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Research Article

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Evaluation of neurotoxicity and testicular toxicity of artificial Butter flavorings

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ABSTRACT

Diacetyl (Artificial Butter Flavoring) is used as an aroma carrier for foods And therefore, it is considered to be used by the food industry for adding aroma in several types of food products. The goal of this study was to investigate how artificial butter flavoring can affect the brain in terms of neurotransmissions, level of testosterone and histological changes in the testes of albino rats. It was dissected brain to measure neurotransmitters and testicular to histological study. The results of the study indicated that daily diacetyl consumption at 25 mg/kg b.wt was responsible for decreasing dopamine (DA), gamm-aminobutyric acid (GABA) along with serotonin (5-HT) and norepinephrine(NE) levels in different parts of the brain. The back of testicular tissue condition is regularly associated with lower testosterone hormonelevel confirmed histological changes in the testis necrosis of some cells , a significant decrease sperm mature and the tubular deficit. It can be concluded that diacetyl has sparked neurotoxicity and damagethe testicular tissue..

Keywords*: Diacetyl ,neurotransmitters,testosteronehormone,testicular tissue*

INTRODUCTION

Exposure to diacetyl can significantly affect the different parts of the human brain by damaging the neurotransmitter process. The damage is known to be fatal [1]. It primarily affects the central nervous system and consequently, it becomes vulnerable to xenobiotics [2]. The mechanism by which these effects occur is not known. However it should be noted that existing literature suggests that it affects the neurotransmitters and monoaminergic effect is known to have a crucial role during the development process, which is known as synthetic [3].

Diacetyl liquid yellow powder (2,3-butanedione; CAS #431–03–8), is a food flavoring agent and is used to enhance the flavor of butter. It is found naturally in foods and be used as an artificial flavoring agent and aroma agent for wide ranging food products including dairy products, butter, coffee and caramel. It is best known as seasoning in buttered popcorn, responsible for the smell and taste the butter in the microwave popcorn [4-5].

Low levels of consumption of diacetyl did not prove exist in the food to make a risk to human health. Inhaling large concentrations of diacetyl occurs only in a small number of professional places. Diacetyl is toxic in nature and therefore, it was inhaled by the young staff operating in the microwave popcorn packaging factory. Consequently, they experienced inflamed bronchioles and fatal lung disease. Histopathological evaluation revealed tissue inflammation and necrosis assessment of purulent nasal epithelium multifocal necrosis of the airway epithelium in the bronchioles of the people[5]. Metabolites of diacetyl frequently are active in terms of toxicity levels and therefore, it is processed swiftly and is expelled in the form of carbon dioxide. In some cases, Acetoin can also affect the respiratory system and can affect the lungs by increasing toxicity in combination with primary protein amino acid groups [6-

7].This study aims at investigation and evaluation of the impact of diacetyl on the brain, the levels of testosterone and male testes of male albino rats.

MATERIALS AND METHODS

Materials

Chemicals

Diacetyl (2,3-butanedione), CAS#431–03–8, had been bought by the researcher from the chemical company.

Animals

The animals used in the experiment were albino rats and were kept in the environment that had standard light, temperature and humidity levels (22 to 25 °C). The animals were given water and food.

Design of experimental

The animals were randomly divided in to 4 groups.

Group1 (G1): This group consisted of 6 rats was used as controland killed at the beginning of the experiment.

Group2(G2):: (n=24) was orally administered with diacetyl at dose (25 mg/kg b.wt) [8]via oral tube for 4 weeks and six rats were decapitated after1, 2, 3 and 4 weeks post treated to examination the leve of norepinephrine (NE), dopamine (DA), gamm-aminobutyric acid (GABA) and serotonin (5-HT)

Group3(G3):(n=12) was orally administered diacetyl at dose (25 mg/kg b.wt) via oral tube for 4 weeks and six rats were decapitated after 2 and 4 weeks post treated for examination level of testosterone.

Group4(G4): (n=12) was orally administered diacetyl at dose (25 mg/kg b.wt) via oral tube for 4 week and six rats were decapitated after 2 and 4 weeks after treated for histological examination.

Animal Treatment

The rats from the second group were decapitated after the treatment of 4 weeks. They were decapitated suddenly. They were then dissected and their brains were taken out and placed on dry ice glass plate. According to the method designed by [9], their brain was dissected in order to expose their brain segments: cerebellum, cerebral cortex, hippocampus, brain stem and striatum. A filter paper was used to dry out the brain tissues. They were weighed. According to the method identified by [10-11], GABA, 5-HT, DA and NE were taken out from the samples. GABA was measured according to the method identified by [12].Jenway 6200 flurometer had been used to evaluate the fluorescence levels in the samples.

Blood Sampling

The blood samples had been collected by the researcher at two stages after the treatment: blood samples collected at week 2 and blood samples collected at week 4. Immunoassay technique and Spectra Testosterone 1 kits were used to determine the levels of testosterone in according to the instructions provided by the manufacturer. In this case, the manufacturer was Merck. [13]

Histological Studies

The animals were killed and there testes were cut small portions and placed in formalin regulator concentration of 10% has been followed standard methods of dewatering Dehydraion and clarification clearing and imbedding in paraffin wax was the work of sectors histological cross sections thickness of 3 microns of tissue samples control and treatment. And painted Hematoxylin & Eosin [14] Sectors have been used textile dyed with H & E for the testicle to the following studies:Recording morphological and histological pathological changes in the testes to determine the level of growth histological testes of rats.

Statistical Analysis

The data had been analyzed using statistical analysis for control and treated animals and had been done by using paired student't' [15]

RESULT

The results are tabulated in Table 1, which shows the dosage of the diacetyl given on daily basis to the animals. It shows that NE content experienced a decline after the start of the second week inside the whole brain till the duration of the experiment. The results indicate that the maximum decrease in the NE content was estimated to be -80.73 % after 2 weeks had passed in stratium.

Table 2 shows the decrease in the DA from the whole brain after the second week had started and till the end of the experiment. Diacetyl was given to the sample on daily basis. The decrease in the DA content after 4 weeks ended in the hypothalamus was estimated to be -59.81 %.

Table 3 shows that serotonin content in the brain after third and fourth week experienced a decline. The maximum decline in serotonin content inside the cerebellum was estimated to be -44.15 % after the 4 weeks.

Table 4 shows the decrease in GABA after second week inside the whole brain and till the end of the experiment. The decrease of GABA after the 4 weeks was estimated to be -69.49% in the hypothalamus.

Table (1): Effect of chronic oral administration of Diacetyl (25 mg/kg b.wt.) on norepinephrine (NE) content in the different brain areas of male albino rat.

Time of decapitation		Cerebellum mean ± S.E.	Striatum mean ± S.E.	Cerebral cortex mean ± S.E.	Hypothalamus mean ± S.E.	Brain stem mean ± S.E.	Hippocampus mean ± S.E.
1 week	C	95.382 ± 0.845	511.473 ± 1.803	56.203 ± 0.225	596.997 ± 3.242	390.050 ± 0.831	292.540 ± 1.536
	T	95.897 ± 0.575	513.011 ± 1.042	56.543 ± 0.755	604.942 ± 1.580	386.165 ± 1.882	292.647 ± 0.544
	%	0.54	0.30	0.60	1.33	-1.00	0.04
2 weeks	C	95.358 ± 0.857	511.118 ± 1.648	54.443 ± 1.898	605.330 ± 9.485	390.490 ± 0.484	292.527 ± 1.531
	T	60.537 ± 6.713	98.500 ± 1.746	41.667 ± 0.760	487.333 ± 1.667	103.000 ± 1.238	100.000 ± 0.632
	%	-36.52 *	-80.73 *	-23.47 *	-19.49 *	-73.62 *	-65.82 *
3 weeks	C	98.688 ± 0.274	495.653 ± 1.445	55.493 ± 0.105	604.906 ± 2.337	394.485 ± 0.942	283.178 ± 0.817
	T	47.500 ± 0.764	105.333 ± 0.422	42.667 ± 0.667	456.833 ± 1.014	152.333 ± 0.882	102.833 ± 0.946
	%	-51.87 *	-78.75 *	-23.11 *	-24.48 *	-61.38 *	-63.69 *
4 weeks	C	98.485 ± 0.271	495.780 ± 1.443	55.525 ± 0.127	604.623 ± 2.261	394.618 ± 0.944	282.998 ± 0.841
	T	43.500 ± 0.764	97.500 ± 0.764	40.793 ± 0.873	402.667 ± 1.085	105.000 ± 1.390	87.333 ± 0.715
	%	-55.83 *	-80.33 *	-26.53 *	-33.40 *	-73.39 *	-69.14 *

- Statistical analyses were performed between control (C=6) and treated (T=6) animals by using paired *t* ' test.

% : Percentage of change from control. * : Significant at p<0.05.

Table (2): Effect of chronic oral administration of diacetyl (25 mg/kg b.wt.) on dopamine (DA) content in the different brain areas of male albino rat.

Time of decapitation		Cerebellum mean ± S.E.	Striatum mean ± S.E.	Cerebral cortex mean ± S.E.	Hypothalamus mean ± S.E.	Brain stem mean ± S.E.	Hippocampus mean ± S.E.
1 week	C	146.755 ± 0.818	473.948 ± 0.856	60.488 ± 0.044	734.223 ± 2.111	451.288 ± 0.633	243.147 ± 0.863
	T	145.631 ± 0.541	476.248 ± 1.273	60.578 ± 0.736	726.235 ± 3.554	453.979 ± 1.850	241.425 ± 0.658
	%	-0.77	0.49	0.15	-1.09	0.60	-0.71
2 weeks	C	145.648 ± 0.914	482.312 ± 3.336	61.240 ± 0.214	739.237 ± 4.314	451.541 ± 1.947	244.597 ± 1.448
	T	123.500 ± 0.764	401.500 ± 0.563	54.831 ± 0.166	552.833 ± 0.946	400.833 ± 0.401	199.333 ± 0.333
	%	-15.21 *	-16.76 *	-10.47 *	-25.22 *	-11.23 *	-18.51 *
3 weeks	C	146.977 ± 0.942	474.115 ± 0.911	60.715 ± 0.259	734.057 ± 2.258	451.606 ± 0.591	242.968 ± 0.843
	T	122.167 ± 0.872	222.833 ± 0.946	53.761 ± 0.505	353.167 ± 0.792	215.833 ± 0.477	156.167 ± 0.401
	%	-16.88 *	-53.00 *	-11.45 *	-51.89 *	-52.21 *	-35.73 *
4 weeks	C	146.100 ± 1.156	482.780 ± 3.194	62.328 ± 0.946	738.215 ± 4.439	451.637 ± 1.987	244.905 ± 1.544
	T	117.156 ± 1.807	196.167 ± 0.910	50.203 ± 0.317	296.667 ± 0.667	217.333 ± 0.843	157.333 ± 0.558
	%	-19.81 *	-59.37 *	-19.45 *	-59.81 *	-51.88 *	-35.76 *

- Statistical analyses were performed between control (C=6) and treated (T=6) animals by using paired *t* ' test.

% : Percentage of change from control. * : Significant at p<0.05.

Table (3): Effect of chronic oral administration oral of Diacetyl (25mg/kg b.wt.) on serotonin (5-HT) content in the different brain areas of male albino rat.

Time of decapitation		Cerebellum mean ± S.E.	Striatum mean ± S.E.	Cerebral cortex mean ± S.E.	Hypothalamus mean ± S.E.	Brain stem mean ± S.E.	Hippocampus mean ± S.E.
1 week	C	192.457 ± 0.799	171.652 ± 0.450	57.247 ± 0.385	432.828 ± 0.319	118.155 ± 0.197	214.787 ± 1.321
	T	182.756 ± 4.869	168.301 ± 1.634	56.723 ± 0.267	431.746 ± 1.004	117.798 ± 0.163	213.043 ± 0.298
	%	-5.04	-1.95	-0.92	-0.25	-0.30	-0.81
2 weeks	C	193.045 ± 0.719	171.496 ± 0.522	57.374 ± 0.463	433.106 ± 0.485	118.368 ± 0.364	215.100 ± 1.229
	T	182.925 ± 4.810	158.554 ± 0.429	54.049 ± 1.950	432.680 ± 0.413	116.000 ± 0.365	204.858 ± 4.789
	%	-5.24	-7.55 *	-5.80	-0.10	-2.00 *	-4.76
3 weeks	C	192.326 ± 0.203	173.444 ± 1.705	57.287 ± 0.176	430.635 ± 0.928	117.401 ± 0.079	216.757 ± 0.943
	T	170.018 ± 7.740	152.288 ± 1.447	44.333 ± 0.333	365.000 ± 0.577	104.945 ± 0.712	194.500 ± 1.544
	%	-11.60 *	-12.20 *	-22.61 *	-15.24 *	-10.61 *	-10.27 *
4 weeks	C	192.276 ± 0.075	173.669 ± 1.772	57.771 ± 0.023	430.951 ± 0.267	118.248 ± 0.380	215.868 ± 1.275
	T	107.381 ± 0.313	145.679 ± 0.781	40.005 ± 0.195	385.847 ± 5.326	92.950 ± 0.584	192.869 ± 1.830
	%	-44.15 *	-16.12 *	-30.75 *	-10.47 *	-21.39 *	-10.65 *

- Statistical analyses were performed between control (C=6) and treated (T=6) animals by using paired *t* ' test.

% : Percentage of change from control. * : Significant at p<0.05.

Table (4): Effect of chronic oral administration of diacetyl (25 mg/kg b.wt.) on gama-butyric acid (GABA) content in the different brain areas of male albino rat.

Time of decapitation		Cerebellum mean ± S.E.	Striatum mean ± S.E.	Cerebral cortex mean ± S.E.	Hypothalamus mean ± S.E.	Brain stem mean ± S.E.	Hippocampus mean ± S.E.
1 week	C	192.457 ± 0.799	171.652 ± 0.450	57.247 ± 0.385	432.828 ± 0.319	118.155 ± 0.197	214.787 ± 1.321
	T	192.349 ± 0.538	171.688 ± 1.024	57.919 ± 0.149	431.393 ± 2.515	116.809 ± 0.958	215.267 ± 0.938
	%	-0.06	0.02	1.17	-0.33	-1.14	0.22
2 weeks	C	192.544 ± 0.759	171.662 ± 0.447	57.374 ± 0.463	432.939 ± 0.370	117.868 ± 0.237	214.933 ± 1.269
	T	137.833 ± 0.307	99.667 ± 0.333	41.833 ± 1.740	253.500 ± 0.764	83.333 ± 0.760	110.333 ± 0.422
	%	-28.41 *	-41.94 *	-27.09 *	-41.45 *	-29.30 *	-48.67 *
3 weeks	C	193.611 ± 0.781	175.423 ± 1.783	57.849 ± 0.675	437.968 ± 1.007	118.436 ± 0.231	216.865 ± 0.870
	T	142.833 ± 0.946	92.333 ± 0.843	40.833 ± 0.401	202.333 ± 0.843	72.500 ± 0.764	85.500 ± 0.563
	%	-26.23 *	-47.37 *	-29.41 *	-53.80 *	-38.79 *	-60.57 *
4 weeks	C	193.379 ± 0.440	171.744 ± 1.615	57.713 ± 0.935	437.849 ± 0.198	118.118 ± 1.398	215.234 ± 1.053
	T	140.500 ± 0.342	69.667 ± 12.341	34.000 ± 0.816	190.500 ± 0.342	65.667 ± 0.211	65.667 ± 0.333
	%	-27.34 *	-59.44 *	-41.09 *	-56.49 *	-44.41 *	-69.49 *

- Statistical analyses were performed between control (C=6) and treated (T=6) animals by using paired *t* ' test.
% : Percentage of change from control. * : Significant at p<0.05.

The data presented in Table 5 shows that the use of diaceytl was responsible for descreasing testosterone in the blood of the male albino rats. The maximum decrease was estimated to be -26.16 percent after 2 weeks.

Table (5): Effect of chronic oral administration of the diacetyl (25 mg/kg bw) on testosterone level in serum blood of male albino rat.

Time of decapitation		Testosterone (ng/ml) mean ± S.E.
2 week	C	0.818 ± 0.001
	T	0.604 ± 0.001
	%	-26.16 *
4 weeks	C	0.818 ± 0.001
	T	0.696 ± 0.002
	%	-14.99 *

Histological analysis of the samples are given for Plate 1(1) and 1(2). The results shows that there seminiferous tubules that are small and appear to be normal and majority of them do not have lumen, are enclosed by a tissue layer that is fibrous in nature. High magnification showed the dark spermatogonium, sertoli cells and pachytene spermatocytes. The myoid cell layer was found to be enclosed and there was intertublar space that had leydig cells.

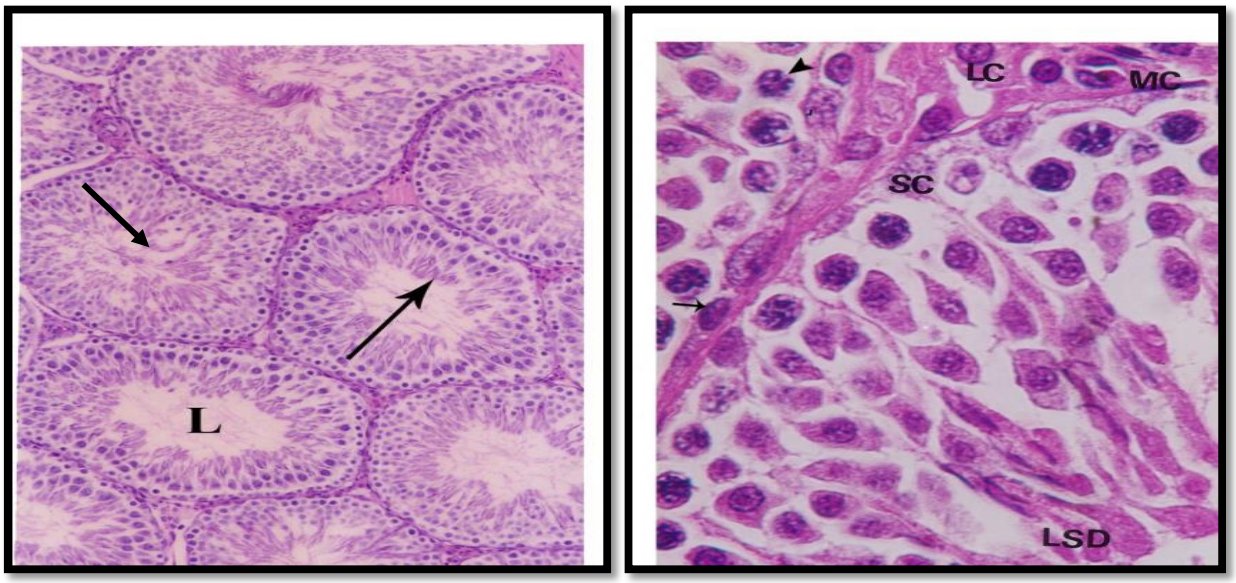

Plate1 (1-2): Transverse sections (T.S.) of tests of male rate for control group (G_1).

(1): Note, enlarged seminiferous tubules populated by spermatocytes and late spermatids (↗) surround the tubular Lumen (L) (8 weeks of age (H & E; x 100).

(2): High power from 2c showing Light (▲) and dark (↗) spermatogonium adjacent to basal Lamina; late spermatids (LSD) with elongated head directed towards Sertoli cells (Sc). Note, myoid cell (Mc) and Leydig cells (LC) (PAS, x 400).

The results of Plate 2(1, 2, 3, 4,5and 6) demonstrate that diacetyl administration after one week in the male albino rat decreased the spermatogenesis and tubules and mature therefore, sperm cells reduced

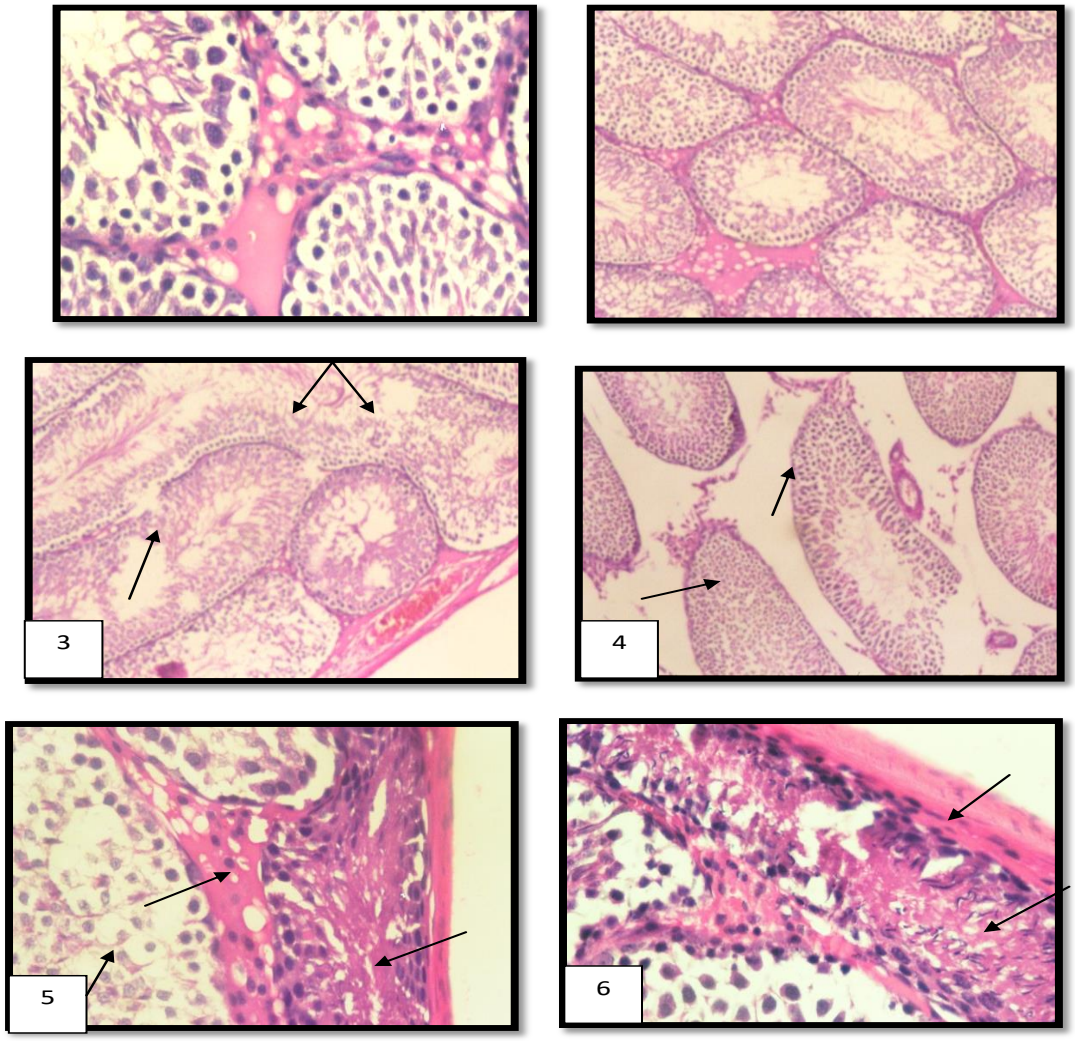

Plate 2 (1-6)Transverse sections (T.S.) of tests of male rate for tretment group after 1 week..

1- degenerating spermatocytes (arrows) with their condensed cytoplasm, round spermatids, supranuclear cape and irregular basal lamina) and edema in the interstitial tissue) (H & E x 400). 2- necrosis of spermatocytes with pyknotic nuclei and homogeneously condensed cytoplasm (H. & E., x 100) 3- showing deformed St with loss and necrosis of spermatocytes sloughing and lysis of spermatocytes into wide lumen (H. & E., x 100) 4- Showing seminiferous tubules with irregular surface and contain sloughed in lumen. Note interstitial tissue and basal lamina spermatogonia still attached to some tubules (H. & E., x1 00) 5- nuclei of necrotic spermetocytes) interstitial tissue edema seminiferous epithelium in basal lamina and myoid cell layers around and deformed transformed spermatids (H & E, x 400).

6 -demonstrate thickened basal lamina lyzed Myoid cell layers around Sts and widen intercellular space with abnormal (H & E, x 400).

The results of the Plate 3(1,2, 3,4, 5 and 6) show that mature sperms and germ cell hypoplasia were not found with the condition of early arrest. The architecture had been disrupted and along with the tubules show that spermatogenesis was absent. The necro-degenerative changes showed inflammation, interstitial fibrosis and vascular injury and congestion. It also showed that atrophy existed in the interstitial cells and germ cells. Results indicate that diacetyl is responsible for the increment of tubular dysfunction as well as early arrest in the testicles,

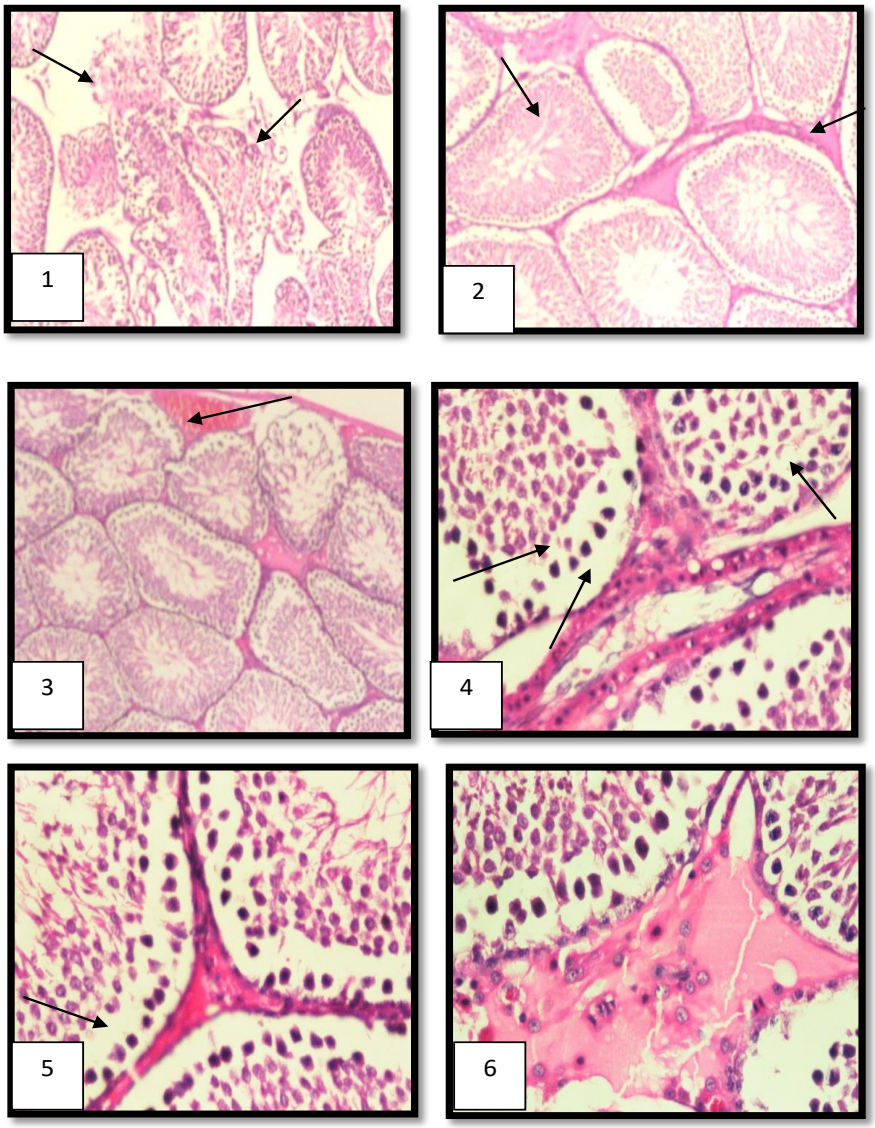

Plate 3(1-6) Transverse sections (T.S.) of tests of male rate for treatment after 2 weeks.

1- damaged, nuclei of germ cell showing deformed Seminfierous tubules with loss and necrosis of spermatocytes sloughing and lysis massive degeneration and wide lumen (H & E x 100).2- Atrophoid tubules., some spermatocytes and odema (H & E x 100) 3- red blood cell stasis in the blood vessels (H & E x 100) 4- Showing germ cell necrosis deformed Sertoli cells and focal lysis of germ cells. Fragmented advanced spermatids (H & E, x 400). 5- Seminfierous tubules with deformed germinal epithelium and irregulat surface (H & E, x 400). 6- oedema inter the seminiferous tubules (H & E, x 400)

DISCUSSION

The results of the study show there is decrease in the levels of NE, GABA, DA and 5-HT in the brain area after the experiment had ended. The results also show that testosterone level reduced in the serum blood of the albino rats after they were given diacetyl orally on daily basis.

The diacetyl of the common flavors used in many foods and it can estimated to cross the blood brain barrier due to the presence of viable compounds soluble in fat research has shown that the high amount of flavor industrial butter in intake of foods cause cognitive decline affects memory It was found that the industrial flavor of butter a detrimental effect on the brain, through the influence on the content of neurotransmitters and outputs metabolic interactions in brain regions that play an important role in various physiological processes in the human body[7].The diacetyl cause of low production of neurotransmitters in the cells produced both norepinephrine , dopamine , GABA and serotonin, which has an important role in learning and memory[16].

All that compound diacetyl located in artificial butter flavoring has an important role high incidence of Alzheimer's and showed disease previous studies that eating foods that contain flavor industrial butter working on occurrence of learning difficulties and weakness incidence of Alzheimer's disease by inducing oxidative damage in the brain and lack of concentration glutathione and antioxidants [16-17-18]. found That a bilateral diacetyl structure similar to the substance that makes beta proteins amyloid accumulate in the brain, causing Alzheimer's disease, which increases the rate of amyloid in the brain, lack of nerve immunizations content is linked to the lack of proteins and protective called glyoxalase working on nervous system, leading to a long-term neurotoxicity of dual-eating diacetyl found the lack of activity of antioxidant enzymes in rats exposed to the substance diacetyl. [6] .

Both noradrenaline and serotonin are known to protect the brain. The mitogen activated protein kinase cascade viability and the β-adrenergic plus 5HT1 serotonergic receptors have the ability to work with the help of mitogen activated protein kinase cascade. These pathways are known to play a vital role in improving the neuronal survival of the system. Diacetyl and its derivatives are known to affect the testicles negatively. It can affect the electron procedure of transfer, can create oxygen species that are reactive in nature and can change the cell signaling events. It can also increase the toxicity levels in the cell membrane of the tissues of the testicles [19-20].

The results of the study indicate that testosterone levels in the serum of the male albino rat decreased when exposed to diacetyl on daily basis. It has the ability to produce oxygen species that are reactive in nature and can produce α-dicarbonyl during the transference of electrons. The results also show that spermatogenesis and tubules were in the condition of late partial arrest after the one week and the mature sperms started to reduce. After the second week, the tubular dysfunction increased and the early arrest was shown. Histopathological analysis shows that necrosis was presented in the proximal tubes of the kidney[21]. . Hepatocytes experienced inflammation. Exposure to diacetyle can lead to death since it can affect the respiratory system. It can cause epithelial necrosis and inflammation in the trachea, bronchi and larynx [22-23-24].

CONCLUSION

Diacetyl is natural compound that is commonly found in foods. However, it is also used as flavoring agent and aroma adding agent in different foods such as dairy products, coffee, caramel and butter. It can be used to treat Parkinson diseases and Alzheimer diseases since it can improve the efficiency of the central nervous system.

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