MISJ-Journal of Medical Research and Allied Sciences
Available Online at http://ijmraas.misj.net Volume 01|Issue 02 (**May-August**)|2023 Page: 80-94
Original Research Paper

PROTECTIVE ROLE OF GARLIC TO ENHANCE SEMEN QUALITY OF RABBITS TREATED WITH TRAMADOL

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Article Received: 01-05-2023 Revised: 13-05-2023 Accepted: 05-06-2023

ABSTRACT:
Garlic contains more than 200 chemicals. It contains sulfur compounds (allicin, alliin and agoene), volatile oils, enzymes (allinase, peroxidase and miracynase), carbohydrates (sucrose and glucose), and minerals (selenium). Tramadol is a synthetic centrally active opioid analgesic used to manage moderate to severe pain. It has dual mechanism of action. It works by binding to μ-opioid receptors in the brain and spinal cord. Therefore, the present experiment was undertaken to determine the effectiveness of garlic in alleviating the toxicity of tramadol on body weight, reproductive performance and testesteron of male rabbits. Animals were assigned to 1 of 4 groups: control; 50mg tramadol/kg bw; 40 mg garlic/kg bw; tramadol (50 mg/kg bw) plus garlic (40 mg/kg bw), respectively. Results showed that live body weight (LBW), testes weight (RTW), and serum testosterone were significantly reduced ($P<0.05$) by treatment with tramadol. tramadol treatment also decreased ($P<0.05$) ejaculate volume, sperm concentration, total sperm output, sperm motility index, and semen initial fructose concentration. The negative effects of tramadol on semen characteristics were dose-dependent. Treatment with garlic increased ($P<0.05$) LBW, TW, serum testosterone concentration, improved semen characteristics, and alleviated the negative effects of tramadol. tramadol treatment increased ($P<0.05$) the numbers of abnormal and dead sperms in a dose-dependent manner. Treatment with garlic alleviated the negative effects of tramadol during treatment. Results demonstrated the beneficial influences of garlic in reducing the negative effects of tramadol on production and reproduction of male rabbits.

Keywords: Tramadol, Rabbits, Semen, Garlic

INTRODUCTION:

Garlic *(ALLIUM SATIVUM)* increases antioxidant defence mechanism in animals[1]. Supplementation of garlic oil at 0.5 g/kg of diet has a positive effect on testes weight, antioxidant status, and testosterone hormone in rabbits[2]. [3] reported that garlic improved the immunity responses and lowered the lipid profile in blood, lipid peroxidation in liver, and increased hepatic antioxidant activity in treated rabbits. The major bioactive components in garlic such as Allicin are mainly responsible for the positive effects of garlic [4]. Productive and reproductive performances as well as physiological parameters were improved significantly by addition of garlic powder to rabbit diet [5]. [6] reported that significant increase observed in the activities of antioxidant enzymes and selenium level could possibly be associated with consumption of high garlic diet by the rabbits. In this study, we therefore sought to determine the effects of acute tramadol (therapeutic dose) use on male reproductive and testesteron and to determine if garlic can prevent or ameliorate the tramadol-induced dam. Tramadol is a synthetic centrally active opioid analgesic used to manage moderate to severe pain. It has dual mechanism of action. It works by binding to μ-opioid receptors in the brain and spinal cord. These receptors are responsible for both the painrelieving effects and at higher doses, the euphoric effects that abusers seek. In addition, it works as aserotonin-norepinephrine reuptake inhibitor, thereby increasing brain levels of serotonin and norepinephrine [7]. There have been several reports of abuse and toxicity of tramadol in recent times especially by young adult males [8]. This abuse includes the use of tramadol as an aphrodisiac for men with premature ejaculation and the use of tramadol and alcohol to ease the effects of manual labour. The main symptoms of tramadol toxicity include

central nervous system depression, nausea, vomiting, seizures and tachycardia [9]. There are also reports of tramadol abuse that resulted in death due to cardiopulmonary arrest, 81ypoglycaemia and liver failure [10]. The dependence on tramadol could be due to the euphoria and mood elevation caused by increased brain levels of serotonin and norepinephrine [11]. In addition to these, histological damage to the testicular seminiferous tubules, sertoli and leydig cells have been reported [12] Tramadol is a widely abused drug, especially among the youthful population, for alleged purpose of sexual enhancement. However, there is dearth of information on the effects of its short term use on male reproduction. Antioxidant supplements are observed to improve rabbit reproduction [13].

MATERIALS AND METHODS:

In this study tramadol and garlic were used. tramadol was purchased from pharmacy alsalam hospital in El - Beida-Libya. Garlic oil was purchased from public market for medicinal herbs in Al-Bayda city. Mature male New Zealand White rabbits (age of 6 months and initial weight of (1.892 ± 50.79 Kg) were used. Animals were individually housed in cages and weighed weekly throughout 6- weeks experimental period. Feed and water were provided ad libtum. Rabbits fed pellets which consisted of 30 % berseem (Trifolium alexandrinum) hay, 25 % yellow corn, 26.2% wheat bran, 14 % soybean meal, 3 % molasses, 1 % $CaCl_2$, 0.4 % NaCl, 0.3 % mixture of minerals and vitamins, and 0.1 % methionine. The vitamin and mineral premix per kg contained the following IU/gm for vitamins or minerals: vit A-4000,000, vit D3-5000, 000, vit E-16,7 g, K-0.67 g, vit B1-0.67 g, vit B2-2 g, B6-0.67 g, B12-0.004 g, B5-16.7 g, Pantothinc acid-6.67 g, Biotein-0.07 g, Folic acid-1.67 g, Choline chloride-400 g, Zn-23.3 g, Mn-10 g, Fe-25 g, Cu-1.67 g, I-0.25 g, Se-0.033 g, and Mg-133.4 g (Rabbit premix produced by Holland Feed Inter. Co.). The chemical analysis of the pellets [14] showed that they contained 15.8 % crude protein, 11.3 % crude fiber, 3.7 % ether extract, 7.2 % ash, 92.9 % organic matter and 62.4 % nitrogen free extract % as DM basis. Twenty mature male rabbits were randomly divided into four equal groups (each five rabbits) as follows:- Group I: Rabbits were used as control daily for 6successive weeks. Group II: Rabbits were treated with garlic. Garlic was given daily by gavage at a dose of 40 mg/kg B.W, [15] for 6 successive weeks. Group III: Rabbits were treated daily with tramadol by gavage at a dose of 50 mg/kg B.W/day [16]. Group IV: Rabbits were given with tramadol daily at a dose of 50 mg/kg B.W./day by gavage like group III and given the garlic concurrently daily at a dose of 40 mg/kg B.W./day by gavage like group II for 6 successive weeks. The doses of the tramadol and garlic were calculated according to the animal's body weight on the week before dosing. The tested doses of tramadol and garlic were given daily for 6 weeks. Body weight of each animal was recorded weekly throughout the 6-week of the experimental period. The weight measurements were carried out in the morning before access to feed and water. At the end of treatment period, all animals of each group were slaughtered. Weights of liver, lung, heart, kidney, spleen and testis were also recorded. These organs were individually identified and kept frozen (–20°C) until assays performed. Blood samples were collected from the ear vein of all animals every other week throughout the 6-weeks experimental period. Blood samples were obtained in the morning before accesses to feed and water and placed immediately on ice. The blood samples were collected in tube containing heparin to obtain plasma. Semen collection was done weekly and continued throughout the 6-weeks experimental period, so 60 ejaculates obtained per treatment. Ejaculates were collected using an artificial vagina and a teaser doe. The volume of each ejaculate was recorded (using a graduated collection tube) after removal of the gel mass. A weak eosin solution[17] was used for evaluation of sperm concentration by the improved Neubauer haemocytometer slide (GmbH + Co., Brandstwiete 4, 2000 Hamburg 11, and Germany). Total sperm output calculated by multiplying semen ejaculate volume and semen concentration. Determination of initial fructose concentration in seminal plasma was determined immediately after semen collection according to [18]. Assessments of dead and normal spermatozoa were performed using an eosin-nigrosine blue staining mixture[19]. The percentages of motile sperm were estimated by visual examination under low-power magnification (10x) using light microscope. Total number of motile sperm was calculated by multiplying the percentage of motile sperm and total sperm output. Reaction time was determined as the moment of subjecting a doe to the buck until the completion of erection; it was measured in seconds. Initial hydrogen ion concentration (pH) was determined immediately after collection using pH cooperative paper (Universalindikator pH 0-14 Merck, Merck KgaA, 64271 Darmstadt, Germany). Packed sperm volume (PSV) was recorded. Total functional sperm fraction (TFSF) was calculated as the product of total sperm output (TSO), sperm motility (%), and normal morphology (%) [20]. Statistical analysis: Where applicable, statistical analysis was carried out in Minitab software (version17) statistical significance was assessed using ANOVA analysis with Tukey multiple comparison test after detection normal distribution to the information and suitable $P < 0.05$ consider critical.

RESULTS:

Observation of animals tramadol-fed rabbits showed varying degrees of clinical signs few days after dose. the testicular weight in group tramadol was significantly lower than control group (Figure. 1).

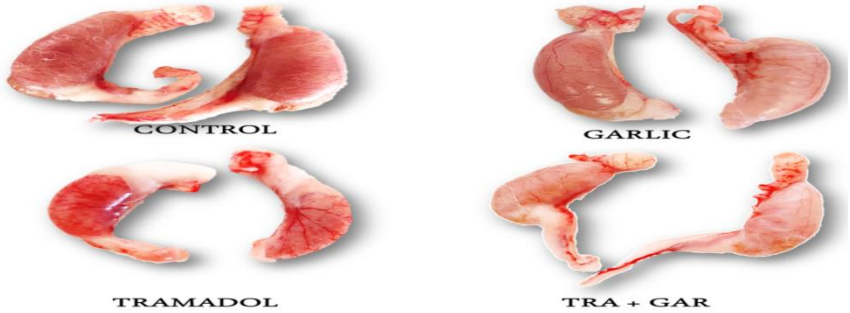

Figure 1: Morphology effect of testes weight during treatment of male rabbits with garlic , tramadol, and/or their combination.

Body weight (BW) relative weight of testes and testosterone were significantly ($P < 0.05$) decreased in rabbits treated with tramadol compared to control animals (Table 1). Results obtained showed that tramadol significantly ($P < 0.05$) decreased libido (by increasing the reaction time), ejaculate volume, sperm concentration, total sperm output, sperm motility (%), total motile sperm per ejaculate (TMS), packed sperm volume (PSV), total functional sperm fraction (TFSF), normal and live sperm and semen initial fructose. While initial hydrogen ion concentration (pH) and dead and abnormal sperm were increased ($P < 0.05$). Live body weight (LBW) and relative weights of testes (RTW) were significantly ($P < 0.05$) decreased. Concentrations of thiobarbituric acid-reactive substances (TBARS) were significantly ($P < 0.05$) increased in plasma of rabbits treated with tramadol compared with control (Table 2).

Table 1: The overall means (±SE) of body weight, relative testes weight , blood plasma testosterone concentration and Thiobarbituric acid-reactive substances (TBARS) in plasma and testes during treatment of male rabbits with tramadol, garlic .

Parameters	Groups			
	Control	Garlic	Tramadol	Tramadol+Garlic
BW (g)	1892 ± 50.79^{a}	1918 ± 39.84^{a}	1529 ± 64.85^{b}	1886 ± 42.25^{a}
RTW (g/100 g BW)	4.432 ± 0.486^{ab}	6.880 ± 0.730^{a}	2.774 ± 0.424^{b}	4.880 ± 0.521^{ab}
Testosterone (ng/mL)	1.570 ± 0.063^{b}	2.857 ± 0.194^{a}	0.987 ± 0.112^{c}	1.682 ± 0.084^{b}
TBARS (nmol/ml)	2.673 ± 0.025^{a}	2.462 ± 0.051^{b}	3.113 ± 0.087^{a}	2.601 ± 0.038^{b}
Testes TBARS (nmol/g tissue)	14.7 ± 1.50^{b}	10.5 ± 0.21^{b}	27.3 ± 2.64^{a}	15.28 ± 1.64^{b}

[abc] Within row, means with different superscript letters differ significantly ($p < 0.05$).

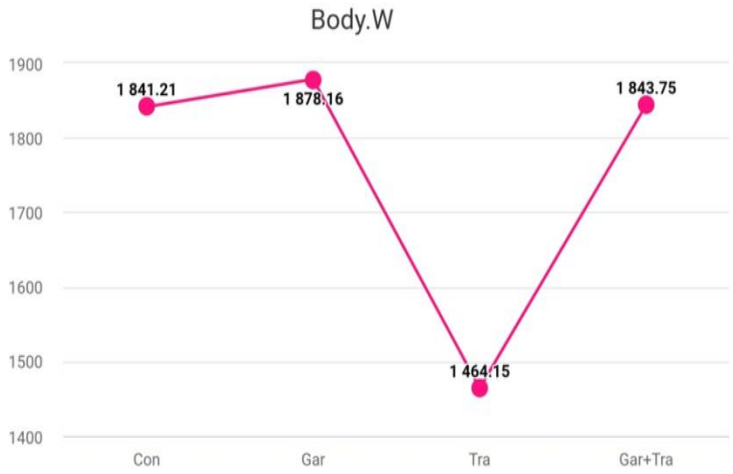

Figure1. Change in body weight during treatment of male rabbits with garlic , tramadol, and/or their combination.

Figure2. Change in testes weight during treatment of male rabbits with garlic , tramadol, and/or their combination.

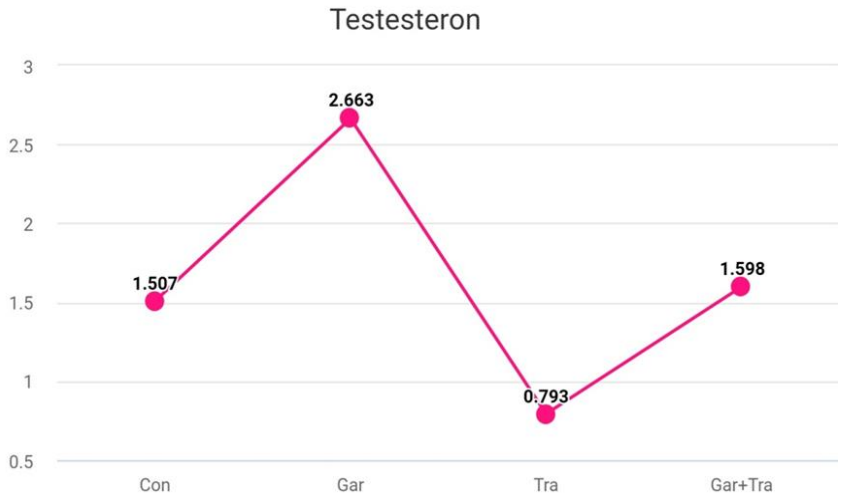

Figure 3. Change in Testesteron during treatment of male rabbits with garlic , tramadol, and/or their combination.

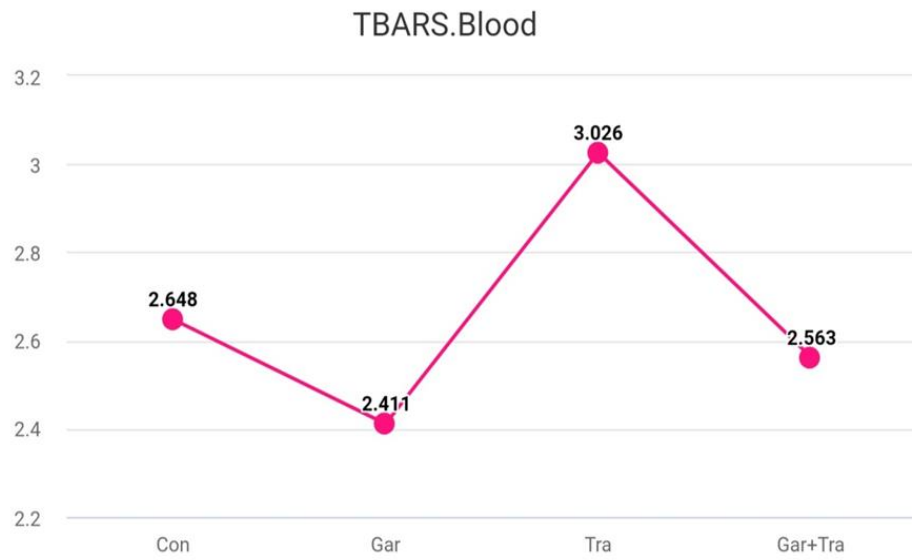

Figure Error! No text of specified style in document. **Change in the TBARS of plasma during treatment of male rabbits with garlic , tramadol, and/or their combination.**

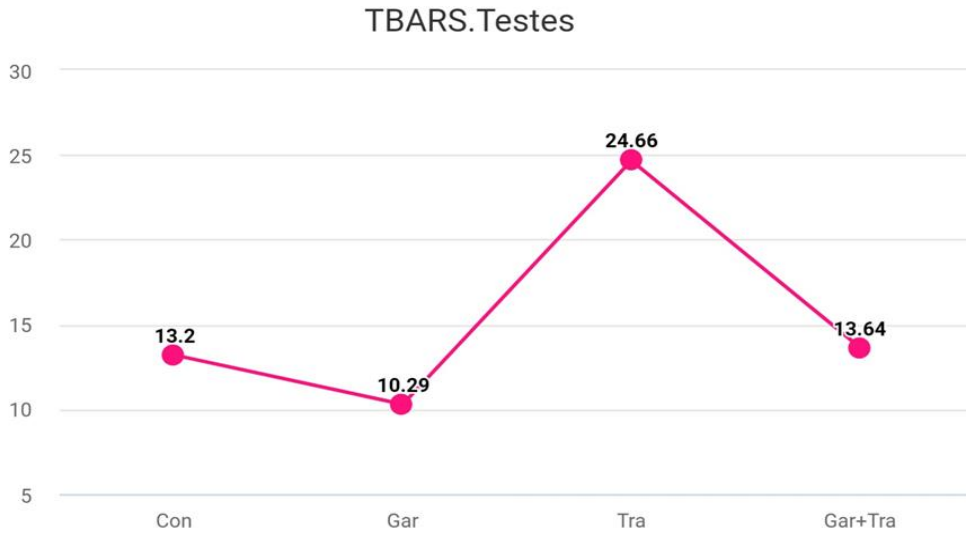

Figure5. Change in the activity of testes TBARS during treatment of male rabbits with garlic , tramadol, and/or their combination.

Table 2: The overall means (±SE) of semen characteristics during treatment of male rabbits with tramadol.

Parameters	Animal Groups			
	Control	Garlic	Tramadol	Gar + Tra
Ejaculate volume (ml)	0.75 ± 0.024^{ab}	0.80 ± 0.022^{a}	0.70 ± 0.024^{b}	0.72 ± 0.025^{ab}
PH	7.84 ± 0.032^{b}	7.75 ± 0.054^{b}	8.08 ± 0.074^{a}	7.90 ± 0.041^{b}
Reaction time (s)	4.00 ± 0.79^{b}	3.68 ± 0.17^{b}	5.13 ± 0.33^{a}	4.30 ± 0.15^{b}
Packed sperm volume (%)	14.6 ± 0.17^{ab}	15.4 ± 0.35^{a}	13.34 ± 0.22^{c}	14.1 ± 0.22^{bc}
Sperm concentration ($\times10^6$ ml^{-1})	248 ± 5.7^{b}	278 ± 6.4^{a}	237 ± 5.3^{b}	250 ± 5.8^{b}
Total sperm output ($\times10^6$)	185 ± 6.5^{b}	222 ± 5.3^{a}	166 ± 6.9^{b}	181 ± 5.7^{b}
Sperm motility (%)	$66.2. \pm0.95^{b}$	70.3 ± 1.3^{a}	62.5 ± 1.1^{b}	65.7 ± 0.9^{b}
Total motile sperm ($\times10^6$)	123 ± 5.0^{b}	157 ± 4.9^{a}	104 ± 4.6^{b}	119 ± 3.9^{b}
Live sperm (%)	73.7 ± 1.2^{b}	77.0 ± 1.0^{a}	65.8 ± 1.24^{a}	73.5 ± 0.8^{a}
Dead sperm (%)	26.3 ± 1.19^{b}	23.0 ± 0.99^{b}	34.2 ± 1.24^{a}	26.5 ± 0.80^{b}
Normal sperm (%)	82 ± 0.5^{a}	83 ± 0.4^{a}	78 ± 0.9^{b}	82 ± 0.4^{a}
Abnormal (%)	18 ± 0.2^{b}	17 ± 0.3^{b}	22 ± 06^{a}	18 ± 0.3^{b}
Total functional sperm fraction ($\times10^6$)	100 ± 4.2^{b}	131 ± 4.3^{a}	82 ± 42^{c}	97 ± 3.2^{bc}
itial fructose (mg/dl)	264 ± 5.2^{b}	271 ± 5.4^{a}	229 ± 5.8^{a}	228 ± 5.4^{b}

^{abc}Within row, means with different superscript letters differ significantly ($p < 0.05$).

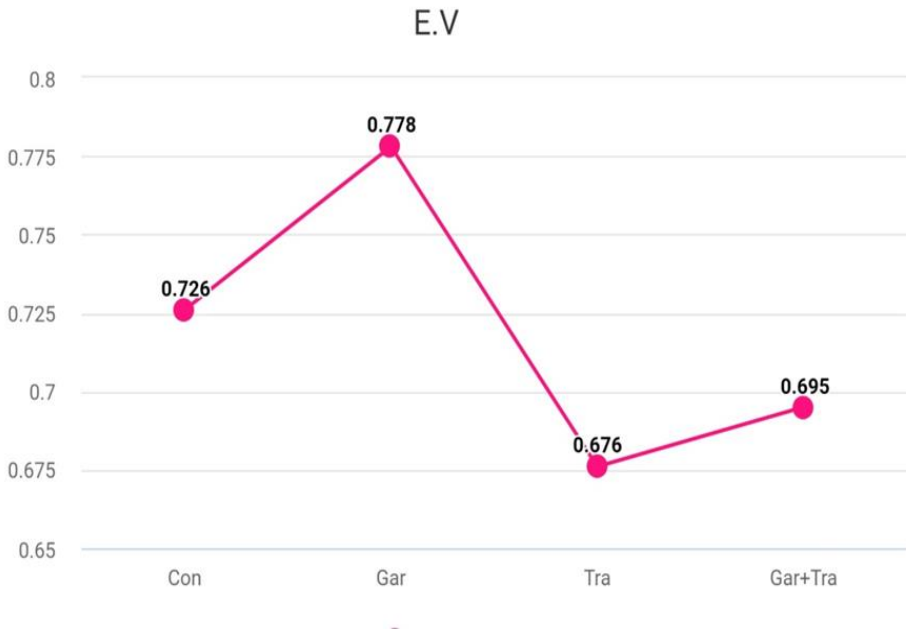

Figure 6. Change in ejaculate volume during treatment of male rabbits with garlic , tramadol, and/or their combination.

Figure 7. Change in initial hydrogen ion concentration during treatment of male rabbits with garlic , tramadol, and/or their combination.

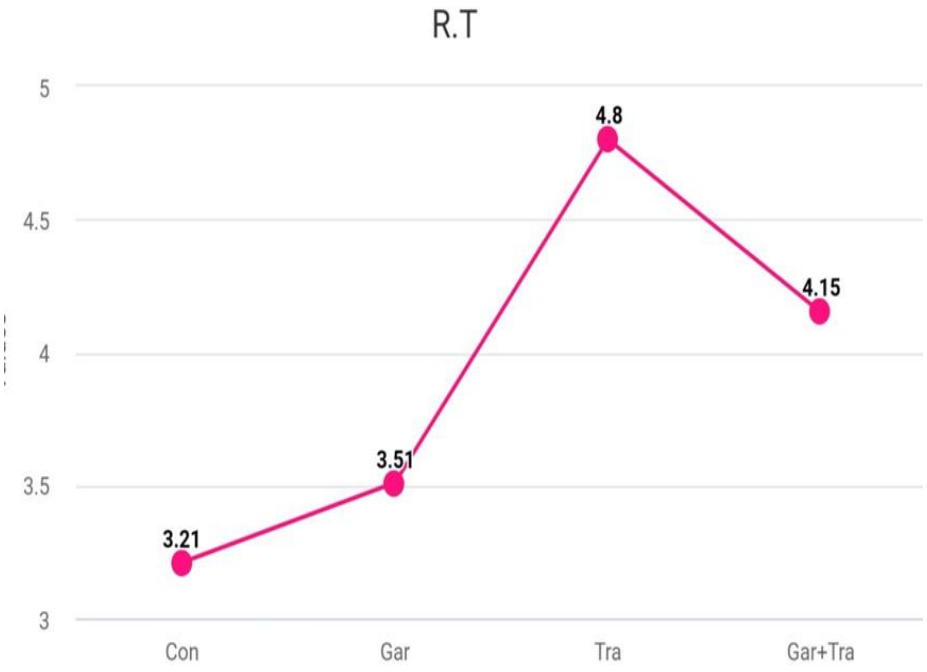

Figure 8. Change in reaction time during treatment of male rabbits with garlic , tramadol, and/or their combination.

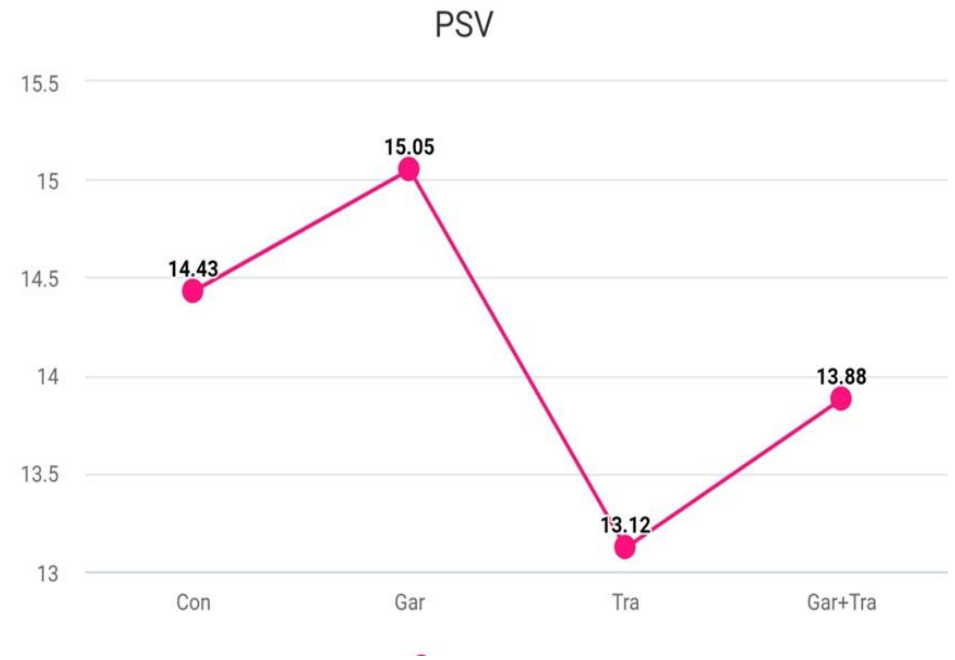

Figure 9. Change in packed sperm volume during treatment of male rabbits with garlic , tramadol, and/or their combination.

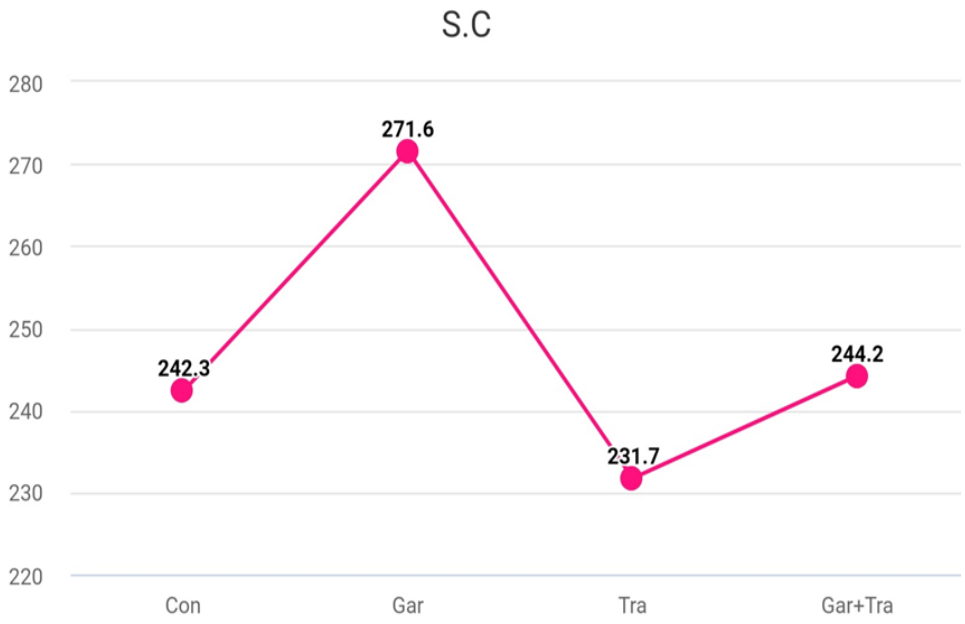

Figure 10. Change in sperm concentration during treatment of male rabbits with garlic, tramadol, and/or their combination.

Figure 11. Change in total sperm output during treatment of male rabbits with garlic , tramadol, and/or their combination.

Figure 12. Change in sperm motility during treatment of male rabbits with garlic , tramadol, and/or their combination.

Figure 13. Change in total motile sperm during treatment of male rabbits with garlic , tramadol, and/or their combination.

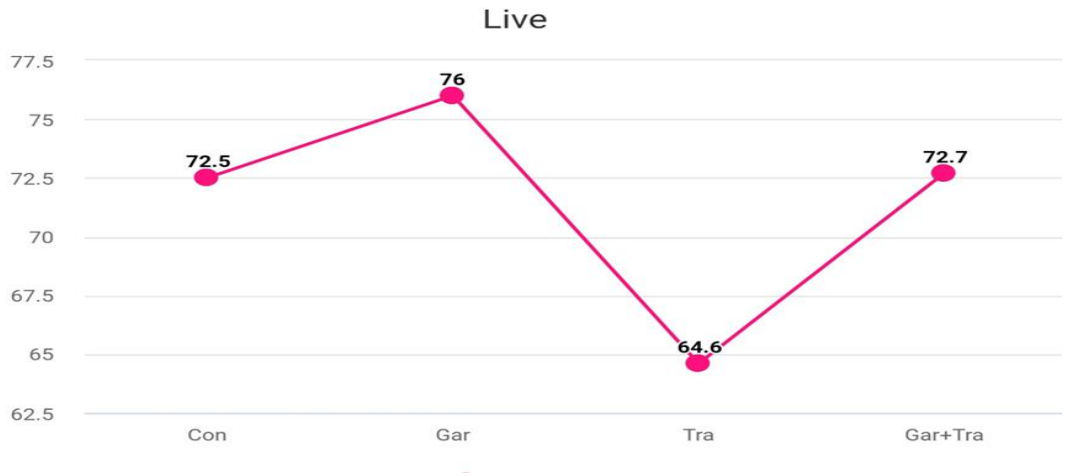

Figure 14. Change in Live sperm during treatment of male rabbits with garlic , tramadol, and/or their combination.

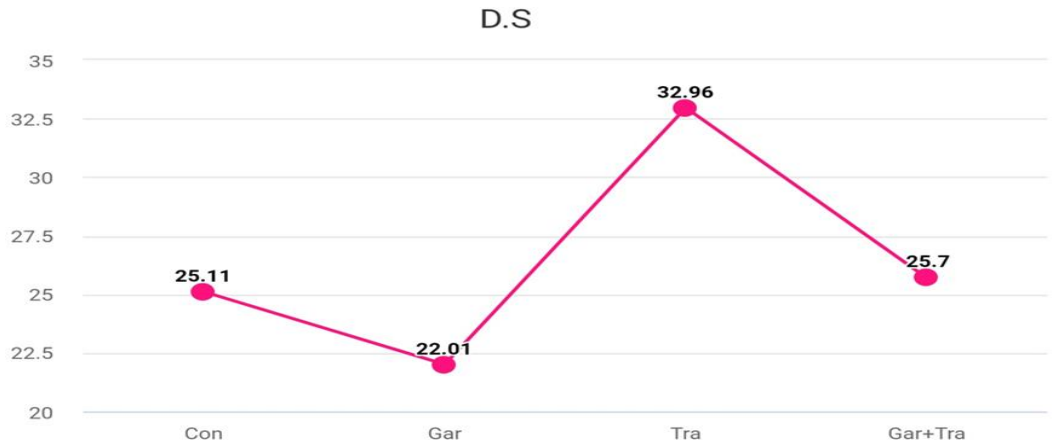

Figure 15. Change in Dead sperm during treatment of male rabbits with garlic , tramadol, and/or their combination.

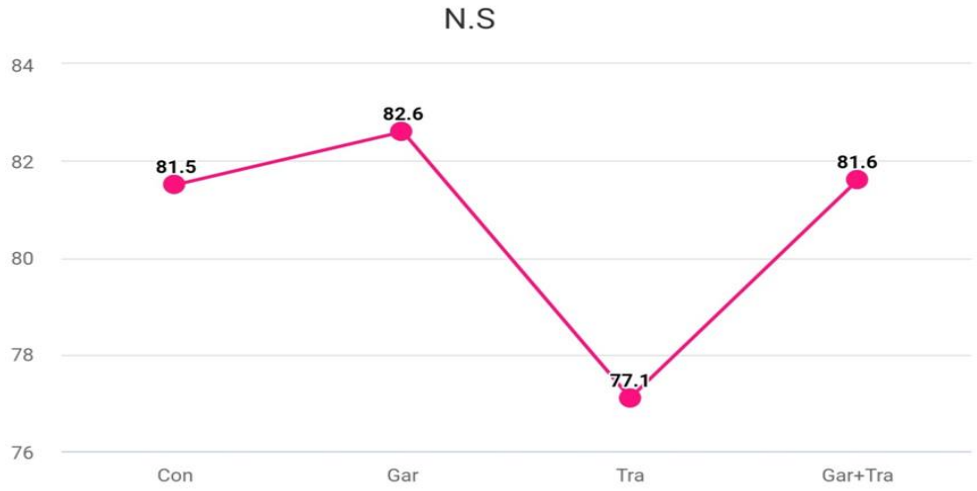

Figure16. Change in Normal sperm during treatment of male rabbits with garlic , tramadol, and/or their combination.

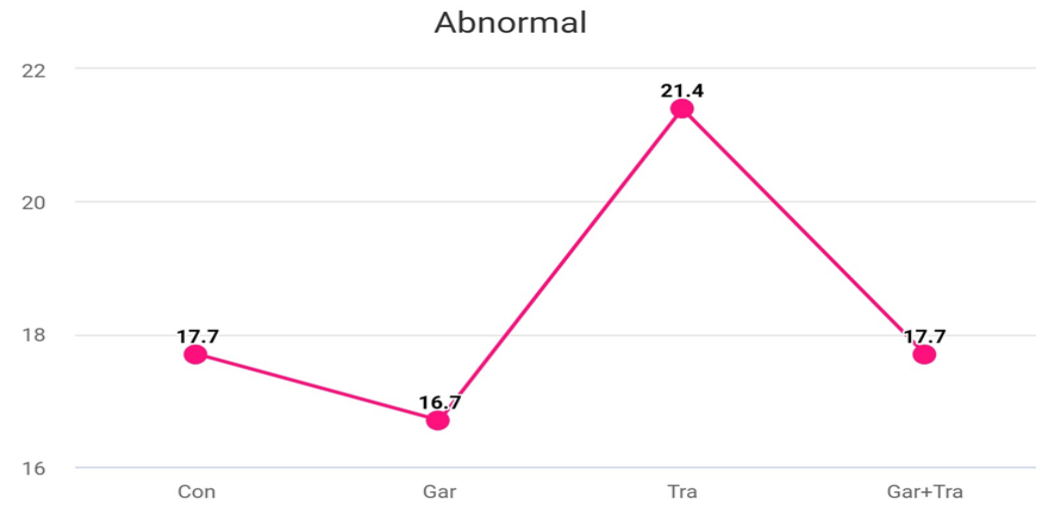

Figure 17. Change in Abnormal sperm during treatment of male rabbits with garlic , tramadol, and/or their combination.

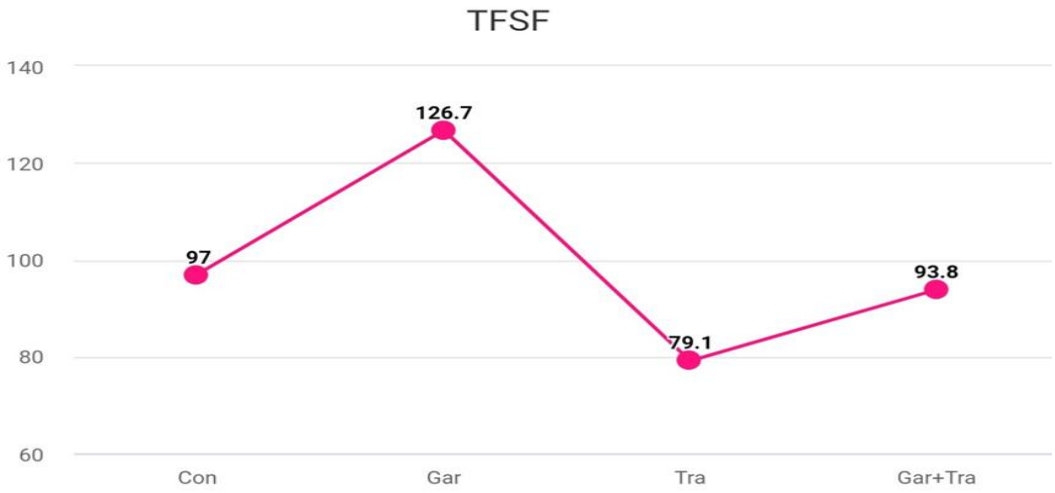

Figure 18. Change in total functional sperm fraction during treatment of male rabbits with garlic , tramadol, and/or their combination.

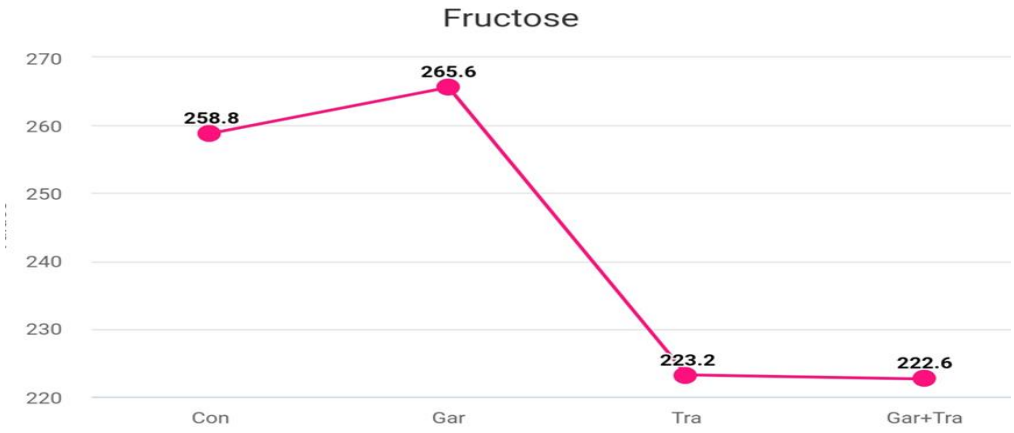

Figure 19. Change in semen initial fructose during treatment of male rabbits with garlic , tramadol, and/or their combination.

DISCUSSION:

The present results indicate that treatment with tramadol caused significant reductions in body weight (BW) and testes weight (TW) (Table 1 and Figure 1 to 3). The reduction in BW and TW of the tramadol treated rabbits is in agreement with those reported in previous studies [21-23]. Also, This study has shown that the treatment of rabbits with tramadol caused significant suggests that tramadol increases the catabolism of lipids in the adipose tissue, resulting in significant reduction in body weight of rabbits at a later stage during the treatment period. Similar results were reported by [24] in Persea americana leaf extractstreated rats. The present study was agreed with the study of [25] who reported that final body weight was increased in garlic supplemented group compared to control group. The increased body weight was observed in this study strengthen the findings of[26,27] who found significant increase of body weight gain of rat and broiler fed a mixture of Garlic. The enhanced body weight gain of the rabbit show positive nutritive effect of these natural feed additives. This enhancement in growth rate of rabbits fed garlic supplemented diets compared to the control is in agreement with the findings of [25] who reported increase in weight gain of rabbits. This improvement in growth rate of rabbits fed garlic supplemented diets compared to the control is in line with the findings of [28,29] who reported increase in weight gain of rabbits and broilers fed garlic supplemented diets respectively. Tramadol addiction and abuse is now a major public health menace in libya. The rampancy of tramadol abuse and the consequential leap in crime in libya led to Government banning the manufacture, sale and use of tramadol . The design of the current study investigated the short- and long-term implications of tramadol abuse on semen quality indices using an animal model. Tramadol abuse has increased in the Middle East region, tramadol use was common among adolescents and over one third of tramadol users had drug-related problems [30].

The present study showed that the dose of garlic (40mg/kg body weight) elicited increases in the ejaculate volume, concentration, the total output (Table 2), the wave motions, the motility percentages (Table 6 to19), the percentages of live spermatozoa and the percentages of abnormal spermatozoa (Table 2).[31] who found that quality of semen parameters was significantly higher after adding garlic to male rabbit diets. The present results are also consistent with the results of[32] who found that treatment of rabbits with row garlic restored lead-induced decrease in sperm speed, motility and viability. Antioxidants are able to abrogate kidney damage by reduction of lipid peroxidation through enhancement of scavenging ability of antioxidant defense system. Inhibition of endogenous antioxidant defense system and increased oxidant levels increased the kidney damage[33] . Testosterone hormone was significantly higher in garlic-fed male rabbit [34] . [35] attributed the garlic-induced increase in testosterone level to the elevation of sex hormone binding globulin, which binds more testosterone, and consequently, oblige the testis to excrete more male sex hormone in plasma. [36] suggested that garlic supplementation might enhance protein anabolism and suppress protein catabolism due to hormonal regulation by the stimulation of steroid hormones, leading to greater testis testosterone content and lower plasma corticosterone concentration. [37] suggested that garlic compounds are responsible for the significant increase in testosterone levels by affecting the performance of steroidgenerating enzymes, testosterone hormone and its metabolites. They concluded that garlic supplementation likely increases testicular testosterone content due to the stimulation of LH secretion from the pituitary gland, which stimulate the testes to increase its testosterone production. [38] who found that the intake of herbs resulted in an increase in serum antioxidant enzyme activities and a decrease in MDA concentration.The concentration of liver MDA is an indicator for evaluating antioxidant systems. The prevention of lipid oxidation (MDA) in muscle-based foods can be achieved by the supplementation of natural antioxidants, such as garlic and turmeric, as dietary supplements. Toxic effects of opioids at cellular level may be explained by lipid peroxidation. Biological membranes contain large amount of poly-unsaturated fatty acids, which are particularly susceptible to peroxidative attacks by oxidants resulting in lipid peroxidation. Therefore, lipid peroxidation has been used as an indirect marker of oxidant- induced cell injury [39]. A significant increase in lipid peroxides was reported in rats receiving an acute dose of cocaine[40]. Similarly, lipid peroxides were found significantly increased among heroin users[41]. These findings are in agreement with the present results which showed significant increase in serum MDA levels in both tramadol groups compared to control group, indicating an increase in lipid peroxidation.

CONCLUSION:

The findings of the study provide substantial evidence that tramadol has an adverse effect on sperm profile and reproductive organs of male albino rabbits in terms of body weight, weight of testes, TBARS, sperm count, sperm viability, sperm motility as well as sperm head abnormalities, it is recommended that moderation should

be exercised in the consumption of this drug by those taking it for therapeutic purpose.

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