen, LH, and FSH in ethanol treated rats (Group III) as compared to control group animals (Group I). There was a significant ($p<0.05$) decrease in estrogen, LH, and FSH in N-Miracle (Polyherbal formulation) treated rats (Group IV) as compared to ethanol induced animals (Group III). Rats treated with ethanol and N-Miracle (Polyherbal formulation) co-treatment group (Group V), there was a significant ($p<0.05$) decrease in estrogen, LH, and FSH as compared to Group III, which indicated the combined effect of N- Miracle (Polyherbal formulation).

Table 3: Testosterone, Estrogen, LH, and FSH in Different Experimental Groups of Rats.

Groups	Testosterone (ng/mL)	Estrogen (pg/mL)	LH (µIU/mL)	FSH (µIU/mL)
I	5.11±0.20[a]	24.70±2.0[a]	4.10±0.15[a]	1.22±0.07[a]
II	5.10±0.18[a]	25.10±3.2[a]	3.97±0.16[a]	1.28±0.08[a]
III	3.25±0.10[b]	26.73±1.80[b]	6.70±0.10[b]	2.20±0.05[b]
IV	5.05±0.21[a]	24.80±2.80[a]	4.07±0.21[a]	1.24±0.07[a]
V	5.02±0.18[a]	24.95±0.56[a]	4.17±0.92[a]	1.20±0.05[a]

Values were mean ± SD of six rats

Values not sharing a common superscript differ significantly at $P < 0.05$

DISCUSSION

Enzymatic Antioxidant in Liver Tissues in Different Experimental Groups of Rats

The decrease of this endogenous antioxidant was obviously connected with ethanol-induced oxidative stress, which was characterized by the generation of toxic acetaldehyde and other reactive molecules in the cell. The obtained result agrees with the findings of Hussain et al., 2001[29] and Molina et al., 2002[30] who reported that chronic ethanol treatment caused a significant decrease in hepatic antioxidant enzymes level. In the present study, ethanol treatment induced a significant decrease in SOD, CAT, glutathione peroxidase activities in the liver of rats when compared to the control group. These changes were markedly reversed by treatment with N-Miracle (Polyherbal formulation). The reduction in the activities of these antioxidant enzymes might be due to the inhibition of their synthesis by some reactive molecules generated during ethanol metabolism. It could also be as a result of oxidation of the enzymatic proteins by the generated reactive oxygen species. Glutathione-S-transferase (GST) was a very important enzyme that plays a crucial role in the detoxification and metabolism of many foreign and endobiotic compounds.[31]

Increase in GST activity was likely a defensive response to detoxify the toxic metabolites produced in the course of ethanol metabolism. When compared with rats treated with ethanol alone, co-treatment of rats with N-Miracle (Polyherbal formulation) and ethanol significantly reduced the hepatic GST activity. The reduction in the hepatic GST activity might be attributed to the antioxidant properties associated with the phenolic and flavonoid compound in N-Miracle (Polyherbal formulation), which enabled it to protect the liver against the dilapidating effect of ethanol. The result of the current study is in agreement with the findings of Ighodaro and Omole study.[32] The positive alteration of enzymatic antioxidant in liver

tissue might be due to presence of *Lycopodium clavatum* (one of herb present in our polyherbal formulation) and this was supported by Banerjee et al.[33]

Enzymatic Antioxidant in Kidney Tissues in Different Experimental Groups of Rats

Catalase catalyses the dismutation of hydrogen peroxides. Its activity increased after of ethanol exposure. Decrease in the activity of GPx in the present investigation might be due to exhaustion or inactivation of the enzyme by reactive oxygen species after chronic ethanol treatment for longer duration. The kidney antioxidant defence system of the rat reacts positively to the ethanol toxicity by increasing the GST activity. The result obtained in the current study was in agreement with Das et al.[34] Dinu et al.[35] proposed that GST activity was increased after a longer period of treatment. The kidney antioxidant defence system of the rat reacts positively to the ethanol toxicity by increasing the GST activity. The results of the current study indicates that there was only slight increase in GST because this enzyme plays an essential role only in liver in comparison with kidney tissue, this was once again in agreement with Das et al.[34]

Testosterone, Estrogen, LH, and FSH in Different Experimental Groups of Rats

Generally elevated testosterone levels enhance sexual behaviour. Muthusami and Chinnaswamy, 2005[36] revealed that testosterone which was essential for spermatogenesis was decreased by chronic consumption of alcohol. Alcohol has a direct toxic effect on the testis which leads to decreased seminiferous tubular function. Alcohol's effects on the anterior pituitary gland produced a decrease in the production of LH and FSH. The finding of the current study correlates with Ren et al., 2005.[37]

Oremosu and Akang,[38] states that alcohol causes testicular toxicity by increasing free radicals or reactive oxygen species and lipid peroxidation. Maneesh et al., 2006[39] reported that the oxidative stress created in the Leydig cells as a consequence of chronic alcohol exposure diminishes the steroidogenic capacity of the testes, lowering circulating testosterone levels.

The decreased testosterone in ethanol induced rats in the current study might due to oxidative stress created in Leydig cell due to adverse effect of ethanol on this type of cells. The present result demonstrated significant increase in testosterone in N-Miracle (Polyherbal formulation) treated rats might be due to antioxidative effect of the Polyherbal formulation. In normal physiology, FSH stimulates spermatogenesis and LH stimulates synthesis and release of

testosterone. Testosterone causes an increased blood flow and stimulates the growth of the target tissues. Testosterone cause direct stimulation of spermatogenesis. Our results also show that there was increase in spermatogenesis and increase in weight of sexual organ in treated group as comparison to control group. The improvement of in-vivo sperm count suggests an improved spermatogenic activity of N-Miracle (Polyherbal formulation) and the current study was in accordance with Sharma et al., 2009.[40]

Banerjee et al., 2014[33] suggested that *Lycopodium clavatum* was most effective homeopathic remedy in treating impotency of young men, and the herb was one of the active component of N-Miracle (Polyherbal formulation) and the curative efficacy of N-Miracle (Polyherbal formulation) in male infertility might be due to the presence of *Lycopodium clavatum* present in N-Miracle (Polyherbal formulation).

In conclusion, infertility was the failure of a sexually active, non-contracepting couple to achieve pregnancy in one year. Over the past years the number of persons suffering from infertility was increasing. Ethanol was generally regarded as a reproductive toxin. Humans have consumed alcoholic beverage since prehistoric times for a variety of reasons. Alcoholic beverages were found to affect different system of the human body including reproductive system. It had been observed that chronic alcohol was common among infertile men. The testis had been shown to be highly susceptible to ethanol as it crosses the blood testis barrier. Chronic ethanol consumption causes sexual defunct. Early histological studies indicated that testis might be even more sensitive to ethanol than the liver. Animal experiment in the current study unveiled the protective efficacy of N-Miracle (Polyherbal formulation) in ethanol induced infertility in male albino rats in respect to enzymatic antioxidants and hormonal profile. Thus, N-Miracle (Polyherbal formulation) can be used for male infertility treatment. Further study is required to demonstrate the anti-infertility efficacy of N-Miracle (Polyherbal formulation) in patients with infertility.

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