

CURCUMIN PROTECTION FROM REPRODUCTIVE TOXICITY CAUSED BY TRAMADOL IN MALE RABBITS.

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ABSTARCT

Curcumin may be a shining yellow chemical created by plants of the *Curcuma longa* species. It is the vital curcuminoid of turmeric (*Curcuma longa*), a part of the ginger family, Zingiberaceae. It is sold as a homegrown supplement, beauty care products ingredient, food flavoring, and nourishment coloring. Tramadol may be a manufactured centrally dynamic opioid pain relieving utilized to oversee direct to extreme pain. It has double component of activity. It works by official to μ -opioid receptors within the brain and spinal line. Hence, the display explore was attempted to decide the viability of curcumin in lightening the harmfulness of tramadol on body weight, regenerative execution and testosterone of male rabbits. Animals were allotted to 1 of 4 bunches: control; 50mg tramadol/kg bw; 15 mg curcumin /kg bw; tramadol (50 mg/kg bw) also curcumin (15 mg/kg bw), separately. Comes about appeared that live body weight (LBW), testicles weight (RTW), and serum testosterone were significantly diminished ($P<0.05$) by treatment with tramadol. tramadol treatment moreover diminished ($P<0.05$) ejaculate volume, sperm concentration, add up to sperm yield, sperm motility list, and semen introductory fructose concentration. The negative impacts of tramadol on semen characteristics were dose-dependent. Treatment with curcumin expanded ($P<0.05$) LBW, TW, serum testosterone concentration, moved forward semen characteristics and reduced the negative impacts of tramadol. tramadol treatment expanded ($P<0.05$) the numbers of irregular and dead sperms in a dose-dependent way. Treatment with curcumin reduced the negative impacts of tramadol amid treatment. Comes about illustrated the advantageous impacts of curcumin in lessening the negative impacts of tramadol on generation and generation of male rabbits.

Keywords: Tramadol, Rabbits, Reproductive, Curcumin

INTRODUCTION

Turmeric (*Curcumin longa*) could be a therapeutic plant broadly utilized and developed in tropical locales. Plant extricates were found to have antifungal, imunomodulatory and antioxidative[1] as well as antimutagenic exercises [2]. Turmeric powder could be a wealthy source of useful phenolic compounds: the curcuminoids, where three primary curcuminoids, curcumin, demethoxycurcumin and bisdemethoxycurcumin [3] have been confined from turmeric. Supplementation of turmeric powder at 0.20 and 0.40 g/kg to the commercial count calories for rabbits emphatically influenced the body weight pick up in rabbit does [4]. On the other hand,[5] famous that dietary consideration of turmeric powder at 0, 0.15 and 0.30% had no useful impacts on blood parameters and meat characteristics of developing rabbits raised beneath summer stress. Be that as it may, data approximately ideal level of curcumin and turmeric powder as development promoters and normal cancer prevention agents in developing rabbit is rare. In this think about, we subsequently looked for to decide the

impacts of intense tramadol (restorative dosage) utilize on male regenerative and hormones and to decide on the off chance that curcumin can avoid or enhance the tramadol-induced dam. Tramadol could be a manufactured centrally dynamic opioid pain relieving utilized to oversee direct to serious torment. It has double instrument of activity. It works by authoritative to μ -opioid receptors within the brain and spinal rope. These receptors are mindful for both the pain-relieving impacts and at higher dosages, the euphoric impacts that abusers look for. In expansion, it works as aserotonin-norepinephrine reuptake inhibitor, subsequently expanding brain levels of serotonin and norepinephrine [6]. There have been a few reports of mishandle and harmfulness of tramadol in later times particularly by youthful grown-up males[7]. This mishandle incorporates the utilize of tramadol as an sexual enhancer for men with untimely ejaculation and the utilize of tramadol and liquor to ease the impacts of manual work. The most indications of tramadol poisonous quality incorporate central apprehensive framework sadness, sickness, heaving, seizures and

tachycardia [8]. There are too reports of tramadol mishandle that come about in passing due to cardiopulmonary capture, hypoglycaemia and liver failure[9]. The reliance on tramadol may be due to the elation and disposition rise caused by expanded brain levels of serotonin and norepinephrine[10]. In expansion to these, histological harm to the testicular seminiferous tubules, sertoli and leydig cells have been reported[11] Tramadol may be a broadly mishandled sedate, particularly among the energetic populace, for alleged reason of sexual upgrade. In any case, there's shortage of data on the impacts of its brief term utilize on male propagation. Antioxidant supplements are watched to make strides rabbit propagation [12].

MATERIALS AND METHODS

In this study tramadol and curcumin were used. tramadol was purchased from pharmacy alsalam hospital in El -Beida-Libya. Curcumin 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 $(1.892 \pm 50.79 \text{ Kg})$ were used. Animals were individually housed in cages and weighed weekly throughout 6- weeks experimental period. Feed and water were provided ad libitum. 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, Pantothenic 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 [13] 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 6 successive weeks. Group II: Rabbits were treated with curcumin. Curcumin was given daily by gavage at a dose of 15 mg/kg B.W, [14] for 6 successive weeks. Group III: Rabbits were treated daily with tramadol by gavage at a dose of 50 mg/kg B.W/day [15]. 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 curcumin concurrently daily at a dose of 15 mg/kg B.W./day by gavage like group II for 6 successive weeks. The doses of the tramadol and curcumin were calculated according to the animal's body weight on the week before dosing. The tested

doses of tramadol and curcumin 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[16] was used for evaluation of sperm concentration by the improved Neubauer haemocytometer slide (GmbH + Co., Brandstwierte 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 [17]. Assessments of dead and normal spermatozoa were performed using an eosin-nigrosine blue staining mixture[18]. 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 (Universal indicator 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 (%) [19]. Statistical analysis: Where applicable, statistical analysis was carried out in Minitab software (version17) statistical significance was assessed using ANOVA analysis with Turkey multiple comparison test after detection normal distribution to the information and suitable $P < 0.05$ consider critical.

RESULTS

The changes in body weight (BW), brain and testes weight of male rabbits throughout the 6-week experimental period of rabbits treated with curcumin, tramadol and their combination were summarized in

(Table 1 and Figures 1 to 3). Overall means indicated that treatment with tramadol caused significant ($P < 0.05$) decrease in BW, TBARS, brain and testes weight compared to control animals. curcumin alone significantly ($P < 0.05$) increased BW, TBARS, brain

and testes weight compared to control. The combination between curcuma and tramadol caused significant increase in the reduction of BW, brain and testes weight due to treatment with tramadol, and this means that curcumin alleviated its toxicity.

Table 1: The overall means (\pm SE) of body weight, brain , testes weight , blood plasma testosterone concentration and thiobarbituric acid-reactive substances (TBARS) in plasma and testes during treatment of male rabbits with curcumin, tramadol and their combination.

Parameters	Groups			
	Control	Curcumin	Tramadol	Tramadol+ Curcumin
BW (g)	1904 \pm 50.67 ^a	1944 \pm 38.33 ^a	1594 \pm 51.28 ^b	1902 \pm 40.51 ^a
BW (g/ BW)	4.628 \pm 0.146 ^{ab}	6.250 \pm 0.34 ^a	2.570 \pm 0.390 ^b	5.020 \pm 0.425 ^{ab}
TW(g/ BW)	4.692 \pm 0.379 ^{ab}	6.790 \pm 0.309 ^a	2.338 \pm 0.475 ^b	4.954 \pm 0.757 ^{ab}
Testosterone (ng/mL)	1.575 \pm 0.043 ^b	2.772 \pm 0.167 ^a	0.994 \pm 0.112 ^c	1.6853 \pm 0.062 ^b
TBARS (nmol/ml)	1.732 \pm 0.024 ^a	1.495 \pm 0.043 ^b	1.945 \pm 0.059 ^a	1.739 \pm 0.033 ^b
Testes TBARS (nmol/g tissue)	13.8 \pm 0.69 ^b	11.2 \pm 0.23 ^b	24.0 \pm 1.17 ^a	14.3 \pm 1.70 ^b

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

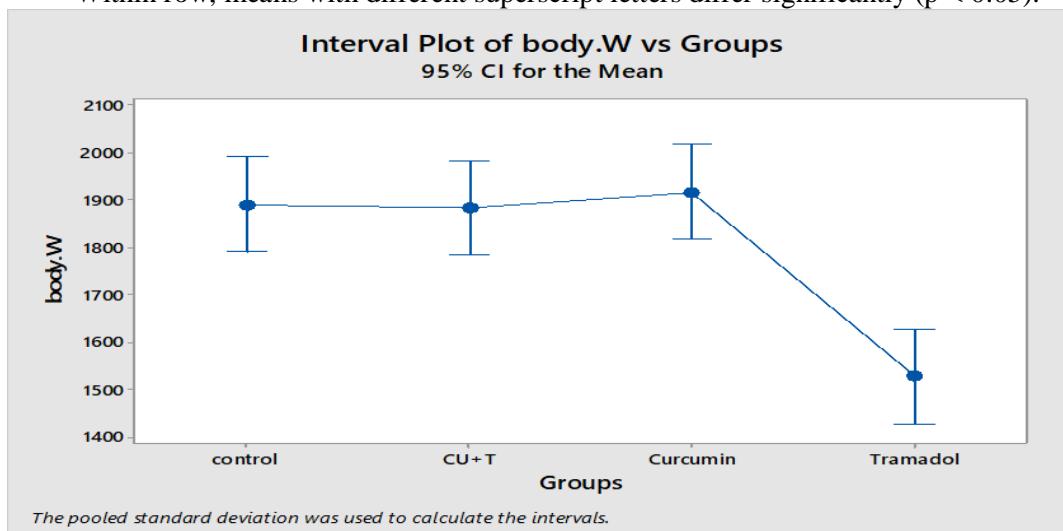


Figure1. Change in body weight during treatment of male rabbits with curcumin, tramadol, and/or their combination.

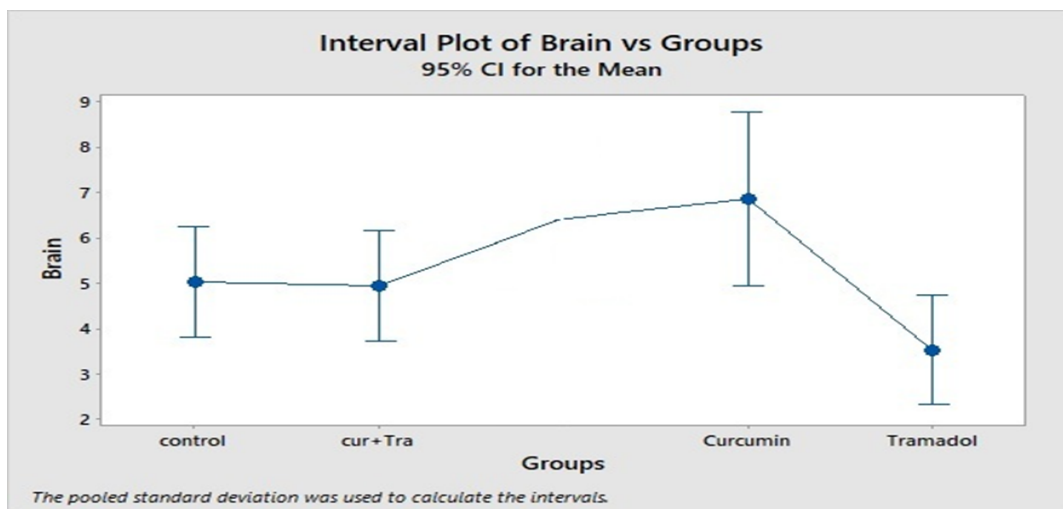


Figure2. Change in brain weight during treatment of male rabbits with curcumin , tramadol, and/or their combination.

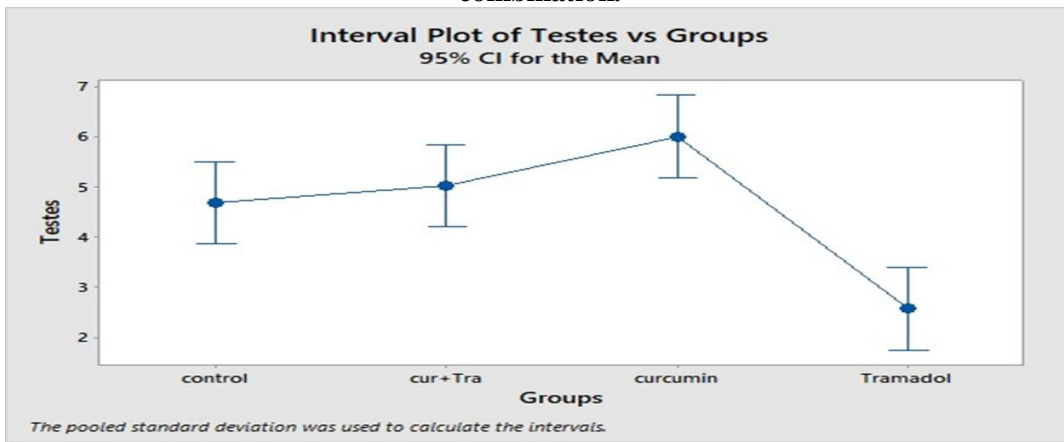


Figure3. Change in testes weight during treatment of male rabbits with curcumin , tramadol, and/or their combination.

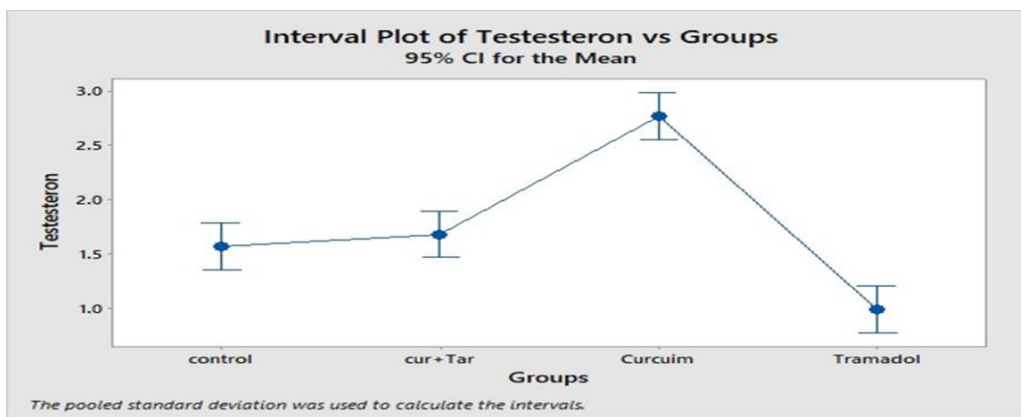


Figure 4. Change in Testosterone during treatment of male rabbits with curcumin , tramadol, and/or their combination.

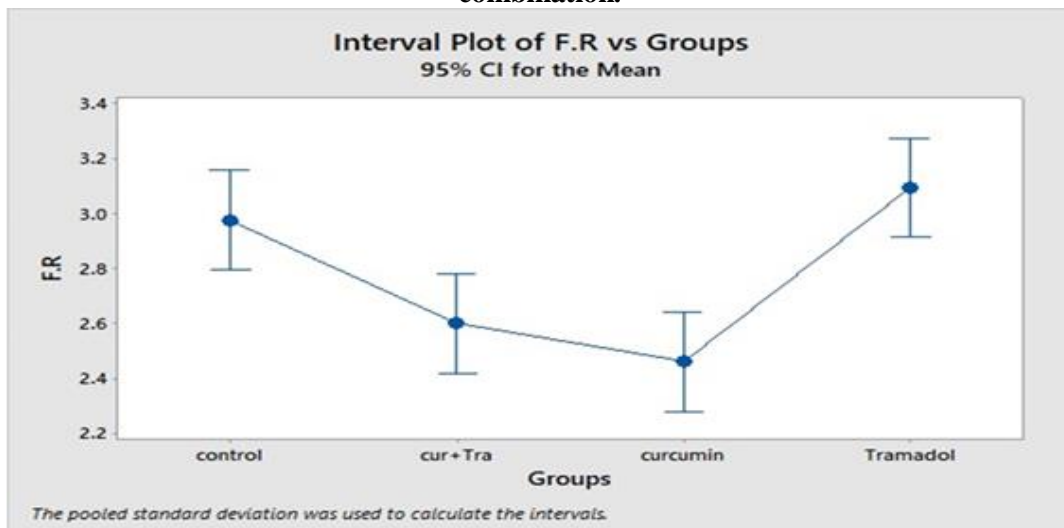


Figure 5. Change in the TBARS of plasma during treatment of male rabbits with curcumin , tramadol, and/or their combination.

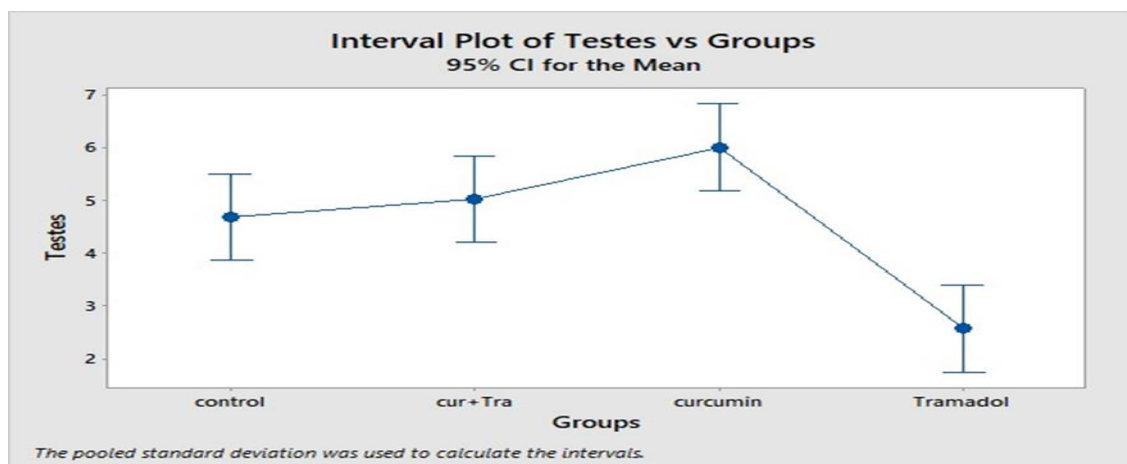


Figure6. Change in the activity of testes TBARS during treatment of male rabbits with curcumin , tramadol, and/or their combination.

Table 2: The overall means (\pm SE) of semen characteristics during treatment of male rabbits with curcumin, tramadol and their combination.

Parameters	Animal Groups			
	Control	Curcumin	Tramadol	Curcumin + Tramadol
<i>Ejaculate volume (ml)</i>	0.76 \pm 0.023 ^{ab}	0.80 \pm 0.020 ^a	0.70 \pm 0.024 ^b	0.76 \pm 0.021 ^{ab}
<i>PH</i>	7.97 \pm 0.079 ^b	7.62 \pm 0.079 ^b	8.20 \pm 0.102 ^a	7.94 \pm 0.054 ^b
<i>Reaction time (s)</i>	4.17 \pm 0.83 ^b	3.45 \pm 0.12 ^b	5.70 \pm 0.33 ^a	4.33 \pm 0.15 ^b
<i>Packed sperm volume (%)</i>	14.7 \pm 0.16 ^{ab}	15.4 \pm 0.34 ^a	13.34 \pm 0.22 ^c	14.1 \pm 0.22 ^{bc}
<i>Sperm concentration ($\times 10^6$ ml⁻¹)</i>	248.3 \pm 5.7 ^b	278.3 \pm 6.4 ^a	237.0 \pm 5.3 ^b	249.7 \pm 5.8 ^b
<i>Total sperm output ($\times 10^6$)</i>	188.6 \pm 4.9 ^b	223.3 \pm 5.1 ^a	166.4 \pm 4.8 ^b	188.6 \pm 4.8 ^b
<i>Sperm motility (%)</i>	67.1 \pm 0.92 ^b	72.4 \pm 1.4 ^a	63.6 \pm 1.1 ^b	65.9 \pm 0.9 ^b
<i>Total motile sperm ($\times 10^6$)</i>	126.0 \pm 3.6 ^b	162.6 \pm 4.9 ^a	106.1 \pm 3.6 ^b	124.3 \pm 3.3 ^b
<i>Live sperm (%)</i>	72.9 \pm 1.1 ^a	77.5 \pm 1.1 ^a	65.8 \pm 1.3 ^b	73.5 \pm 0.8 ^a
<i>Dead sperm (%)</i>	27.7 \pm 1.11 ^b	28.0 \pm 1.07 ^b	27.4 \pm 1.28 ^a	26.6 \pm 0.79 ^b
<i>Normal sperm (%)</i>	82.63 \pm 0.5 ^a	83.97 \pm 0.5 ^a	77.97 \pm 0.9 ^b	81.77 \pm 0.4 ^a
<i>Abnormal (%)</i>	17 \pm 0.4 ^b	16 \pm 0.3 ^b	22 \pm 0.6 ^a	18 \pm 0.3 ^b
<i>Total functional sperm fraction ($\times 10^6$)</i>	103.7 \pm 3.1 ^b	136.9 \pm 4.4 ^a	83.6 \pm 3.2 ^c	101.7 \pm 2.8 ^{bc}
<i>fructose (mg/dl)</i>	264.4 \pm 5.2 ^a	271.3 \pm 4.5 ^a	228.7 \pm 5.8 ^b	228 \pm 5.4 ^b

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

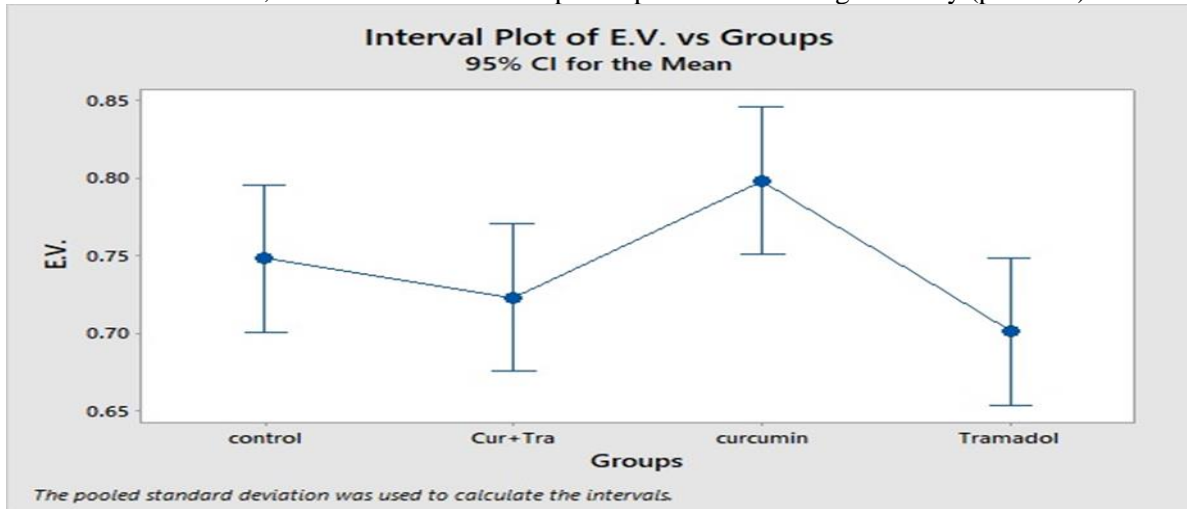


Figure 7. Change in ejaculate volume during treatment of male rabbits with curcumin, tramadol, and/or their combination.

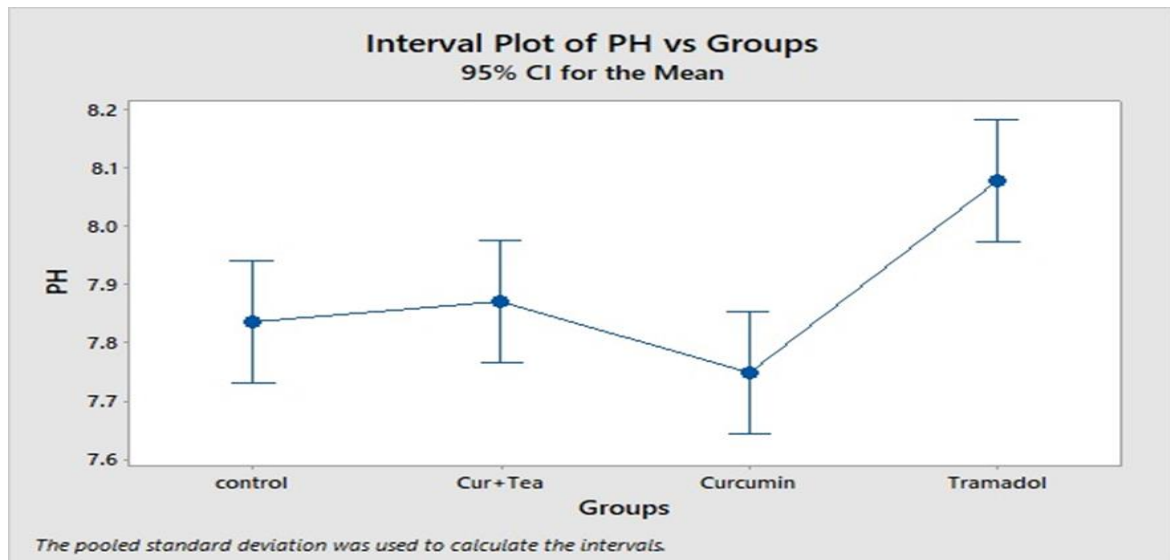


Figure 8. Change in initial hydrogen ion concentration during treatment of male rabbits with curcumin, tramadol, and/or their combination.

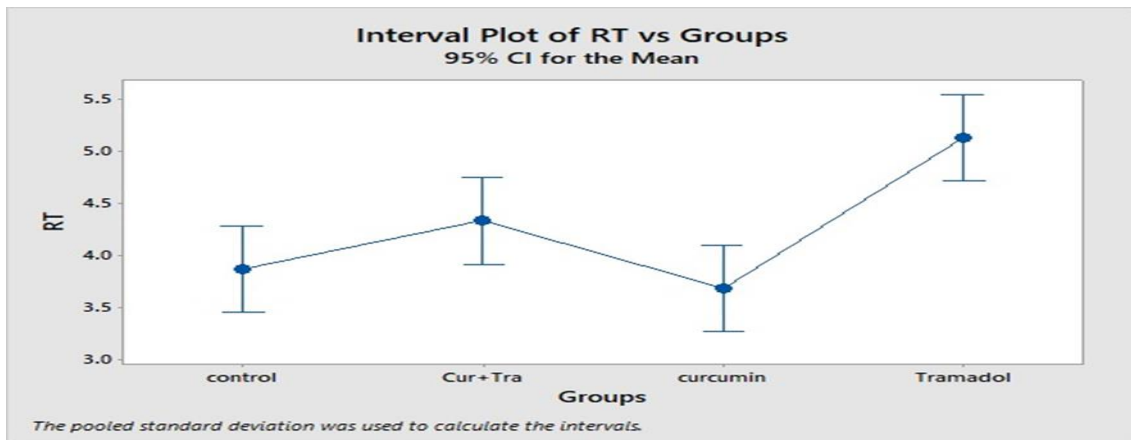


Figure 9. Change in reaction time during treatment of male rabbits with curcumin , tramadol, and/or their combination.

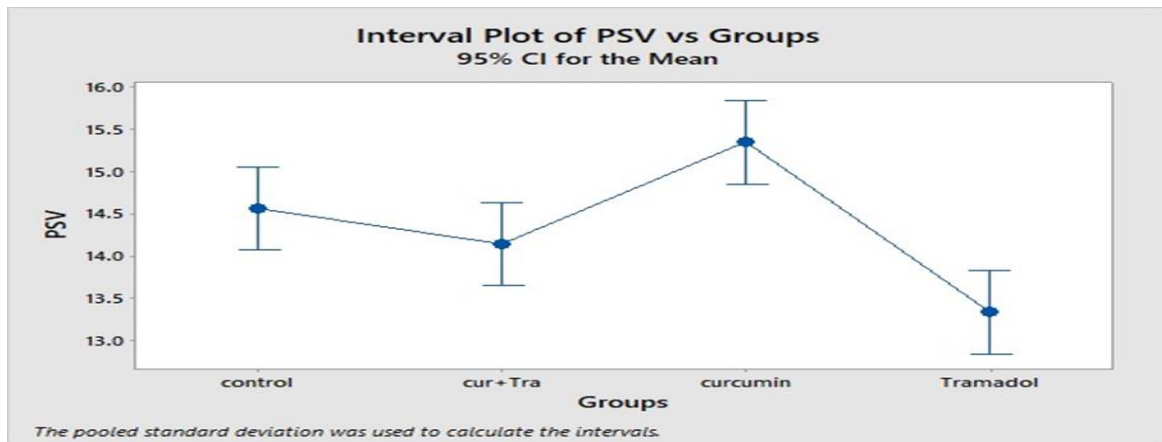


Figure 10. Change in packed sperm volume during treatment of male rabbits with curcumin , tramadol, and/or their combination.

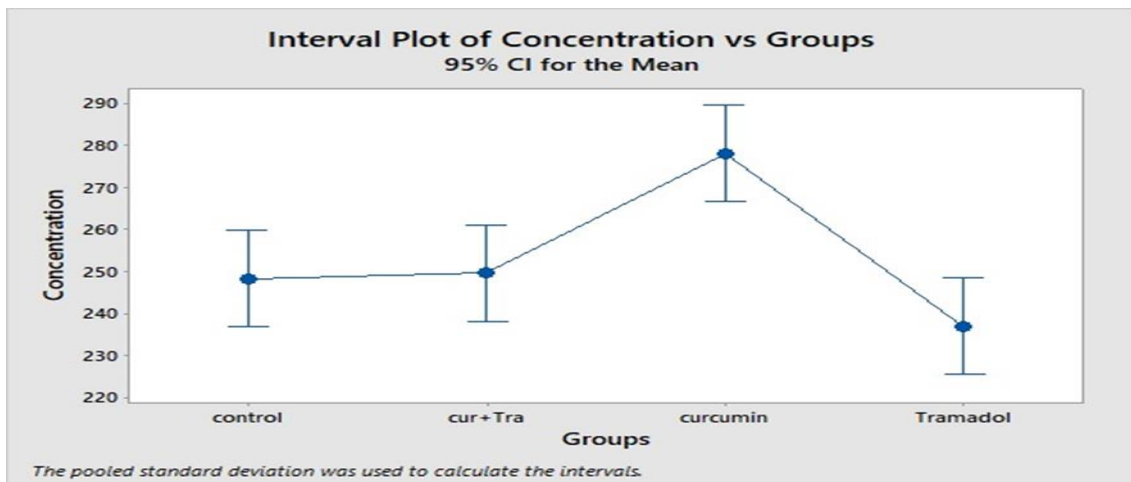


Figure 11. Change in sperm concentration during treatment of male rabbits with curcumin, tramadol, and/or their combination.

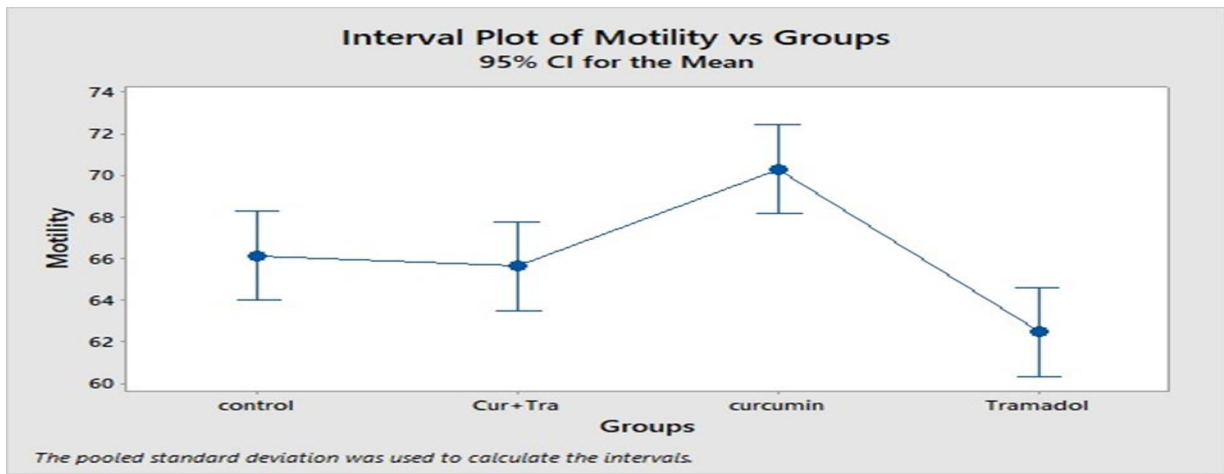


Figure 12. Change in sperm motility during treatment of male rabbits with curcumin, tramadol, and/or their combination.

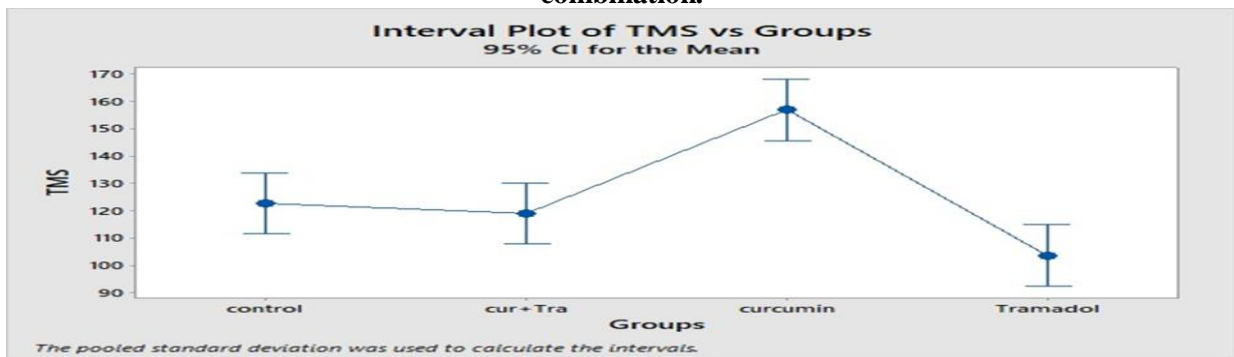


Figure 13. Change in total motile sperm during treatment of male rabbits with curcumin, tramadol, and/or their combination.

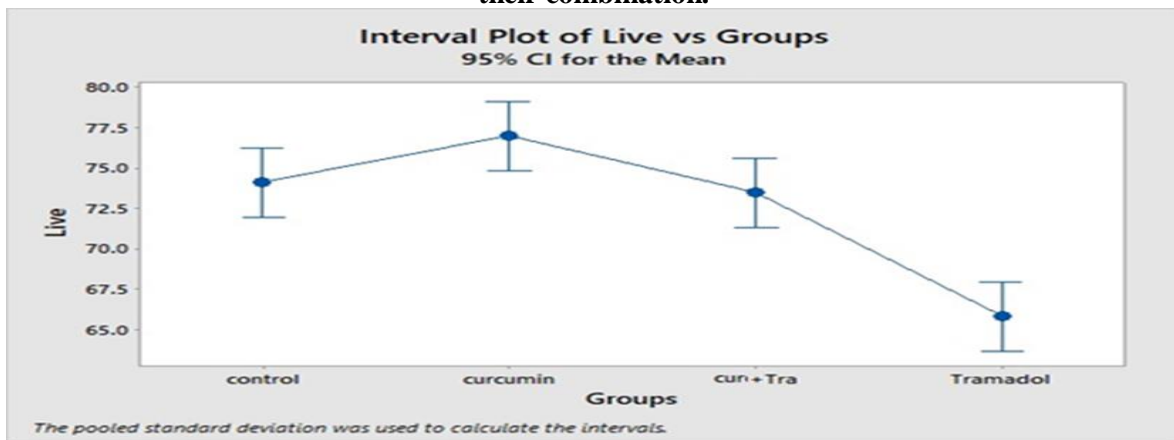


Figure 14. Change in Live sperm during treatment of male rabbits with curcumin, tramadol, and/or their combination.

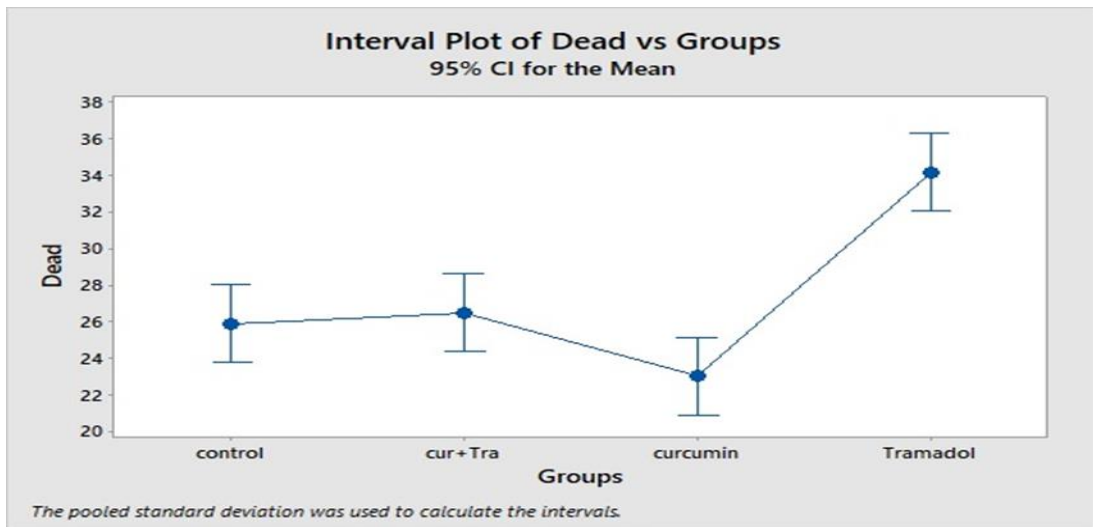


Figure 15. Change in Dead sperm during treatment of male rabbits with curcumin , tramadol, and/or their combination.

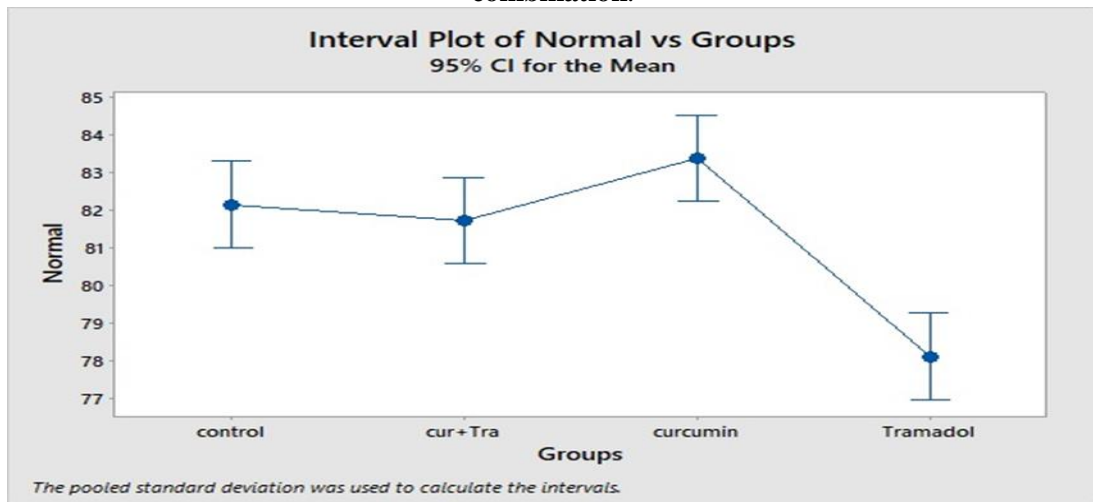


Figure16. Change in Normal sperm during treatment of male rabbits with curcumin , tramadol, and/or their combination.

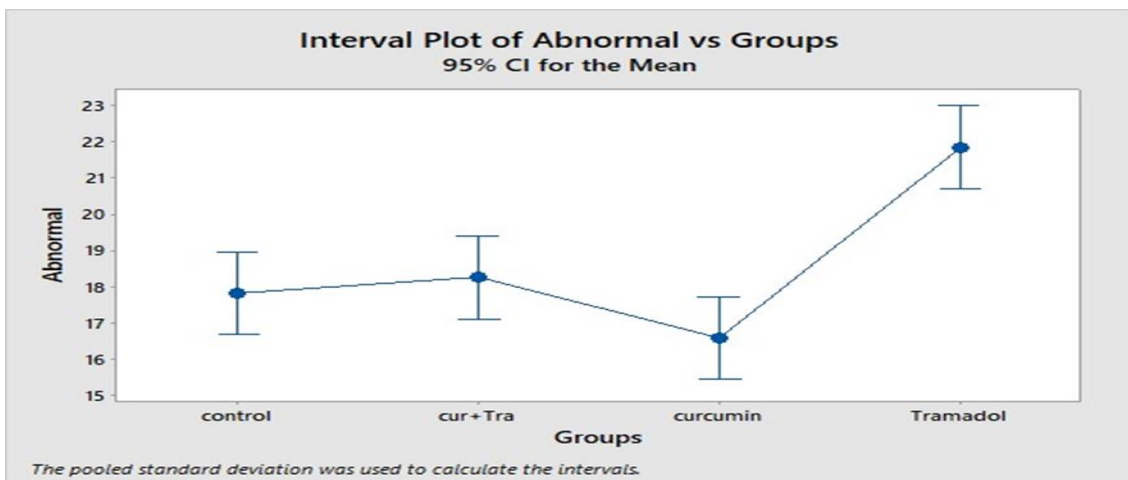


Figure 17. Change in Abnormal sperm during treatment of male rabbits with curcumin , tramadol, and/or their combination.

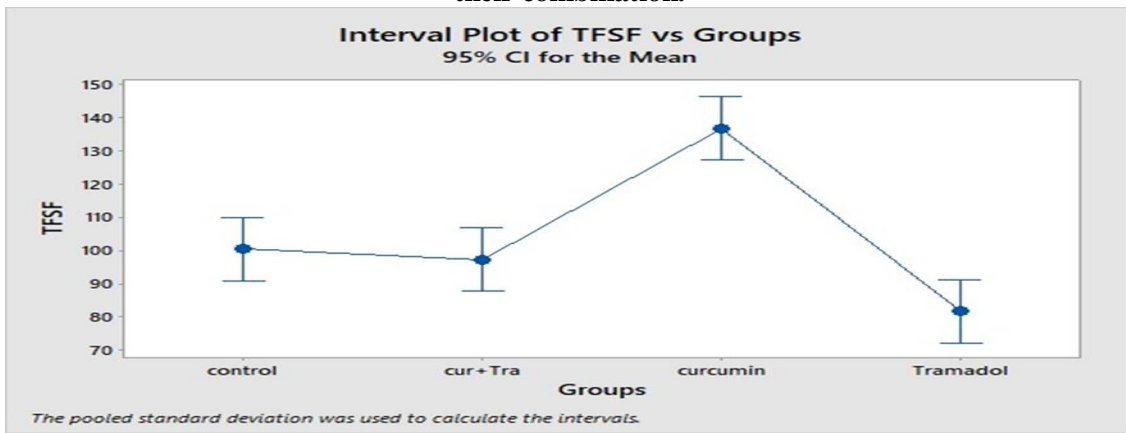


Figure 18. Change in total functional sperm fraction during treatment of male rabbits with curcumin , tramadol, and/or their combination.

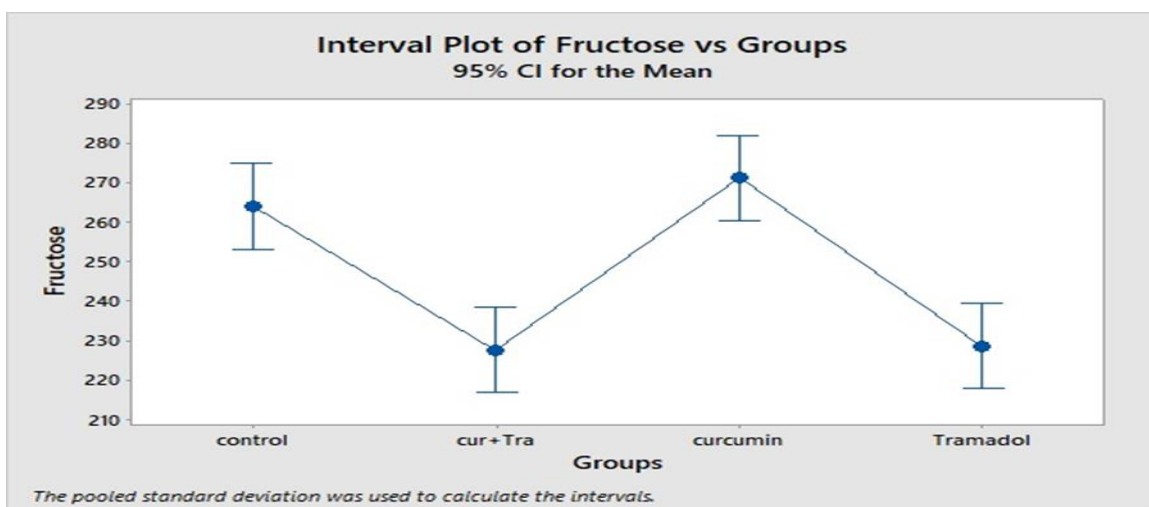


Figure 19. Change in semen initial fructose during treatment of male rabbits with curcumin , tramadol, and/or their combination.

DISCUSSION

The display comes about show that treatment with tramadol caused critical decreases in body weight (BW) and testicles weight (TW) (Table 1 and Figure 1 to 3). The diminishment in BW and TW of the tramadol treated rabbits is in assention with those detailed in past thinks about [20,21]. Moreover, This think about has appeared that the treatment of rabbits with tramadol caused noteworthy recommends that tramadol increments the catabolism of lipids within the fat tissue, coming about in significant lessening in body weight of rabbits at a afterward arrange amid the treatment period. Comparative comes about were detailed by[22] in *Persea americana* leaf extract streated rats. *Curcuma longa* Linn, a part of Zingiberaceae family, commonly known as turmeric, begin in tropical and subtropical districts of India and China. Restorative properties of *Curcuma longa* have been credited essentially to curcuminoids, which are located in the plant rhizome. Curcumin

(diferuloylmethane) is the foremost vital division of *Curcuma longa*[23]. Polyphenol curcumin, extricated from dried rhizomes of the plant, acts through hindrance of mitogen-activated protein kinases[24]. In spite of the fact that turmeric is expended each day in Asian nations, no poisonous impact on the wellbeing of populace was found [25]. Positive impact of *Curcuma longa* powder to the count calories (Table 1 and Figure 1) was moreover found in broiler chickens. Higher weight pick up was watched within the fowls bolstered the slim down containing *Curcuma longa* at level of 0.5 %, compared to the winged creatures accepting 0.25 %, 1 % and control birds[26]. [26] credited the increment within the body weight pick up to the antioxidant action of *Curcuma longa*. Additionally, [27] detailed altogether positive impact of curcuma at the level of 0.5 % on weight pick up of fowls. It was moreover appeared, that curcumin included to the slim down of kids amid the hot summer months essentially moved forward the ultimate live

body weight and normal day by day body pick up of kids compared to the control [28]. On the other hand, additionally to our comes about no critical impact of the supplementation of curcuma powder within the broiler rabbit[5] and within the broiler chicks nourish a blend [29] were detailed.

Cancer prevention agents are able to repeal kidney harm by decrease of lipid peroxidation through upgrade of rummaging capacity of antioxidant defense framework [30]. [31] detailed that supplementation with *Curcuma longa* diminishes oxidative stretch and constricts the improvement of greasy streaks in rabbits bolstered a tall cholesterol slim down. Harmful impacts of opioids at cellular level may be clarified by lipid peroxidation. Organic films contain huge sum of poly-unsaturated greasy acids, which are especially vulnerable to peroxidative assaults by oxidants coming about in lipid peroxidation. Subsequently, lipid peroxidation has been utilized as an circuitous marker of oxidant- initiated cell damage [32]. A noteworthy increment in lipid peroxides was detailed in rats getting an intense dosage of cocaine[33]. Essentially, lipid peroxides were found altogether expanded among heroin users[34]. These discoveries are in assention with the display comes about which appeared critical increment in serum MDA levels in both tramadol bunches compared to control gather, showing an increment in lipid peroxidation. Tramadol habit and manhandle is presently a major open wellbeing threat in libya. The rampancy of tramadol manhandle and the noteworthy jump in wrongdoing in libya driven to Government prohibiting the make, deal and utilize of tramadol. The plan of the current think about examined the brief- and long-term suggestions of tramadol manhandle on semen quality lists utilizing an creature demonstrate. Tramadol manhandle has expanded within the Center East locale, tramadol utilize was common among youths and over one third of tramadol clients had drug-related issues [35]. The display consider appeared that the measurements of curcumin (15mg/kg body weight) evoked increments within the ejaculate volume, concentration, the overall yield , the wave movements, the motility rates, the rates of live spermatozoa and the rates of unusual spermatozoa (Table 2). On the other hand, the higher dosages (9 and 27 mg/kg body weight) appeared diminishes within the ejaculate volumes, the wave movements and the motility rates, but expanded concentrations and anomalous spermatozoa. Curcumin (1 mg/kg) actuated antioxidant impacts and made strides CdCl₂-caused oxidative harm. The epididymis sperm concentration and sperm motility essentially diminished in CdCl₂-exposed gather and the sperm characteristics were recuperated after treating with curcumin. In expansion, CdCl₂ organization brought about in vacuities, edema, hemorrhage, and acute inflammatory cells penetration

within the interstitial space. Organization of curcumin after CdCl₂ poisonous quality diminished the seriousness of histopathological changes [36].

CONCLUSION

The discoveries of the consider give significant prove that tramadol has an unfavorable impact on sperm profile and regenerative organs of male rabbits in terms of body weight, weight of testicles, TBARS, sperm number, sperm viability, sperm motility as well as sperm head anomalies, it is prescribed that balance ought to be worked out within the utilization of this medicate by those taking it for helpful reason.

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