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# 'Toxicological Studies of Drugs of Abuse: Sub-Chronic and Chronic Toxicity Study of Benzocaine-Caffeine Combination'

[1]Shivaramakrishnan B*, [2]Ramachandra Setty S,
[1]*Department of Pharmacology, JSS College of Pharmacy, Ootacamund-643001, India*
[2]*Department of Pharmacology, Government College of Pharmacy,Bangalore-560 027, India*

***Abstract:*** *Drug abuse and drug dependence causes drastic medical complications in the body that needs to be averted. New psychoactive drug combinations are emerging globally; pose a severe threat to mankind. An abused formulation sold online under tradename 'Magic', was confirmed to contain- Benzocaine & Caffeine by GC-MS study. In purview of the OECD guidelines, the assessment of sub-chronic& chronic toxicities of benzocaine and caffeine in combination was accomplished in three treatment groups at dosage regimens of-therapeutic dose (TD), Enhancing dose (Enh D) and 5-times therapeutic dose (5X TD). The assessment of toxicity of study drug combination in all the dosage regimens was evaluated in four aspects - Gross Behaviour, Haemotology, Clinical Biochemistry & Histopathological studies with respect to Control. After 180 days of treatment, TD and Enh D regimens of benzocaine and caffeine combination enhanced the body weight in male animals. Treatment with TD and Enh D regimens for 90 and 180 days increased the WBC counts, MCV, RDW only. Increase in the levels of SGPT, SGOT, ALP is indicative of hepatic damage and biliary obstruction. Treatment with Enh D and 5X TD arrested the maturation of testis leading to hypo-spermatogenesis is due to increased testosterone level.*

***Keywords:*** *Benzocaine, Caffeine, Drug abuse, Toxicity study, Testosterone*

## I. Introduction

Drug abuse, also called substance abuse, is a disorder that is characterized by a destructive pattern of using a substance that leads to significant problems or distress. Drug addiction, also called substance dependence or chemical dependency, is a disease that is characterized by a destructive pattern of drug abuse that leads to significant problems such as euphoria, paranoia, severe depression or suicidal thoughts.

The 'World Drug Report 2012' states an estimate of 230 million people used illicit substances at least once a year; which represents about 1 in 20 persons in the age group of 15 and 64. Approximately 1 in every 100 deaths among adults is attributed to illicit drug use [1] [2]. It is a tedious task for a drug monitoring and regulatory body of a nation to battle such illegal and devastating activity.

Drug abuse and addiction have a negative impact on an individual in particular and the society in general. Long-term abuse causes changes in anatomical and physiological functions of the body leading to complications; which may or may not be reversible. Addiction is influenced by a combination of factorsincluding individual's biology, his/her social environment, and age/stage of development [3]. Hence, the problem of drug abuse and drug addiction is a global solemn concern with commonly abused substances like alcohol, opiates, cannabinoids, cocaine, and amphetamines etc. [1] [2].

Drug trafficking organizations are constantly evolving and continue to adapt modern manufacturing strategies and innovative marketing to avoid detection. The era of the internet, technological advancements, access to scientific literature and inexpensive organic synthesis techniques; have all accelerated the development of abuse of psychoactive substances from their existing medicinal usage. Many of these new substances were marketed as 'legal highs' and act as substitutes for illicit stimulant drugs such as cocaine or'ecstasy' [4]. Therefore, abusive drug manufacturing and marketing are posing a threat to the society and a new challenge to drug control authorities worldwide.

The European Monitoring Centre on Drugs and Drug Addiction (EMCDDA) and National Advisory Committee on Drugs (NACD), Ireland have recognized the severity of this emerging situation. NACD and Dublin Institute of Technology (DIT) Ireland jointly have identified and published the new psychoactive drugs and their combinations. Few such drugs are: mephedrone, flephedrone, naphyrone, benzocaine, caffeine, lidocaine, procaine etc. [5].

These drugs come from unregulated sources and are often dubbed as research chemicals with label-'Not for human consumption' [5] [6]. These substances are known to be potential candidates for mimicking the effects of the monitored drugs of abuse.

Thus, the need to curb the use of such substances and educate the community regarding the toxic and long term complications of such drugs is eminent. However, toxicity profiles of above mentioned drugs are incomplete or not yet established. In this regard, the survey showed that an abusive combination of benzocaine

and caffeine is available in the online market under the trade name- "*magic*" [5]. But the toxicity profile of this combination is not available and yet to be documented. Further, literature study indicated that caffeine is combined with local anesthetics by drug abusers. The exact mode of action of such combinations is not clear. Therefore, in the present it was planned to assess the toxicity of this combination in '*Rats'* [7] [8]. With the toxicological study of such drugs of abuse either in mono or poly drug usage helps in understanding the physiological and psychological complications that arises in and after exposure. The present study was intended with the objective of establish a toxicological profile of benzocaine-caffeine combination on a sub-chronic duration and chronic duration in varying doses in rodent model-albino rats.

## II. Methodology

Wistar albino rats of 6 weeks of age of both the sexes males (M) and females (F) were used so as to meet the essential criteria of toxicity study on rodents as per the OECD guidelines (OECD TG 452) [9]. The animals were allowed for ten days to get acclimatized to light-dark cycle, to diet and water ad libitum. The animals possessing normal behavioural patterns, exhibiting normal food & water consumption and excretory activities, normal body weight were chosen for the study. Benzocaine and Caffeine being slightly soluble and sparingly soluble, in water respectively; Acacia 2% w/v suspension was used as a vehicle in the study.

The adult oral dose of benzocaine and caffeine are 60mg and 100mg respectively [10] [11]. These doses were converted to rat dose by Surface Area Ratio method [12]. Further the drug abusers are reported to increase the dose sequentially from these therapeutic doses to a higher range. With this hypothesis in mind, the dosing schedule for the animals of the abusive group was designed so as to raise the dose sequentially from the therapeutic dose up to 5X therapeutic dose. The dosing regimen of the abusive group was increased to 2X, 3X, 4X till 5X sequentially along with the necessary evaluations namely animal observations -body weight & food consumption, Haemotology, Clinical serum biochemistry & Histopathological study [13].

All the data and values collected was expressed as mean ± SEM. Statistical difference in mean will be analyzed using one way ANOVA tests. $P< 0.05$ was considered statistically significant.

## III. Results

### 3.1 Effect Of Treatment on Gross Physical And Behaviour:

In the present study, the effect of benzocaine and caffeine on the animal observations of body weight and food consumption was assessed for three treatment groups by comparing with the control. After 180 days of treatment, the male rats of therapeutic dose (TD-M) and enhancing dose (Enh D-M) groups showed a significant increase in the body weight when compared to control males (Table 1). Even the third treatment regimen (5X TD) did not alter the body weight of both sexes. After 180 days of treatment, the rats of all the treatment groups showed substantial increase in food consumption.

### 3.2 Effect of Treatment on Haemotological Parameters:

Effect of benzocaine and caffeine on haemotological parameters were assessed in three groups. After 180 days of treatment, an assessment in haemotology revealed WBC count of males of therapeutic dose and enhancing dose showed significant increase in the count when compared to the control. The platelet count of therapeutic dose males exhibited a noticeable increase in the count when compared to control. The rats (both sexes) of all three treatment groups showed a unanimous significant increase in mean corpuscular volume as in comparison with control group. The rats (both sexes) of all three treatment groups showed a marked significant increase in the Red cell volume Distribution Width (RDW) when compared to the control group. The results of the haemotology assessed 180 days of treatment are depicted (Table 2).

### 3.3 Effect of Treatment on Clinical Biochemistry:

The effect of study drug combination on clinical biochemical parameters was assessed to ascertain the possibility of specific organ toxicity. After 90 days of treatment, there was a significant increase in the levels of SGOT in all the treatment groups compared with the control. The levels of ALP and SGPT are elevated significantly in the male rats of 5X-TD groups as shown in table 3. After 180 days of treatment; several alterations in the levels of serum biomarkers were observed. An increase in level of SGPT was significant in male rats of all the treatment groups. The same treatment group showed significant increase in levels of SGOT.

It is also noted that the increase in levels of SGOT was more marked. A significant unanimous increase in levels of ALP was evident when compared to the control groups. The Cholesterol levels were found to be significantly increased in the rats of 5X TD treatment group when compared to control. Treatment with benzocaine and caffeine did not alter the levels of creatinine, glucose, total proteins and urea assessed parameters – creatinine (CR), glucose, total proteins (TP) and urea in all the treatment groups after 0 day, 30 days, 90 days and 180 days (Table 3 & 4).

**3.4 Effect of Treatment on Histopathological parameters on organs:**

After the treatment of 180days with the test drug combination, organs such as brain, heart, liver, kidney and gonads were examined by histopathological study for any sign of damage. Of which, brain heart remained unscathed, however liver kidney and male reproductive organs showed varying levels of cellular debris as well as inflammatory cells (Fig.1, 2 & 3).

**Table 1. Effect of Benzocaine and Caffeine on Body Weight (in grams) at designated check points**

| After treatment | Cont | | TD | | Enh-D | | 5X-TD | |
|---|---|---|---|---|---|---|---|---|
| | Males (M) | Females (F) | Males | Females | Males | Females | Males | Females |
| 0 day | 103.0 ± 2.13 | 84.50 ±1.57 | 105.0 ± 2.23 | 84.00 ±1.24 | 107.0 ± 2.13 | 84.50±1.38 | 107.0 ± 2.13 | 84.00 ±1.63 |
| 7 days | 106.0 ± 2.66 | 89.00 ± 1.24 | 109.0 ± 2.70 | 93.50 ± 1.83 | 108.0 ± 2.49 | 90.50 ± 1.57 | 108.0 ± 2.49 | 90.00 ± 1.29 |
| 14 days | 109.0 ± 2.33 | 90.00 ± 1.29 | **117.0 ± 2.13*** | 97.00 ± 1.10 | 112.0 ± 2.00 | 93.00 ± 1.52 | 110.0 ± 2.10 | 92.00 ± 1.52 |
| 21 days | 119.0 ± 3.05 | 95.00 ± 1.29 | **131.5 ± 4.08*** | 107.0 ± 2.13 | 123.5 ± 3.16 | 99.00 ± 2.21 | 115.0 ± 2.23 | 98.00 ± 2.00 |
| 30 days | 128.5 ± 3.16 | 104.0 ± 1.63 | **139.0 ± 4.58*** | 112.0 ± 2.00 | 130.0 ± 3.57 | 104.0 ± 1.63 | 121.0 ± 2.33 | 105.0 ± 1.66 |
| 60 days | 138.0 ± 3.88 | 112.0 ± 2.49 | **153.0 ± 4.23*** | 121.0 ± 2.76 | 140.0 ± 4.71 | 113.0 ± 2.60 | 127.0 ± 3.00 | 112.0 ± 2.00 |
| 90 days | 160.0 ± 3.65 | 127.0 ± 2.13 | **173.0 ± 3.66*** | 130.0 ± 2.98 | 161.0 ± 5.04 | 126.0 ± 2.66 | 146.5 ± 3.07 | 124.0 ± 1.63 |
| 180 days | 212.0 ± 6.79 | 201.5 ± 6.32 | **281.0 ± 10.30 ***** | 214.0 ± 6.70 | **284.0 ± 10.35 ***** | 230.0 ± 7.45 | 223.0 ± 5.97 | 224.0 ± 4.98 |

**Table 2. Effect of Benzocaine and Caffeine on Haemotology after completion of treatment**

| Groups | Sex ↓ | RBC ($10^6$/µl) | WBC ($10^3$/µl) | PLT ($10^3$/µl) | LYM (%) | Hb (g/dL) | MCV (fL) | RDW (%) |
|---|---|---|---|---|---|---|---|---|
| Cont | M | 7.75 ± 0.15 | 5.08 ± 0.52 | 1154 ± 136.5 | 73.25 ± 1.94 | 14.67 ± 0.53 | 48.85 ± 0.80 | 13.85 0.59 |
| | F | 6.92 ± 0.36 | 4.51 ± 0.52 | 1058 ± 124.9 | 75.45 ± 1.94 | 15.07 ± 0.21 | 50.08 ± 0.60 | 13.18 ± 0.84 |
| TD | M | 7.21 ± 0.28 | **9.65 ± 0.39 ***** | **2024 ± 201.7 **** | 67.55 ± 1.94 | 14.80 ± 0.20 | **57.63 ± 0.34***** | **17.52 ± 0.29***** |
| | F | 6.01 ± 0.42 | 5.88 ± 0.61 | 1374 ± 182.1 | 72.50 ± 0.83 | 15.28 ± 0.20 | **61.75 ± 1.31***** | **16.90 ± 0.17***** |
| Enh-D | M | 7.74 ± 0.10 | **9.41 ± 0.62 ***** | 1861 ± 120.0 | 70.40 ± 2.82 | 15.43 ± 0.25 | **56.70 ± 0.72**** | **17.82 ± 0.27***** |
| | F | 6.24 ± 0.70 | 4.80 ± 0.57 | 1076 ± 178.1 | 70.78 ± 3.75 | 14.25 ± 1.74 | **62.32 ± 1.40***** | **18.22 ± 0.30***** |
| 5X-T D | M | 6.41 ± 0.58 | 6.88 ± 0.87 | 1449 ± 169.5 | 69.00 ± 2.56 | 13.83 ± 0.87 | **60.45 ± 0.83***** | **17.52 ± 0.33***** |
| | F | 6.33 ± 0.21 | 5.71 ± 0.66 | 1267 ± 140.8 | 70.83 ± 1.45 | 14.28 ± 0.13 | **61.52 ± 2.61***** | **17.45 ± 0.31***** |

**Table 3. Effect of Benzocaine and Caffeine on Clinical Biochemistry after 90 days of treatment**

| Groups | Sex | SGPT (U/L) | SGOT (U/L) | ALP (U/L) | CHOL (mg/dL) | CR (mg/dL) | GLUCOSE (mg/dL) | TP (g/dL) | UREA (mg/dL) |
|---|---|---|---|---|---|---|---|---|---|
| Cont | M | 33.42 ± 1.845 | 62.12 ± 3.671 | 101.7 ± 3.839 | 50.40 ± 1.36 | 0.474 ± 0.014 | 93.5 ± 1.69 | 6.22 ± 0.12 | 40.40 ± 1.72 |
| | F | 30.05 ± 1.54 | 76.97 ± 3.587 | 98.40 ± 3.276 | 55.50 ± 2.02 | 0.457 ± 0.014 | 93.8 ± 2.11 | 6.68 ± 0.13 | 41.02 ± 1.82 |
| TD | M | 41.19 ± 1.72 | **95.87 ± 1.528 ***** | 121.3 ± 8.096 | 48.48 ± 1.91 | 0.492 ± 0.012 | 92.2 ± 3.39 | 6.25 ± 0.12 | 42.31 ± 4.69 |
| | F | 34.77 ± 1.88 | **89.81 ± 2.084 *** | 100.6 ± 3.089 | 64.58 ± 3.69 | 0.482 ± 0.018 | 87.0 ± 2.31 | 6.66 ± 0.10 | 39.10 ± 4.58 |
| Enh-D | M | 42.87 ± 2.19 | **89.46 ± 2.426 ***** | 120.5 ± 7.462 | 50.14 ± 1.61 | 0.477 ± 0.023 | 91.1 ± 2.97 | 6.47 ± 0.16 | 39.40 ± 1.96 |
| | F | 39.16 ± 2.37 | **92.50 ± 2.371 **** | 102.3 ± 4.211 | 64.21 ± 2.82 | 0.481 ± 0.012 | 86.4 ± 2.24 | 6.36 ± 0.15 | 37.60 ± 0.92 |
| 5X-T D | M | 44.23 ± 3.39 * | **93.17 ± 2.026 ***** | **143.7 ± 7.172 ***** | 50.08 ± 1.85 | 0.489 ± 0.019 | 93.3 ± 2.63 | 6.33 ± 0.13 | 44.44 ± 1.52 |
| | F | 38.48 ± 1.68 | **95.88 ± 2.147 ***** | 113.9 ± 5.493 | 54.10 ± 1.83 | 0.473 ± 0.020 | 84.6 ± 2.29 | 6.24 ± 0.14 | 42.51 ± 1.82 |

**Table 4. Effect of Benzocaine and Caffeine on Clinical Biochemistry after 180 days of treatment**

| Groups | Sex | SGPT (U/L) | SGOT (U/L) | ALP (U/L) | CHOL (mg/dL) | CR (mg/dL) | GLUCOSE (mg/dL) | TP (g/dL) | UREA (mg/dL) |
|---|---|---|---|---|---|---|---|---|---|
| Cont | M | 34.50 ± 3.50 | 106.7 ± 5.92 | 150.5 ± 10.48 | 50.40 ± 1.93 | 0.4770 ± 0.01 | 98.4 ± 2.63 | 5.81 ± 0.15 | 40.53 ± 1.67 |
| | F | 32.69 ± 4.14 | 107.9 ± 4.53 | 126.8 ± 5.40 | 56.20 ± 2.47 | 0.4880 ± 0.01 | 97.9 ± 2.25 | 6.22 ± 0.18 | 38.28 ± 1.01 |
| TD | M | **69.94 ± 5.84*** ** | **167.6 ± 11.71** ** | **637.8 ± 58.78*** ** | 61.20 ± 3.50 | 0.5490 ± 0.02 | 130.3 ± 8.62*** | 6.65 ± 0.20 | 43.32 ± 1.97 |
| | F | 39.12 ± 3.77 | 161.1 ± 7.14* | **469.6 ± 67.74*** ** | 64.30 ± 3.09 | 0.5530 ± 0.01 | 91.1 ± 3.59 | 7.03 ± 0.14 | 46.37 ± 4.30 |
| Enh-D | M | **67.79 ± 7.24*** ** | **174.4 ± 12.81** ** | **715.4 ± 51.21*** ** | 62.60 ± 3.37 | 0.5180 ± 0.01 | 101.8 ± 5.68 | 6.38 ± 0.19 | 38.50 ± 1.66 |
| | F | 37.87 ± 3.01 | 151.6 ± 16.00 | **556.4 ± 48.04*** ** | 65.90 ± 2.51 | 0.5610 ± 0.01 | 85.3 ± 2.82 | 6.77 ± 0.26 | 37.60 ± 0.92 |
| 5X-T D | M | **67.56 ± 6.18*** ** | **178.9 ± 14.99*** ** | **674.1 ± 58.76*** ** | **67.70 ± 3.01*** ** | 0.5000 ± 0.01 | 107.6 ± 3.86 | 5.97 ± 0.18 | 50.29 ± 2.04 |
| | F | 52.96 ± 5.27 | 157.0 ± 13.53 | **561.5 ± 51.84*** ** | **71.10 ± 1.69** ** | 0.5380 ± 0.01 | 96.4 ± 3.17 | 6.72 ± 0.15 | 38.77 ± 3.09 |

**Figure 1. Effect of treatment on histopathology of Liver**

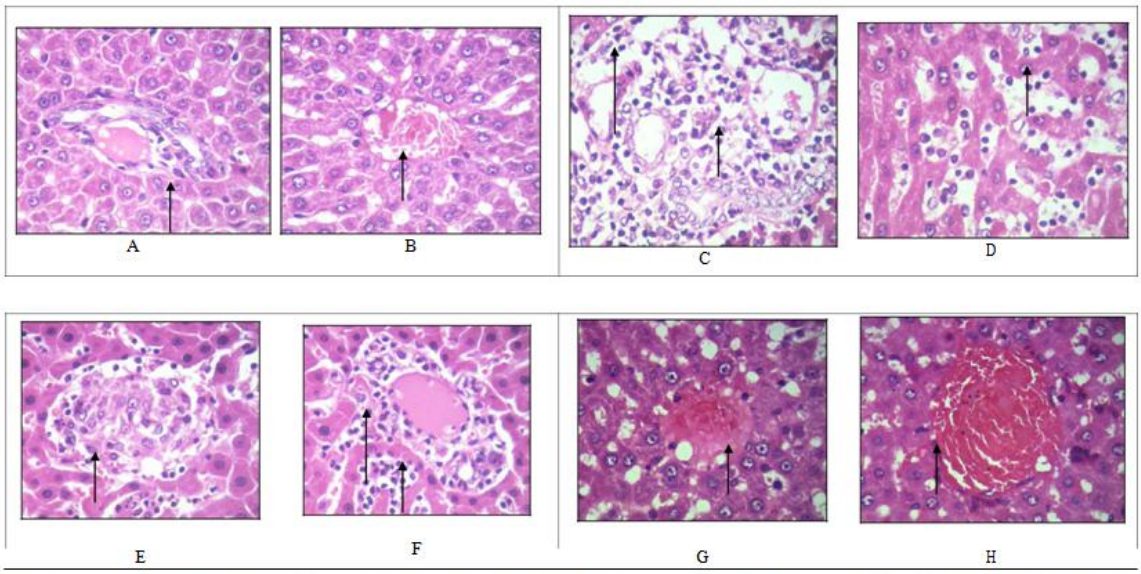


Fig 1. On comparison with control (A & B), Treatment groups TD (C&D), Enh-D (E&F) and 5X-TD (G&H) shows marked dense inflammatory infiltration [Short-Arrow] along with damaged hepatocytes [Long arrow]

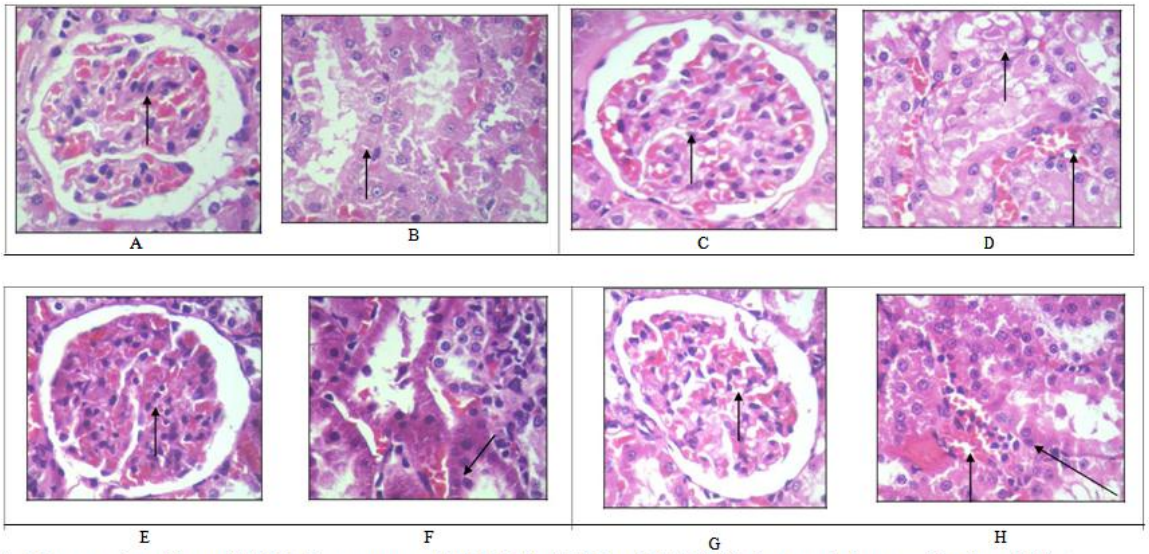


Fig 2. On comparison with control (A & B), Treatment groups TD (C&D), Enh-D (E&F) and 5X-TD (G&H) shows marked congested blood vessels [Short-Arrow] along with aggregates of dense inflammatory cells [Long arrow]

**Figure 3. Effect of treatment on histopathology on Testis**

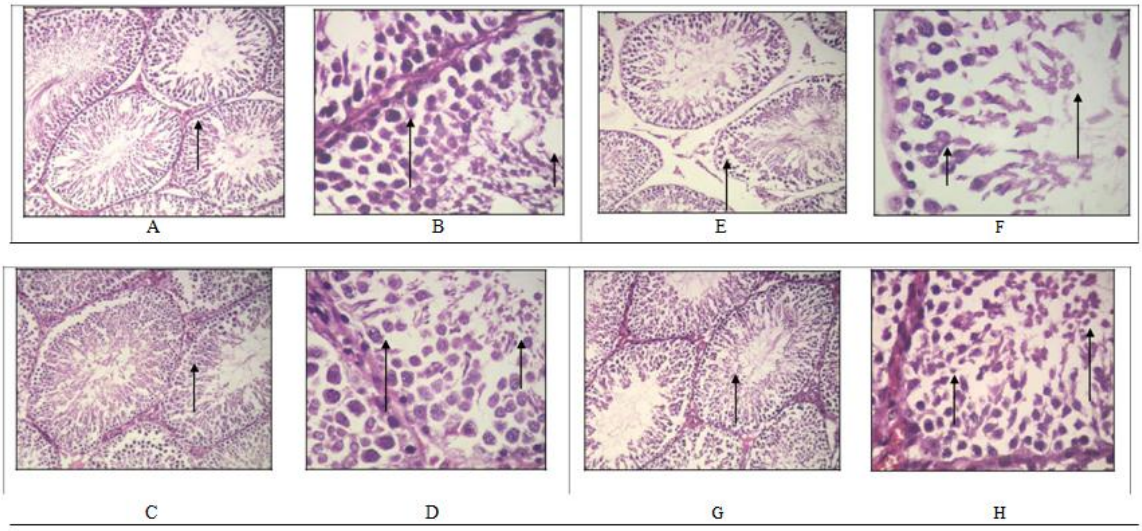


Fig 3. On comparison with control (A & B), Treatment groups TD (C&D) showing spermatogenesis, Enh-D (E&F) showing maturation arrest and 5X-TD (G&H) showing hypospermatogenesis.

## IV. Discussion

### 4.1 Influence of abusive drug combination on Gross Behaviour:

The influence of study drug combination on gross behaviour was made at 0day, 7$^{th}$ day, 14$^{th}$ day, 21$^{st}$ day, 30$^{th}$ day, 60$^{th}$ day, 90$^{th}$ day and 180$^{th}$ day of treatment. It is observed that after the administration of study drug combination, all the animals (both males and females) were highly active. However, increase in body weight of male animals of TD and Enh D group was more pronounced than females. It was surprising to note that the animals treated with 5X TD did not alter the body weight till 180 days of treatment. After 180 days of treatment, there was slight increase in food consumption in this group. Various factors that predominantly influence the progression of body weight are: thyroid hormones, growth hormones, anabolic steroids like testosterone [14] [15] [16]. In the present study it is noted that the rate of progression of body weight upon treatment with TD and Enh D affected only in male animals. If treatment increased due to the thyroid hormones, it would have influenced the progression of body weight in both males and females. Similarly effects (if increase in body weight) in both males and females would have been seen if growth hormone is the cause of increased body weight. But the results are indicating that the body weight in enhanced in only male animals. Hence, the above mentioned factors can be ruled. Since testosterone like anabolic steroids and are male specific hormones which are reported to increase body mass by the increase in protein synthesis [17]. It is hypothesized that treatment with study drug combination might have enhanced the testosterone levels in males. But the treatment with 5 X TD regimens did not have any significant influence on body weight of males and females. This may be due to toxic influence of study drug combinations at high doses [18] [19].

### 4.2 Influence of abusive drug combination on Haemotological parameters:

The treatment of abusive drug combination at 3 different dose regimens after 90 days of treatment with TD and Enh D significantly increased the WBC levels in male animals; whereas RBC count and Hb levels are were not altered. In contrast treatment with 5X TD did not alter any of the haemotological parameters. Since there is some change observed in male rats, it was decided to undertake the assessment on complete band of haemotological parameters- WBC, RBC, PLT, % LYM, Hb, MCV, MCH, MCHC, RDW etc. after 180 days of treatment.

After 180 days of treatment, it was observed that animals treated with TD and Enh D increased the levels of WBC in male animals only; whereas RBC, PLT, % LYM, Hb, MCH, MCHC was not altered significantly. In contrast, 5 X TD treatments did not alter the any of these parameters in either males or females. It is further observed that 180 days of treatment with TD, Enh D and 5X TD significantly increased levels of MCV and RDW % in both males and female animals; whereas MCH and MCHC did not alter in any of treatment regimen.

It is reported that treatment with caffeine increased the levels of WBC in male rats [20]. In the present study, WBC levels are enhanced due to treatment with benzocaine and caffeine. Since the results are in concurrent with above mentioned report it may be inferred that enhanced levels of WBC in male rats due to the treatment regimen; may be attributed to caffeine.

Similarly the levels of MCV are increased with the treatment with all the dose regimens of study drug combination. There is a report that MCV levels are enhanced during severe liver damages [18] [19]. Therefore, it is hypothesized that given abusive drug may cause hepatotoxicity. At this juncture, it is difficult to correlate and explain clearly the causes for enhanced RDW due to the treatment.

**4.3 Influence of abusive drug combination on Clinical Biochemistry:**

The level of SGPT and SGOT were determined at 0 day and after 30 days of treatment and it was found that levels of these two biomarkers were raised in both male and females to extent of about 20%. However, the levels in all the treatment groups were well within the clinical safety levels.

After 90 days of treatment, SGPT and SGOT altered significantly but well with the tolerable limits. Similarly ALP levels were increased. The other parameters –CHOL, CR, Glucose, Total proteins, Urea did not alter after 90 days of treatment.

After 180 days of treatment it is observed that SGPT levels in males almost doubled whereas with the females this alteration is not significant. Similarly SGOT levels are significantly enhanced both in males and females in the entire treatment group. It is notably mention here the ALP levels are exorbitant. That is about 5-times the control levels after treatment with all the three dose regimens of study drug combination. The other parameters did not significantly alter. It is observed blood glucose levels in males were found tobe little higher than females; but it is not clinically significant as the elevated levels of glucose were within the normal range. There is a report that if the elevation of SGOT and SGPT ratio is 2:1 and this is persistent for more than 6 months. It is indicative of chronic liver disease [21] [22] [23] [24] [25]. This may lead to liver cirrhosis if untreated. In the present study also the SGOT and SGPT are elevated more than 2:1 ratio and persistent for more than 180 days. This is suggestive of chronic liver disease due to treatment with all three dose regimens of study drug combination.

It is observed that exorbitant ALP levels are seen in all the three treatment groups. There is a report stating that if ALP levels are enhanced 5 times than that of normal values, it indicates intrahepatic or extra hepatic obstructive biliary obstruction. Even in the present study, ALP is enhanced about 5 times indicating there is hepatic damage and biliary obstruction. In addition, there is a report that caffeine enhances the levels of testosterone, progesterone, corticosterone [26]. And benzocaine enhances the caffeine pharmacokinetics by inhibiting liver mixed function oxidases (MFO) [27]. Therefore, chronic administration of benzocaine and caffeine combination enhances the levels of testosterone significantly. There is a report that testosterone inhibits gall bladder motility [28] and thereby enhances the biliary obstruction. In the present study, ALP levels increased & suggestive of extra hepatic and intra hepatic obstructive cholestasis this may be attributed to elevated levels of testosterone due to the treatment with all dosage regimen of study drug combination for 180 days.

**4.4 Influence of abusive drug combination on Histopathological study:**

The histopathological observations of liver tissues of animals treated with TD dose regimen of study drug combination for 180 days revealed that periportal hepatic region showed a dense inflammatory infiltration and damaged hepatocytes in both males and females. Dilation of sinusoids and appearances of macrophages were observed. It is very strange to note that there was no change in central veins in males but slight congestion of central veins was seen in the females. Similar observations were made with liver tissue of Enh D treatment regimen.

The histopathological observations of sections of liver tissues obtained from animals treated with 5X TD revealed that hepatic architecture was not altered. This observation could not be explained clearly. Histopathological observations and biochemical observations in case of hepatic damage are in concomitantand suggestive of chronic liver damage due to treatment with study drug combination. As explained, hepatotoxic effect of study drug combination may be attributed to caffeine; which enhances the levels of steroid hormones and inhibitory effect of benzocaine on MFO which metabolises these hormones. This results in elevated levels of steroid hormones. The steroidal hormones are causing intra hepatic and extra hepatic biliary obstruction.

Similarly sections of renal tissues were taken from animals treated with all three dose regimens of study drug combination. Section of renal tissues obtained from TD treatment group revealed that blood vessels are congested in both males and females; where as in males tubules showed eosinophilic material in the lumen.

The sections of renal tissue from animals treated with Enh D revealed glomerular capillary and blood vessels congestion is seen in both males and females. Similar observations were made in section of renal tissues obtained from animal treated with 5X TD. In all these sections cellular architecture was almost intact. Therefore it may be inferred that renal damage is not caused to a significant extent however there is slight influence on the blood vessels innervating the renal tissues. Serum CR levels and Urea levels are not altered. Body weight is not decreased (instead body weight is elevated in males of TD and Enh D). Therefore, it is inferred that treatment with all three dose regimens did not cause any renal toxicity except slight alterations in renal blood supply.

Sections of testes from the males treated with TD of study drug combination revealed that architecture is intact; spermatogenesis is normal or slightly enhanced. These are suggestive that testicular anatomy and physiology is not much influenced by treatment. Whereas the section of testis obtained from males of Enh D of study drug combination showed that distorted testis architecture and loosely packed seminiferous tubules with alteration in germinal epithelium. It is also observed that reduction in spermatocytes and disintegration of spermatids &spermatozoas. It is noteworthy to mention that sperm maturation is arrested in tissues. Similarly section of males of 5X TD regimens for 180 days treatment showed reduces in spermatocytes and hypo spermatogenesis is seen. All these finding indicate that treatment with Enh D regimen and 5 X TD regimens causes testicular toxicity. There is a report that elevated levels of testosterone for chronic period (due to endogenous increase in testosterone or exogenous administration) may reduce testicular function that is hypo spermatogenesis. Reports are indicating that caffeine andbenzocaine enhances the testosterone levels by stimulation of release and inhibition of metabolism. This may cause malfunction of testis observed histopathologically.

## V. Conclusion

Treatment with therapeutic dose and enhancing dose regimens of benzocaine and caffeine combination enhanced the body weight in male animals but not in female animals. In contrast, treatment with 5-times the therapeutic dose regimen did not alter the body weight significantly. This may be attributed to the ability of caffeine to release the testosterone and ability of benzocaine in inhibiting the metabolism of it.

Treatment with therapeutic dose and enhancing dose regimens for 90 days and 180 days increased the WBC counts, mean corpuscular volume(MCV), red cell volume distribution width (RDW) without affecting the other haemotological parameters. This effect is attributed to chronic administration of caffeine.

Treatment with therapeutic dose, enhancing dose and 5-times therapeutic dose regimens for 180 days significantly enhanced the levels of biomarkers- SGPT, SGOT, ALP indicating of hepatic damage and biliary obstruction. This may be attributed to the ability of benzocaine and caffeine in increasing the levels of testosterone.

In addition, treatment with enhancing dose and 5-times therapeutic dose arrested the maturation of testis leading to hypo spermatogenesis. This may be attributed to elevated levels of testosterone. Treatment with all dosage regimens congested the blood vessels in heart which may lead To Ischemic heart disease.

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