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BEHAVIORAL AND NEUROBIOLOGICAL CONSEQUENCES OF HIGH-FAT DIET ON ALCOHOL AND NICOTINE DRINKING IN MALE WISTAR RAT

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ABSTRACT

Consumption of ethanol and nicotine are highly co-consumptive condition for addictive drugs and rate of occurrence between alcohol and nicotine addiction is so high. Alcohol and nicotine shows the synergistic effect to each other with respect to neuronal changes in region of the brain. Consumption of addictive substances influenced on various neurotransmitters like dopamine, serotonin, GABA and acetylcholine. High fat diet (HFD), fat and calorie enriched food; consumption of HFD may consequences in reducing of alcohol and nicotine. High fat diet shows pharmacological results in alcohol use disorder (AUD), Anxiolytic and addictions like symptoms but how it works remains unclear. We first examined the alcohol and nicotine co- consumption in male Wistar rats by 3-bottle choice procedure; we found that rats are more interested in intake of alcohol rather than nicotine. We determined that intermittent and chronic HFD consumption help in reducing the alcohol consumption and nicotine consumption in male Wistar rat with the help of behavioral testing and neurobiological testing. In behavioral testing by light and dark box, high-fat diets also produce anxiolytic effect that spent more time in light side by chronic group and intermittent group of rat and spent more time in open area for same group. In our testing there is significantly correlation of HFD was found on alcohol and nicotine consumption.

KEYWORDS: High fat diet, Alcohol, Nicotine, Alcohol use disorder, Drug dependence.

1. INTRODUCTION

In the present era, Ethanol and nicotine both addictions are vastly co-morbid for consumers **[O'Rourke *at el;* 2016].** The history of Ethanol consumption is too old which is about 3000 BC, used by people on religious festivals as well as beverages for pleasure. Ethanol and nicotine both have toxic as well as psychoactive properties for the consumers. According to last decade survey of WHO, consumption rate of ethanol and nicotine increased in people of developing countries like India, South Africa and others. In adult age of person are highly vulnerable to ethanol, nicotine and other types of drugs addiction. Diseases induced in alcoholics are- Cirrhosis, Gastritis, Pancreatitis, Myopathy, Cerebral degeneration while disorders induced by consuming of nicotine in acute way or chronic way are bronchitis, different types of cancers and lungs diseases and Nicotine mainly enhances the carbon monoxide (CO) inside the blood stream. Due to over consumption of alcohol and nicotine chances of inflammatory disease increased in particular area of the body. According to WHO [2018] 5.1 % burden of disease is only chronic alcohol consumption and 3.3 million death per year occurs due to alcohol intake, this situation of mortality and disease enhancement promoted year to year consistently due to ethanol consumption and nicotine consumption. Consumption of alcohol causes two main syndrome that is Wernicke's syndrome and Korsakoff syndrome in the region of the brain, simultaneously it named as Wernicke-Korsakoff syndrome (WKS), deficiency of thiamine in brain is the etiology for alcohol related WKS. WKS is age dependent syndrome, occurrence of this syndrome is higher in 60+ aged person who intake alcohol in chronic way of consumption **[David et al; 2007].**

Alcohol and nicotine work as depressant for the central nervous system, these are mostly release the inhibitory neurotransmitters like dopamine, serotonin, GABA and acetylcholine. These inhibitory neurotransmitters activities enhances, because these addictive substances down the level of excitatory neurotransmitters that result in enhancement of inhibitory neurotransmitters in body system. These alterations of excitatory and inhibitory neurotransmitters result in symptom of addiction and drug dependence. Alcohol and nicotine both addictive drug excite the ventral tegmental area (VTA) of the mid brain, which further response to Nucleus accumbens (NA) of the limbic system to release more and more dopamine in frontal cortex region of the brain **[Carole et al; 2020**].

Highly palatable food helps in reducing the addictive substances like alcohol and other due to influence of intake behavior. High-fat diet (HFD) mostly fat and calorie enriched feeding material showed diabetic induction like characters and sometime used as a palatable food. However, there is no any model that can exactly mimic all dimensions of hyperphagia in the brain that can introduce about high-fat diet (HFD) rather than normal chow but palatability or affection towards the HFD can influence on intake of alcohol and nicotine consumption spontaneously or simultaneously **[Sirohi et al; 2013].**

Rats or mice are nocturnal mammals and these mammals show less activity in light phase. The dark phase of light session responsible for motor activity in rodents, mostly all neurobiological activity performs in dark phase because rodents are more active and rate of consumption like alcohol and nicotine is higher.

Finally, we hypothesized on intake of HFD would attenuates consumption of alcohol and nicotine, however HFD would enhance the body weight of experimental rats.

2. MATERIAL AND METHODS

2.1 Diet

Entire designed groups of male Wistar rats had ad-libitum access to normal chow throughout the experiments. Protocol accordingly intermittent high-fat diet (HFD) only provided on Tuesday and Thursday while chronic high-fat diets were served on each day of the working week. The HFD feed have approx. 40% of fat value. HFD pellets were prepared by using crushed normal feed, fat (Peanut oil, maze oil, vegetable oil) and milk powder. All types of diets were measured on each day before serving to the animal, which helps in the calculation of feeding amount in different groups of animal.

Each 100 grams high-fat Diet contains-

S. No.	Ingredient Name	Amount in grams (100 gm)
1	Crushed normal feed	68
2	Maize oil	6
3	Dalda ghee	6
4	Milk powder	20

2.2 Animals

All male Wistar rats purchased from **M/S CHAKRABORTY ENTERPRISE (Regd. No.1443/PO/Bt/s/11/CPCSEA**) Kolkata, India. 24 Male Wistar rats were accommodated spontaneously in an environmentally organized vivarium on a reverse light and dark cycle (i.e. lights off at 8:30 a.m.) with normal chow and water freely available ad-libitum in manufactured cages. All experimental protocol on animals was approved by Institutional Animal Ethical Committee **(UIP/IAEC/Nov.-2020/03)** adhered to the Committee for the Purpose of Control and Supervision of Experiment on animals and use guidelines at Prayagraj, India.

2.3 General procedure

Male Wistar rats coordinated for body weight and diet consumption received behavior for initial 1st week of the experiment. On the basis of their feeding and drinking behavior categorized into 4 different -groups, and groups name was accordingly- Positive control, Negative control, Intermittent HFD, Chronic HFD. Drinking tap water and normal chow were freely available ad-libitum to entire experimental animal groups. Different Feeding like normal chow, HFD and water were kept in each cage of animal accordingly and were observed how much amount of water and variants chow were be taken and how much left in cage and refill them on each day. This experimental segment was performed for 6 weeks continuously for all 4 groups.

Entire the experiments performed under the reverse light phenomenon i.e. light off on 8:30 am to 5:30 pm. Red light with 350 lx were used only during the working time. Rats or mice are nocturnal mammals and these mammals show less activity in light phase. The dark phase of light session responsible for motor activity in rodents, mostly all neurobiological activity performs in dark phase because rodents are more active and rate of consumption like alcohol and nicotine is higher. Hence, a good reason behind the work on behavioral testing in dark phase on rats. Literature review on rat behavioral testing indicates some researcher work on reversed light-dark cycle and some researcher do not.

In the end of 6th week of variants Chow and High Fat Diets, water was provided along with Alcohol and Nicotine for 2 weeks to entire experimental animal groups. 20% alcohol and 30µg/ml nicotine solution intake were examined in red light room (350lx). Alcohol (20%) and Nicotine (30µg/ml) was prepared freshly in tap water on each 4th day of experiments. There was no need of pH adjustment and filtration of prepared nicotine liquid. In both ethanol and nicotine solution any sweeteners were not added as masking agent. During the experimental procedure position of Alcohol and nicotine bottle was altered on each day of working. In the end of 8th weeks of experiments Alcohol and Nicotine exposure was stop and re-exposure of diet for 1 week as usual.

2 animals were euthanized from each group by decapitation method and brain region of the rats separated and kept at -20 °C in formaldehyde solution. Homogenize the brain tissue in erythroxylan dye at 1500 rpm for 20 minutes after that centrifuged the homogenized tissues for quantitative analysis of neuronal changes like Dopamine, acetyl-choline, and others with respect to reference drugs. Brain of different group rats were dissected and separated out for histological study by using of microtone apparatus.

2.4 Alcohol and Nicotine drinking exposure

For drinking exposure on animals graduated bottles used for the experimental purpose on different group animal. Both alcohol and nicotine drug was administered in desired liquid form to the animal group.

In case of neurobiological and behavioral experiments 99.9 % pure Ethanol was purchased from Science Corporation, Prayagraj India and the desired amount and 20% concentrated of ethanol were freshly arranged on each 4^{th} day of experiments at least 1 day before exposure of ethanol testing. Generally concentrated Ethanol was mixed with tap water to dilute and found in desire concentration of testing. On day alcohol consumption day, rats were provided with unsweetened and freshly prepared alcohol (20% v/v) solution by using dilution method.

Nicotine liquid purchased from Sigma Aldrich, nicotine liquid was prepared freshly in tap water on each 3^{rd} day of the experiments. The concentration of nicotine liquid was 30 µg/ml.

Ethanol, Nicotine and tap drinking water were used as 3 bottle choice paradigm. During the experimental session bottle positions were alternated. Alcohol and nicotine were kept for 8 hours drinking session and consumption of nicotine, alcohol and water were measured to understand the drinking behavior on Wistar rats.

2.5 Anxiety-like behavior testing

2.5.1 Light and dark apparatus

Anxiety-like behavior was measured using a light and dark apparatus, this apparatus was mostly defined by Crawley and Goodwin (1980). A closed top arena (45×27×27) and walls covered with methacrylate polymers. In this, one compartment was painted black in color named as dark compartment while non-painted compartment named as white or light compartment of the apparatus. Animal (rats) were kept in middle of the box for 5 minutes to observe. After the removal of animal L&D box were cleaned perfectly to remove odor. During the anxiety like behavior testing in the L&D apparatus, defecation and urination were counted. The total quantity of entrances and period spent in the light compartment side were calculated for behavioral statistics.

2.5.2 Elevated plus maze

EPM behavioral model principally works as giving incentive from the outside environment not as inside ones, basically used to examine the anxiety- related behavior model in rats/mice or other rodents. The EPM apparatus consists of a "+" shaped maze and EPM consists of two oppositively closed arms and two opposite open arms, and a center area. The dimensions of both open and closed arms have basically same i.e. 48cm x 10cm. To measure the anxiety level in experimental animal the EPM was elevated up to 50cm height.

Basically two parameters are used in EPM are Memory retention and Transfer Latency. In Transfer latency we keep experimental animal at the edge of open arm and placing of the rat is in the direction of far from the mean of the platform. To measure "Transfer latency" we note down the time spent by the rat to enter one of its closed appendix with all its four legs. In case if rats did not enter into the closed arm within 3 minute, then it is delicately driven into one of the secured arms inside 180 sec, and in this case TL is assigned as 180 sec. At that point of following 15 sec., rodent was permitted for investigate the labyrinth before returning it to its home cage. Memory maintenance is inspected 24 h after the principal day preliminary on the subsequent day. Between every meeting, the labyrinth was painstakingly cleaned with 30% ethanol to expel any olfactory signs inside or on arms of the elevated plus maze.

2.6 Biochemical parameters

After performing the behavioral tests and trials, the experimental animals were sacrificed by decapitation method. Animal brains were collected and wash with tap water gently to remove out debris and other unnecessary substances of the brain. With the help of Teflon, Homogenizer the excluded brain was homogenized in a medium containing (phosphate's PO_4^{3-}) buffer having pH in range of 7.4 i.e. pH of body fluids. After that the sample of brain's homogenate, centrifuged at 1500 rpm for 1200 seconds. For bio- chemical investigation we take brain homogenate supernatant.

2.7 Statistical analysis

The statistical analysis of all pharmacological analysis performed using software Graph Pad prism Two-way ANOVA, version 9.1.2 for windows. The values are represented as Mean$\pm$S. D, for 6 rats data were analyzed by two-way ANOVA analyzed body weight, food ingestion, ethanol consumption, nicotine consumption, total water intake, and ethanol or nicotine preference testing during the exposure of the experiment.

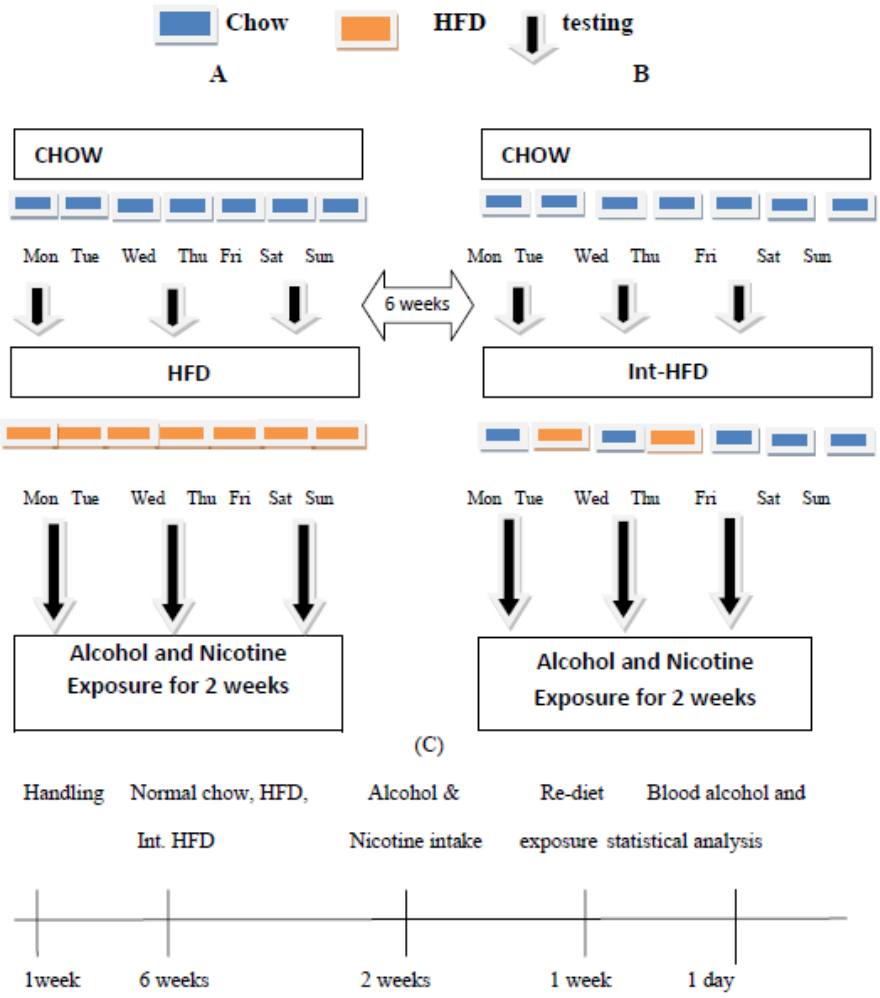


Fig. 1: Schematic description of the HFD variants on body weight during experimental protocol; (A) Rats received intermittent pattern of high-fat diet for 6 week of designed experimental procedure (24 h every Tuesday and Thursday), (B) tested of high-fat diet intake on alcohol and nicotine in Chronic and intermittent group of animal for 2 weeks (12 h drinking procedure). (C) Depict base line experimental procedure.

3. RESULTS

3.1 Consequences of high-fat diet (HFD) intake on body weight

A mixed model Two- way ANOVA analyzed body weight data in male Wistar rats following ten weeks of HFD exposure. Rats of positive control and Negative control receiving normal chow to maintain body weight while group of rats in Chronic HFD and Intermittent HFD receiving limited access of HFD 40% table 4.1 demonstrated a feeding intake fashion. A group of animal exposed to HFD in Chronic (211.00±1.71) and intermittent HFD (237.33±1.05) showed enhancement in the body weight and compared in body weight [F (3, 40) = 417.0, P<0.0001]. But, there was no significantly variation was observed in all group of animals.

3.2 Consequences of high-fat diet on alcohol consumption

A mixed model Two- way ANOVA analyzed ethanol consumption data in male Wistar rats following 6- weeks of chronic and intermittent HFD exposure. Rats of negative control, chronic HFD and intermittent HFD receiving alcohol depicts in Table 4.2. All group of animals those exposed to alcohol and nicotine consumed higher amount of alcohol as compare to nicotine solution. Alcohol consumption is reduced in HFD exposed group as compare to negative control group. Intermittent exposure (4.16 ± 0.75) of HFD group show more significant reduction of alcohol consumption behavior as compare to chronic HFD (3.00 ± 1.09) exposed group. The comparison between Chronic HFD and intermittent HFD for 20% ethanol consumption [F(2,30)=87.66, P<0.0001]. There was no any significant observed in negative control.

3.3 Consequences of high-fat diet on nicotine consumption

A mixed model Two- way ANOVA analyzed nicotine consumption data in male Wistar rats following 6- weeks of chronic and intermittent HFD exposure. Rats of negative control, chronic HFD and intermittent HFD

receiving nicotine depicts in Table 4.3. All three group rats underwent nicotine three bottle choice paradigm. Rate of nicotine consumption in initial week is high in all experimental group of animal (n=6), while till 14th day of nicotine intake exposure, rate of consumption for Int. HFD (2.65 ± 0.08) was not significant in comparison to Chronic HFD (2.36 ± 0.09) and negative control (2.26 ± 0.09). The comparison between Chronic HFD and intermittent HFD for nicotine consumption [F(2,30)=21.13, P<0.0001]. There was no any significant observed in all group of animals.

3.4 Drinking assessment by 3-bottle choice paradigm –

A mixed model Two- way ANOVA analyzed average ethanol, average water and average nicotine consumption data in male Wistar rats following 6- weeks of chronic and intermittent HFD exposure. After 2 weeks, drinking evaluation was started by using Drinking in Dark paradigm. The result showed water intake is normal in all groups whereas ethanol intake is more than nicotine in chronic, intermittent and negative control group except then positive control. Compared water, alcohol and nicotine consumption [F(3,40)=447.2, P<0.0001].

3.5 High-fat diet induced anxiolytic-like behavioral effects

Light and dark apparatus

To determine limited HFD variants (Chronic and intermittent) access impacted anxiety-like behavior, Wistar rats from positive controls (chow), negative control, intermittent and chronic HFD groups were tested for the time spent in the light side and dark side(second) of the light and dark box. A two-way ANOVA identified a main effect of HFD exposure [F(2-30)=44.78, P<0.0001]. The time spent in light side for intermittent HFD group (85.33 ± 1.06), Chronic HFD (73.83 ± 0.91), Positive control (85.33 ± 1.14) and Negative control (64.66 ± 1.04) Wistar rats. These data suggested that HFD intake on Wistar rats induced an anxiolytic condition.

Elevated plus maze apparatus

To determine variants HFD like Chronic and intermittent access impacted on anxiety- like behavior, Wistar rats from positive controls, negative control, chronic and intermittent HFD groups were tested for the time spent in open arm and closed arm in seconds by the elevated plus maze apparatus. A two-way ANOVA identified a main effect of HFD exposure [F(3,40)=417.0 P<0.0001]. The time spent in open arm for Chronic HFD group (107 ± 0.53), Intermittent HFD group (93.83 ± 1.24), Positive control (96.83 ± 1.21) and Negative control (74.83 ± 0.91) Wistar rats. These data suggested that HFD intake induced anxiolytic like condition in male Wistar rats.

Table 1: The data is presented as Mean ±SD for 6 Wistar rats per group. Statistical analysis was performed by using two way ANOVA, ****p value is <0.0001

Experimental Animal	INITIATL WEIGHT (1st day of experiment)	FINAL WEIGHT (8th week of experiment)
Positive Control	156.66±2.01	193.33±1.97
Negative Control	167.5±0.80	208.5± 0.79
Chronic HFD	155.83±2.41	211±1.71
Intermittent HFD	157.5±1.07	237.33±1.05

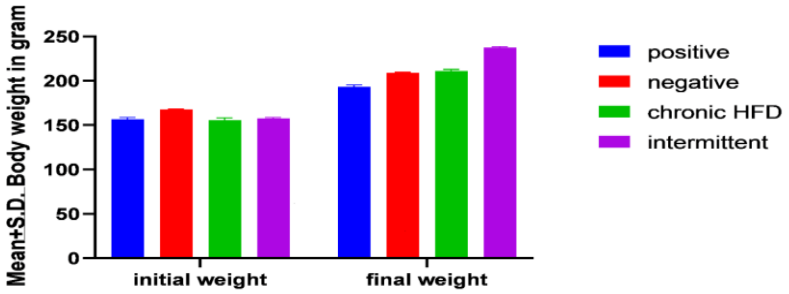


Table 2: The data is presented as Mean±SD 6 Wistar rats per group. Statistical analysis was performed using two way ANOVA *P value is 0.01 and ***P value is 0.001.

GROUP NAME	1ST week of alcohol exposure	2ND week of alcohol exposure
POSITIVE	-	-
NEGATIVE	4.83±1.47	5.33±0.51
CHRONIC	4.16±1.16	3.00±1.09
INTERMITTENT	6.16±0.75	4.16±0.75

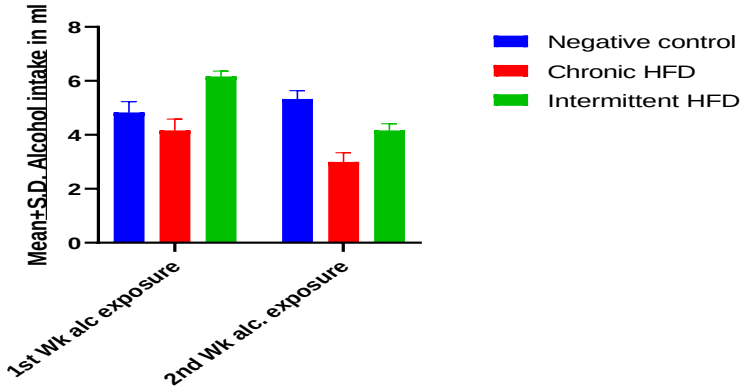


Table 3: The data is presented as mean±SD six rats per group. Statistical analysis was performed by using Two-way ANOVA, P value is ****p<0.0001.

GROUP NAME	1ST week of nicotine exposure	2ND week of nicotine exposure
POSITIVE	-	-
NEGATIVE	2.5±0.12	2.26±0.09
CHRONIC	2.5±0.12	2.36±0.09
INTERMITTENT	2.83±0.14	2.65 ±0.08

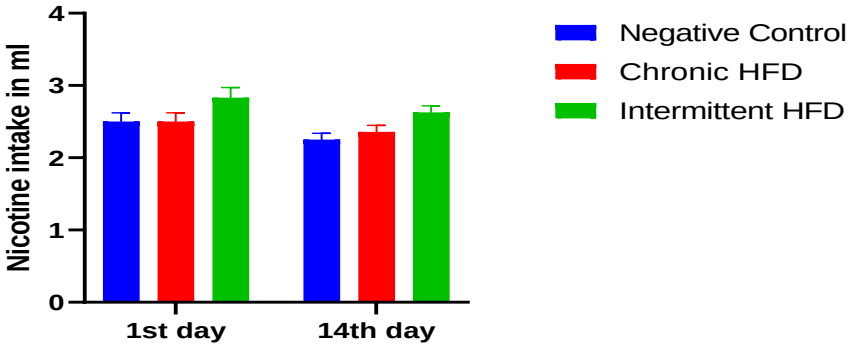


Table 4: The data is presented as Mean±SD for six rats per group. Statistical analysis was done by using two-way ANOVA, p value is ****p<0.0001.

Group Name	Average Water Intake	Average Alcohol Intake	Average Nicotine Intake
POSITIVE	14.00 ±0.65	-	-
NEGATIVE	13.00 ±0.70	5.5±0.5	2.5±0.54
CHRONIC	11.00±1.08	4.2±1.09	2.5±0.54
INTERMIITTENT	12.00±1.21	5.1±0.75	2.8±0.65

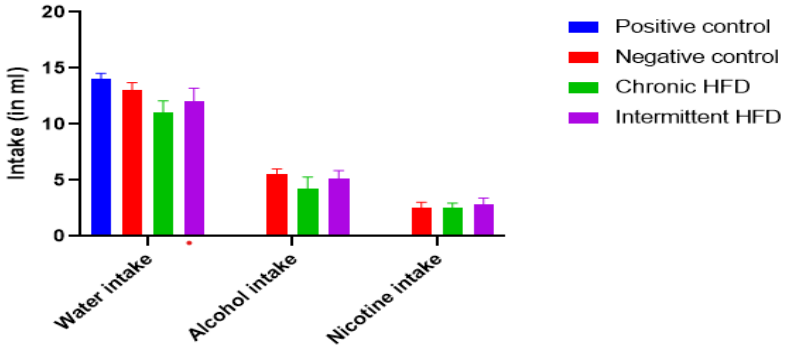


Table 5: The data is presented as Mean±SD for six rats per group. Statistical analysis was done by using two-way ANOVA, p value is ****p<0.0001.

Group Name	Time spent in light phase (Sec.)	Time spent in dark phase (Sec.)
POSITIVE	85.33±1.14	214.66±1.14
NEGATIVE	64.66±1.04	237±1.03
CHRONIC	73.83±0.91	226.16±0.85
INTERMIITTENT	85.33±1.06	214.66±1.04

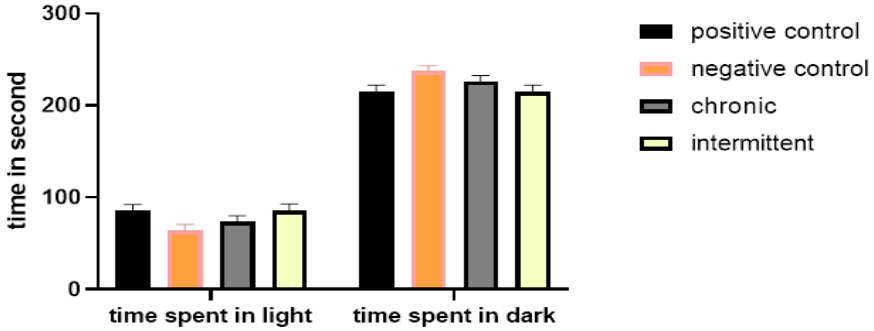
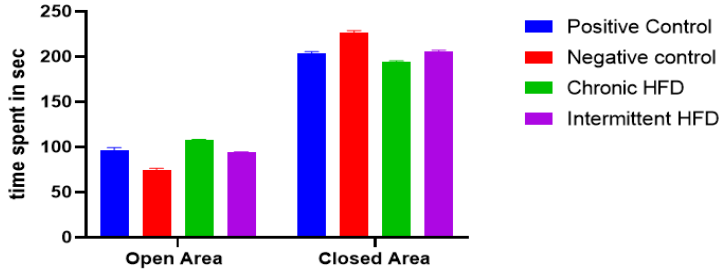


Table 6- The data is presented as Mean±SD for six rats per group. Statistical analysis was done by using two-way ANOVA, p value is ****p<0.0001

Group Name	Time spent in open area (Sec.)	Time spent in open area (Sec.)
POSITIVE	96.83±1.21	203.16±1.09
NEGATIVE	74.83±0.91	226.83±0.95
CHRONIC	107.5±0.53	194.16±1.20
INTERMIITTENT	93.83±1.24	206.16±0.98



4. DISCUSSION

The current data of study help in assessing the consequences of high-fat diet in two different manner that is chronic HFD and intermittent HFD intake on alcohol and nicotine drinking and whether different intake pattern results in reducing the consumption rate of alcohol and nicotine simultaneously following diet exposure.

We found that consumption rate of nicotine in comparison to alcohol was lower and the utilized alcohol was unsweetened in nature. We expose the HFD in Chronic and intermittent group of male rats and found high-fat diet significantly increased the body weight of rat However, in case of control groups not treated with high-fat diet and there was no significant consequences observed in both group of rats. The high-fat diets are fats enriched in nature and rate palatability was higher in comparison to normal chow. In intermittent access rats received more amounts on Tuesday and Thursday while in chronic access rats received slightly less amount and amount maintain consistently during the experimental procedures.(Sirohi et al; 2019)

Various researcher have examined the consequences of high-fat diets on alcohol consumption and they have found both kind of result that increasing and decreasing impact on alcohol drinking in HFD groups of rats in compare to control group. However, when received intermittent HFD pattern rats group was minimized the intake of alcohol in comparison to Chronic and both controls group and in chronic HFD group also had minimum level of alcohol drinking ability. Positive control was non-treated group so there was no impact was found.(Carillo et al;2004)

An important difference between alcohol and nicotine consumption was observed during the session HFD intake in entire group of rats. In intermittent and chronic HFD group of rats showed significance value on intake of nicotine. In alcohol consumption intermittent HFD pattern showed more reduction while in nicotine consumption chronic HFD pattern showed more reduction in comparison to intermittent and controls group. Positive control was non-treated group so there was no impact was found. (Kyu. O, et al; 2016)

Some behavior models also supported the study and showed the significance data of Light and Dark or Elevated plus maze which indicated that rats fed with HFD spent more time in light side as compare to control groups and found more number of entries in open area on EPM during study.

In conclusion, entire data suggest that high-fat diet intake impact on reduction in alcohol consumption in intermittent and chronic group of rats while in case of nicotine consumption, HFD reduced in chronic and intermittent group in comparison to negative control group. Positive control was non-treated group so there was no impact was found. Since, with the help of anxiety behavioral testing model, high-fat diet demonstrated that anxiolytic property in Chronic HFD and intermittent HFD group of rats except in positive and negative group of rat.

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