An Acute toxicity profile of Tramadol on Rattus norvegicus – A case study of up and down regulations and Psychobiotic clinical approach.

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ABSTRACT

In recent years, many opioids are enter into the human life, among them one of the widely used popular opioid, that is extremely recommended for effective analgesic agent to treat more severe acute and chronic pains are named as Tramadol hydrochloride. On using the long-term and higher doses of Tramadol induce seizures might be Serotonin syndrome and acute renal failure etc. The aim of the present study is to investigate the acute lethal dose (LD_{50}) of the Tramadol through oral administration in Rattus norvegicus by Dixon's Up and Down LD_{50} method. This method improves the performances and accurate results for estimating the toxicity and also the notable on reduction of test animals for using the LD_{50} determination. The current study shows the significance of Psychobiotic that could reduce the toxicity of opioid effects. Lactobacillus helveticus - MCC 2036 was shown astonishing mark on reduces lethal toxic effects of tramadol while comparing lethal and control groups. The present investigation, Oral administration of Tramadol LD_{50} dose along with Psychobiotic therapy were compared with control group at weekly intervals of short and long period effects, assess by the changes on behavioral characters with their mortality signs of each animal, Biochemical profiles and Histological modifications on brain, liver and kidney tissues.

Key words: Tramadol, Rattus norvegicus , Psychobiotic therapy, Up and Down LD_{50} estimation, Biochemical analysis and Histological studies.

I .INTRODUCTION

Opioids are referred to as the most potent effective analgesics and have become accepted as an appropriate treatment for acute, cancer and chronic pain [1]. Among the several of Opioids one of a typical analgesic that is regularly preferred by physician named as Tramadol hydrochloride [2], because it provides multimodal analgesia through a dual mechanism of antino- ciceptive action in central nervous system and spinal cord , that is primarily bound to the activation of μ-opioid receptor and then it inhibits the reuptake of neurotransmitter namely Serotonin and Nor-epinephrine [3]. Tramadol is a synthetic opioid, chemically named as ((1RS, 2RS)-2-[(dimethylamino) methyl]-1-(3-methoxyphenyl)-cyclo-hexanol), and commonly known as amino cyclohexanol group [4]. For the management of moderate to moderately severe acute and chronic pains, Tramadol can be

administered through the body by intramuscularly, intravenously, orally, and also intrathecal. Among 195 countries in the world more than 100 countries have authorized Tramadol for moderate to severe pains. It is popularly used for prolonged chronic cancer, noncancer pains mostly rheumatoid arthritis, osteoarthritis neuropathic, fibromyalgia, migraine pain due to trauma, dental, abdominal, labor chronic, post-operative pain and also used in surgeries with combinations of anesthetic drugs [5-7]. Tramadol is recommended for sickle cell diseases, neuropathic pains also [8]. Most of the tramadol metabolism is carried out by the P450 enzyme. It is produced in the liver and the kidneys and it plays a major role in the excretion of by-products of tramadol. It's toxic to some level of more than recommended doses or high doses and prolonged using of animals and humans [9].

Regular usage of Tramadol may also indicate the adverse reactions include hypersensitivity, hallucinations, and convulsion reactions [10]. Tramadol inhibited human Serotonin Transporter at concentrations that are close to clinical drug plasma and brain concentrations [11]. Overdose may induce the seizure, increased Creatinine Phosphokinase (CPK), and acute renal failure. Additionally, most important serotonin syndrome (SS) has been reported due to its overdose [12]. Tramadol acts as a central opioid analgesic to control severe pains, but now-a-days analgesics are among the most popular drugs for being abused [13]. Tramadol was used as an analgesic among youth particularly college students for increasing their study period [14]. There is growing evidence of the non-medical use of tramadol in addition to the high levels of misuse of prescription in some countries namely North Africa and the Near Middle East [15] and also in India has also traced a case on tramadol dependence[16]. Widespread misconceptions regarding tramadol such as energy booster during work, mood enhancer, and has been used in the treatment of sexual dysfunction for longer-lasting erection, premature and delayed ejaculation, etc [17]. Individuals who abuse tramadol, they were taken far above prescribed doses increases the overdose risks. Symptoms like coma, cardiac arrest, collapse, and respiratory depression were observed. Fatal poisoning can occur when tramadol is consumed with other drugs or alcohol [18]. The abuse of tramadol leads to Psychosis and Brain disorders lie inability due to decision making, losing coordination in walking and also most properly double personality disorders [19].Tramadol addiction is recognized to cause adverse and in some cases lethal health effects [20].Tramadol abuse, dependence, as well as related deaths, are due to ingested alone in overdose have been increasingly reported especially in young male adults [21].

Hence we introduced therapy of Probiotic Psychobiotic's are live microorganisms that promotes health benefits to the host at suitable right dosages and yield positive effect on mental health [22]. Serotonin synthesis in the gut could be regulated by the gut microbiota [23]. Among them psychobiotics it's an novel class of Psychobiotic s that have potential applications in treat psychiatric diseases like serotonin syndrome , Alzheimer's etc., [24]. These microbes play major roles in controlling the neural excitatory-inhibitory balance namely mood, memory, and cognitive functions processes by regulate the neurotransmitters and proteins like serotonin, glutamate and gamma-amino butyric acid etc, [25]. Psychobiotics, the most commonly preferred are gram-positive bacteria, namely Lactobacillus and Bifidobacterium families, because the absences of lipopolysaccharide

chains, decreases the probability of an immunological response into the host [26]. Psychobiotics were recommended as best alternative method for treat psychiatric disorders Patients, who rejects to take chemical therapy [27]. Lactobacillus helveticus have ability to decreases cortisol hormone and psychological distress in humans [28]. Lactobacillus helveticus is specified for shown thermophilic tendencies with an optimum growth temperature up to 42 to 45°C and also optimum at pH 5.5 to 5.8 on intestinal secretions and the species have ability to grow as fastidious [29]. The single strain Lactobacillus helveticus can reduce depression, anxiety, and cognitive dysfunction through increased the serotonin, nor epinephrine levels in the hippocampus region of brain [30]. In the previous study, tramadol hydrochloride LD_{50} was given by the 300mg -350/kg body weight for rats but they could not accord an exact concentration [31].

The aim of this work was to investigate, the accurate acute toxicity of Tramadol, LD_{50} based on the Up and Down method, and also prove the impact of acute and chronic administration effects of Tramadol and Psychobiotic significance to reduce toxicity on the Biochemical profiles and Histological studies in the Brain, Liver and Kidney functions in adult male albino rats.

II.MATERIALS AND METHODS

2.1 Animals Treatment

Healthy young male Wister strain albino rat (Rattus norvegicus) (250 ± 20 gm) obtained from Sri Venkateswara traders, Bangalore. These were maintained in polypropylene cage under good laboratory conditions (temperature - 25 ± 28^0C, light and dark - 12:12 hrs, humidity -75%) and feed with standard laboratory chow (Hindustan lever limited, Bombay). The rats chow 45–50gm kcal/day during the experimental period and also normal tap water was provided adlibitum. All rats were maintained in a quite non-stressful laboratory environment for ten days before the beginning of the experimental protocol to overcome the stress possibly incurred during transit. Toxicity of Tramadol was evaluated according to the Dixon's up and down method through this basic period; perceive the body temperature, and also behavioral characteristics of test animals.

2.2 Culture collection and Determination of Colony-Forming Units (CFU/ml)

The Psychobiotic bacterium of Lactobacillus helveticus - MCC 2036 strains were obtained from the Microbial culture collection center from Pune- INDIA. The bacterial strains were grown in MRS Broth M641(Himedia-Mumbai), for 100ml broth with 200mg/l Ampicillin is employed and kept for 37^0C for overnight at 100 rpm shaking incubator. The resistant colonies were picked up and transferred into the sterile fresh MRS broth and finally make the concentration by using the formula: Cfu /ml = (no. of colonies x dilution factor) / volume of culture plate. Therefore Cfu /ml = no. of colonies counted was 30, dilution factor as 10^7(for better colonies grown) and the volume of culture plate is 0.1. Finally, Cfu /ml = 30 x 10^7/0.1 =3 x10^9. Then, the number of bacteria present at 1 ml of the given sample at 10^7 contained 3 x10^9 colonies of Psychobiotic L. helveticus.

2.3 Collection of Drug and Dosage estimation

The pure form of Tramadol hydrochloride was obtained from Sigma-Aldrich-42965 and it's Cass Number - 36282-47-0 (spruce street ST. Louis, MO6103 USA 314-7715765). According to the OECD 425 guidelines administered tramadol in a single dose per day as given orally through gavages based on their body weights as follows that is required dose equal to the weight of the animal (g)/1000g into stranded dose (mg), [32]. The animals were divided and calculated the required dose based on the above guidelines are 250g rat = weight of the animal (g)/1000 g x stranded dose (mg). Therefore 250/ 1000 x 300 =75mg/kg bw, similarly the stranded does 250mg = 62.5/kg bw, 200 mg = 50 mg/kg bw and 150 mg = 37.5 mg/kg bw. Before administration of Tramadol Albino rats weighing 250± 20 gm/kg bw have fasted overnight with ad libitum food but not water should be withheld. Carefully delivered into the oral route by inserting a gavages tube between the tongue and roof of the mouth until a free space was observed into the stomach [33].

2.4 Up and down LD_{50} estimation of Tramadol

To reduce the deaths of experimental animals choose Dixon's Up and down method for estimation of Tramadol LD_{50} concentration. This method was based on the dose of test substances toxicity. The dose either to be the rise or reduce was based on the survival and mortality of first test animal dose concentration. In this process, survival denotes as "O" and mortality refers for "x", then, the score was made according to the Dixons table [34]. The above all experimental animals were observed continuously up to 48 hours for behavioral changes, locomotion, convulsions, and mortality rates before and after dosages to estimation, the tramadol accurate LD_{50} concentration on rats. Presently, the study was approved by the guidelines of the Ethical Committee and Council of the S V University Regd.No.438/01a/CPCSEA,dt:17-07-2001.

After estimation the tramadol accurate LD_{50} concentration on rats , then the animals were kept for investigating by Psychobiotic drug therapy on inducing tramadol lethal effects on albinos . The present study, taken twenty-four male albino rats, Rattus norvegicus (mean weight 250±20 g) were divided into three groups and third group is sub divided into two sub groups, each group may contain six albinos.

Group-1:- Albinos received oral administered normal saline (Nacl-0.9%) at 1ml/ 100 gm/kg bw as the Control group.

Group-2:- Animals received a single LD_{50} dose of 280mg/kg bw of tramadol hydrochloride orally by gavages according to the above up and down method as indicates Tramadol LD_{50} group.

Group-3:- The GroupIII is further subdivided into two sub groups namely IIIA and IIIB.

Sub group 3 A:- Psychobiotic L. helvetius MCC 2036 strains at $3x10^9$ CFU/ml along with tramadol LD_{50} dose per day at Four weeks therapy and

Subgroup 3 B :- Psychobiotic L. helvetius MCC 2036 strains at $3x10^9$ CFU/ml along with tramadol LD_{50} dose per day for Eight weeks therapy

2.5 Biochemical studies of tramadol in albino rats

The biochemical parameters were examined in all groups of albino rats by collect blood into coagulant tubes and centrifuged at 4000 rpm /10 min, Serum was separated (preserved at -20°C analysis) to detect liver and kidney hormonal levels by using the Ensure biotech test kits methods.

2.5.1. Liver function indices

Alanine aminotransferase (ALT) and Aspartate amino transferase (AST) enzyme activities were measured in IU/l by using the method of IFCC [35]. The serum alkaline phosphatase (ALP) enzyme in the IU/l level in mg/100 ml is based on the pNPP Kinetic method [36], and the quantitative determination Bilirubin was measured by the Diazo sulfanilic (DSA) method [37].

2.5.2. Kidney function indices

Serum Creatinine (Sr C) level in mg/dl was determined using the method of Jaffe's modified method, End Point & Kinetic Method [38], Blood urea nitrogen (BUN) measurement in mg/dl was assessed based on the cleavage of urea with according to Berthelot Method [39].

2.6 Histological scrutinizes of tramadol in albino rats

The above-stated groups of albinos were decapitated and separated the organs viz., Brain, Liver, and Kidney of each rat were rinsed immediately into 0.9% NaCl for 30 seconds to avoid bacterial contamination and washed by sterile distilled water then preserved at 10% formalin solution and dehydrated in ascending grades of alcohol. Organs are treated with Xylene, and in paraffin blocks to infiltrate tissue samples with paraffin and replace the water content of tissue by this wax material [40]. Sectioning the organs by using microtome into four to Five-micron thick section cuts and are kept on a water bath maintained at 45°C, then dried at room temp (30-32^0C). Stained the sections by using hematoxylin and eosin (H & E), place a thin glass over the tissue section to enhance the optical evaluation of the tissue. The above unintentional bias was prevented by coding rats' tissue samples. Finally, slides were examined by using a light microscope under the high power field (40X) for histological variations [41].

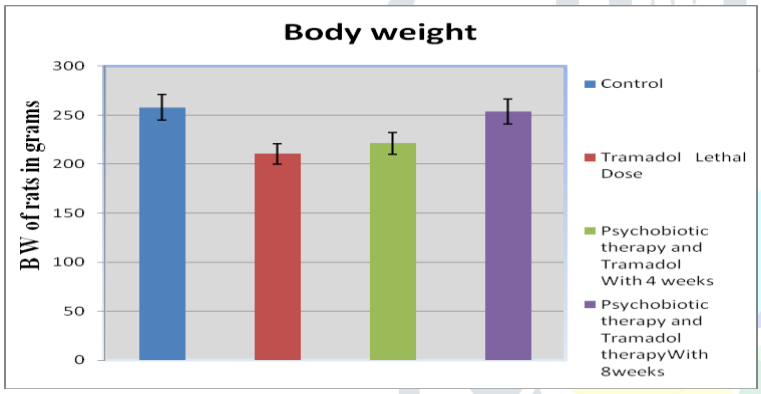
2.7 Statistical evaluation

The values were presented as mean ± SD. The statically Dixon's Up and down table were used for the LD_{50} determination as described below. A comparison of the changes in mortality and live rate in Tramadol treated groups was performed by using two-way ANOVA. $P<0.05$ was considered as significant was calculated SPSS software (version 11.8) and graphs were drawn exert Microsoft excel (2007).

III. RESULTS

3.1 Assessment of body weight and Cognitive behavior

Compared with the control group, Tramadol administered rats showed variation on skin elasticity which thin and slight saggy and the body weight of rats gradually decreased when compared to the control group presented in Graph-1. Tramadol LD_{50} dose along with Psychobiotic Lactobacillus helveticus - MCC 2036 treated group were significantly increased in total body weight, when contrast to the Tramadol group and nearer to control. As a result, the body weight of control (257.91±7.43), albinos treated with *L*. helveticus - MCC 2036 capable of 4 weeks (221.28± 7.208) to 8 weeks (251.33±7.537), exhibited increased body weight when compared to tramadol group (210.447±11.457). Were calculated as average mean values (Mean ± SD) from organs isolated from six animals from each group. The f-ratio value shows 3.325835 and p-value shows less than 0.427515. Therefore result is significant at $p < 0.05$.



Graph.1. Assessment of control and experimental Rattus norvegicus body weights

3.2 Tramadol LD_{50} determination by up and down method

3.2.1. Dixon's Up word regulation LD_{50} estimation

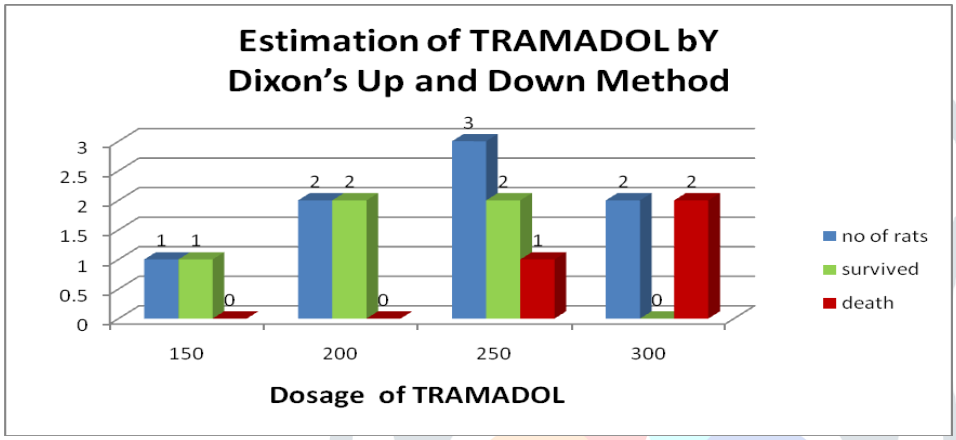
Specified dose was gradual risen up to its lethal of test animal, initial dose starts at 150mg/kg bw tramadol with 2ml sterile water to albinos were survived (O). Then, the secondary dose raised up to 200mg/kg BW tramadol it's also survived (O). The tertiary dose increased up to 250mg/kg bw tramadol indicating tremble, then it's slowly recovered and survived (O). Later, an increasing dose up to 300mg/ kg bw of tramadol inserted albinos died within a 2 - 4 hrs period (x). Then, finally, confirmation LD_{50} estimation test was conducted below.

3.2.2. Dixon's down word regulation LD_{50} estimation

The test animal dose was gradually reduced, up to its lethal to survival. In down words, estimation start at dose of 300mg/ kg bw inserted to albino. It was died within two hours of time (x). Then, secondary dose was reduced

to 250mg/ kg bw tramadol with 2ml sterile water that was nearer to the mortality dose. It was introduced into two rats, kept for observation one was struggled and slowly recovered, survived within 24 hrs (O), another one has died within 16 hours (x). Finally, inserted the lower concentration of the tramadol that was 200mg/ kg bw into one rat and observed for its reaction up to 48 hrs with 2-4hrs intervals of time, it's survived (O).

The Up words LD_{50} estimation dose score indicates, three Survived (O, O, O) and one mortality was denoted (x). Hence, Conformation of Down words test conduct by reducing doses on shows two Survived (O, O) and two mortality (x, x). Final score obtained according to given dosages OOOXOOXX and with this score, the LD_{50} represented in Graph-2.



Graph.2. **Dixon's up and down regulation method of tramadol in Rattus norvegicus**

To calculate the LD_{50} based on the given equation $LD_{50}= X_f \pm kd$, *where* X_f is the final dose administered (2.477). k = -0.142 value obtained from the Dixon's table, and d is the difference between log of initial dose and final dose [(2.477-2.176) = 0.301].

Therefore, LD_{50}=2.477± [(-0.142) x (0.301)],

2.477± -0.042742= 2.4342,

Antilog for 2.43425 is 271.8003(or) 272 mg/kg.

Finally, LD_{50} for Tramadol according to Dixon's Up and Down method is equal to 272>/ 280 mg/kg body weight in albinos represented in Table.1.

Table.1. **Effect of Different doses of oral administration Tramadol by Dixon's Up and Down method ('O' indicates Survival; and 'x' indicates Death).**

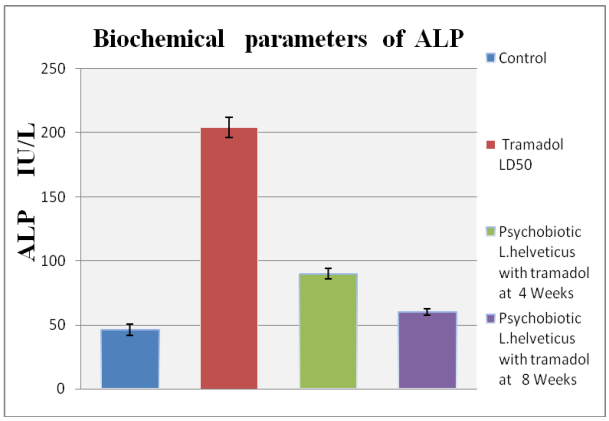
S.No	Body weight of Albino rats	Tramadol Dose administrated to rats (mg/kg/ bw)	Log Dose 0f tramadol	Dixon's Up and Down method Score
1	250	150	2.176	O

2	250	200	2.301	O O
3	250	250	2.397	O O X
4	250	300	2.477	X X

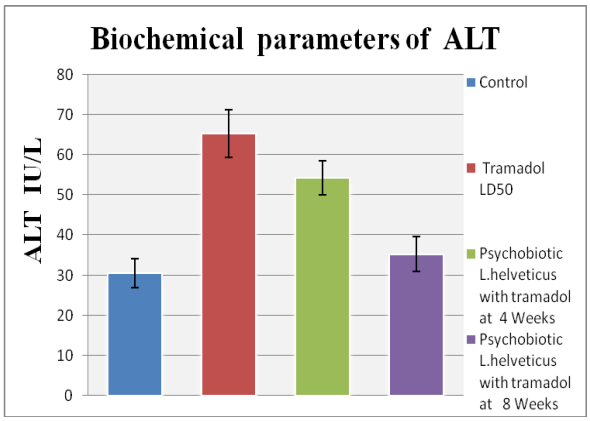
3.3 Biochemical analysis indices

The biochemical parameters of Serum enzymes ALP (alkaline phosphatase), ALT (alanine aminotransferase), AST (aspartate aminotransferase), serum creatinine and blood urea nitrogen (BUN) were documented. The serum ALP was conspicuously raising the leaves on lethal dosages (204.3±7.7 IU/L) in contrast to the control group (Graph.3). However the maximum increase in ALT (65.3±6.0 IU/L) was recorded with the administration of lethal dose 280mg/kg compared to control (Graph.4). The present study revealed a significantly higher ($p< 0.05$) level of AST in LD_{50} experimental groups of rats compared to control and Psychobiotic groups, the highest level AST (78.2±5.9 IU/L) was found with the administration of 280mg/kg tramadol (Graph.5) and Bilirubin is a major factor to identify the liver metabolism. Bilirubin levels are also increased (39.2±3.2) analogy to the Psychobiotic therapy seen in (Graph.6). Significant decrease found in the level of AST in rats treated with Psychobiotic tramadol 4 and 8 weeks period of time (20.7±1.6and 18.2±0.8U/L). Among the above biochemical parameters ALT activity significantly raised more than ($p< 0.05$) in LD_{50} of Tramadol compared with control, 4 weeks and 8 weeks dosages of Psychobiotic therapy. The estimation of serum creatinine and Blood Urea Nitrogen (BUN) is mainly important to determine the functions of the kidney. In additionally serum creatinine level is also moderately affected by factors compared to BUN levels. In the present study, the maximum increase in creatinine (3.43±0.9mg/dl) and BUN (68.4±8.2mg/dl) were recorded significantly different ($p< 0.05$) from lethal to control and Psychobiotic tramadol groups in the Graph.7 and 8.

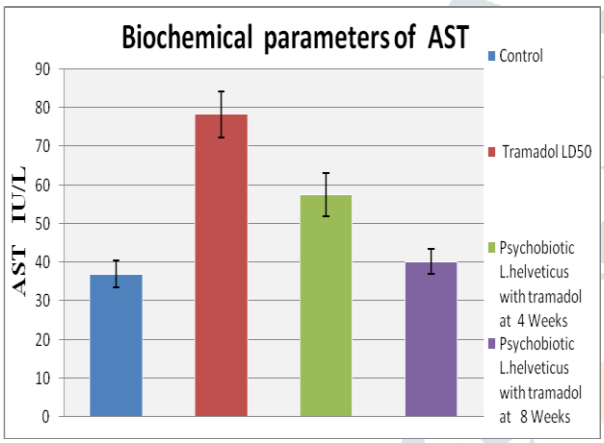
Table.3.Biochemical Analysis of tramadol effect and Psychobiotic therapy on Albino Rat



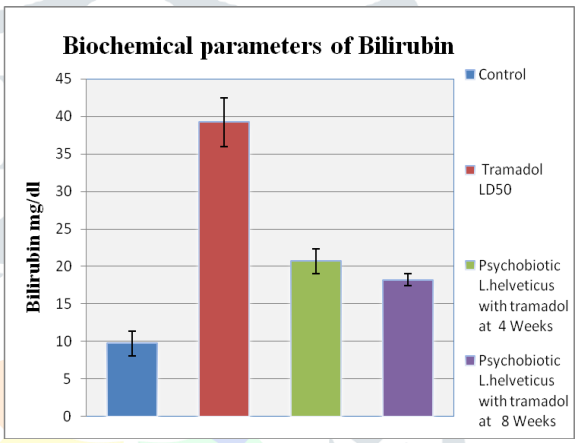
Graph.3



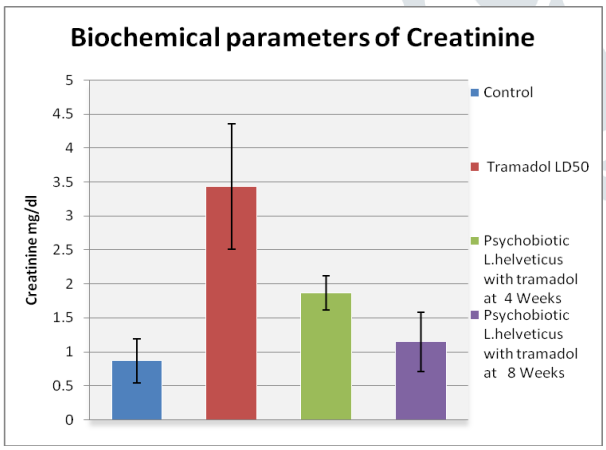
Graph.4



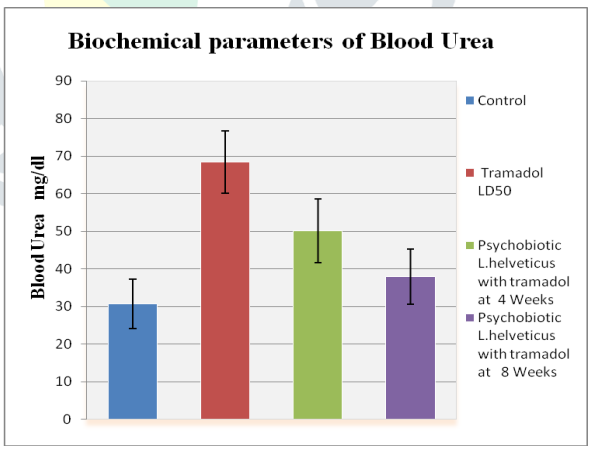
Graph.5



Graph.6



Graph.7



Graph.8

Graphs. 3 to 8. Biochemical analysis of liver and kidney of albinorats with reference to tramadol toxicity and Psychobiotic therapy

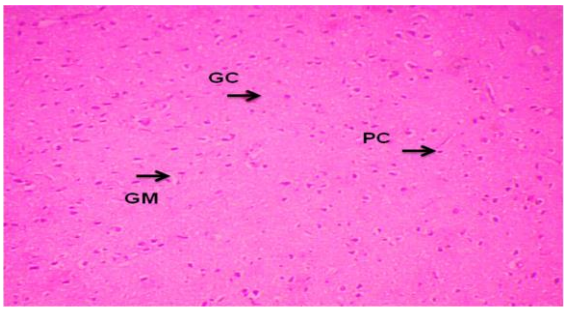
3.4 Histological studies of Brain, Liver, and kidney of the albino rats with reference to Tramadol toxicity and Psychobiotic therapy

The control Group of Brain cerebral cortex region shows with Glial cells(GC) surround neurons provide supporting, normal granular layer with gray matter connected with capillaries and insulation between them indicates by arrow and the Purkinje cells (PC) are surroundings in brain shows in control group at 40X microscopic observations on Group1 (Fig.1).The Tramadol LD_{50} tissues of Brain indicating necrotic changes of cerebral region with granular layer and the distraction of the brain capillaries, gray matter red spots show with of focal inflammatory lesions, the destruction of the Purkinje cells leads to gaps on granular layer were noticed on Group 2 (Fig.2). The Psychobiotic Group.3.A cytoarchitecture of cerebral cortex region brain tissues was mild inflammations are surroundings in the granular region, and the Purkinje cells and glial cells were normal in the granular layer and red spots were randomly decreased compared to lethal dose on the gray matter of brain were shown (Fig.3). The cerebral cortex region of brain tissue has no inflammations lesions were found and normal cytoarchitectural cerebral cortex and red spot inflammation reduction were clearly seen in the granular region of Psychobiotic Group.3.B compared to above groups (Fig.4).

The Liver tissue of control Group.1 indicates the normal structure of central vein and surrounding hepatocytes architecture with hepatic duct and also normal sinusoids be seen on higher Magnification (40X) (Fig.5). The Tramadol LD_{50} Group.2 of liver tissues indicates degenerative changes on dilations of sinusoids, congestion, and necrosis which indicates in apoptosis of hepatocytes with structural destructive hepatic arteries and lipid deposits, dispersion of cell infiltration and congestive central veins, lyses of connective tissue with rupture of portal veins and sinusoids were discerned (Fig.6). Variations in the portal veins (PV) and hepatic sinusoids, its indicates in hepatocytes with inflammatory cell infiltration on connective tissue with mild enlargement of portal veins, sinusoids and congestive veins with slight variations on hepatic parenchyma were seen on Psychobiotic Group.3.A (Fig.7). The mild destruction on sinusoids and on the Hepatocytes and Necrotic changes were reduced and congestion is mild on central veins, congregations of hepatic parenchyma and lipid deposits were noticed the Psychobiotic Group.3.B contrast to above groups (Fig.8).

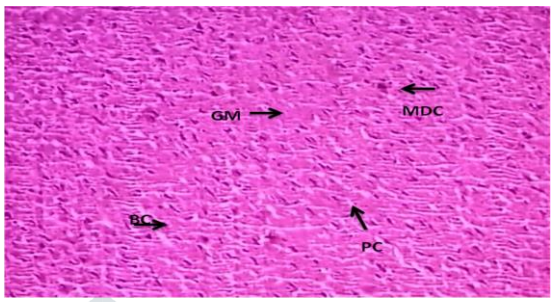
Control Group of kidney cytoarchitecture represents by normal Glomeruli and Mesangial Cells are lined with cuboidal epithelium, (Fig.9). The kidney of tramadol LD_{50} inserted albino indicates Necrotic changes in glomeruli (NC), that contained Vacuolization (V) of glomerular epithelium surrounded by the outer surfaces of the glomerulus. Layers of Bowman's capsule (BC) that could be separated by narrow Bowman's space indicating disruption of the filtration slit and glomeruli tubes cause Degenerative Bowman's Capsule (DBC). Destruction on Mesangial Cells and epithelial cells lining undergoes to focal necrotic changes were detected on Group.2 (Fig.9). The Psychobiotic Group.3.A shows mild degenerative changes appears on glomeruli (G), and cytoarchitecture of parietal layer's on Bowman's capsule (BC) and the space variations are also observation on Glomeruli (G) and Mesangial Cells (MC) lined with cuboidal epithelium with cytoplasm and vesicular central

rounded nuclei were exhibited on compare to above group (Fig.9). Psychobiotic Group.3.B histological structures were nearer to the control Group. It indicating normal glomerular epithelium surrounded by the outer surfaces of the glomerulus, the mild changes on the Bowman's capsule and necrosis reduction on the Glomeruli (G) and Mesangial Cells (MC) were examined under 40x microscopy in (Fig.12).



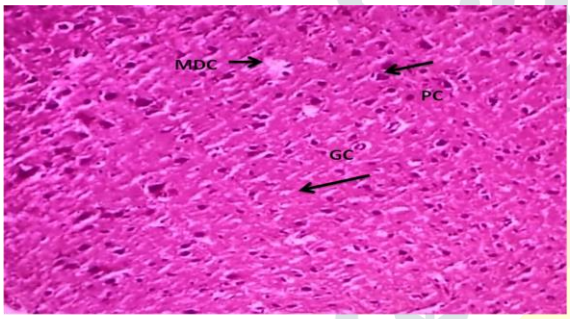
BRAIN CONTROL 40X

Figure 1



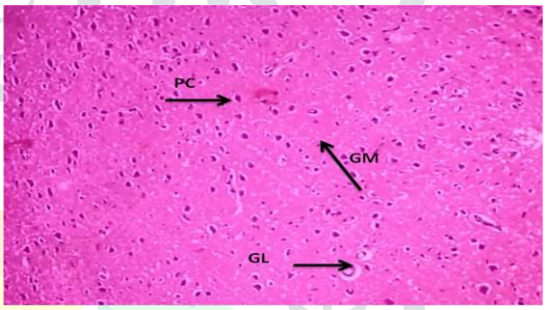
BRAIN Tramadol LD50 280 MG 40X

Figure 2



Psychobiotic *L. Helveticus Therapy with Tramadol at 4 weeks*

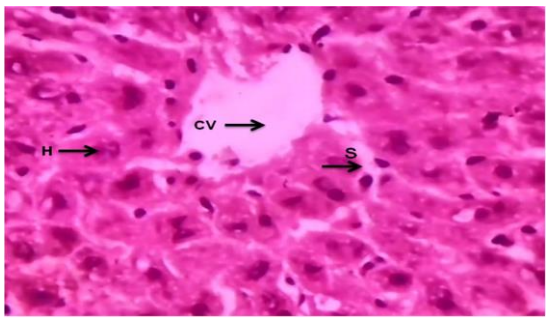
Figure 3



Psychobiotic *L. Helveticus Therapy with Tramadol at 8 weeks*

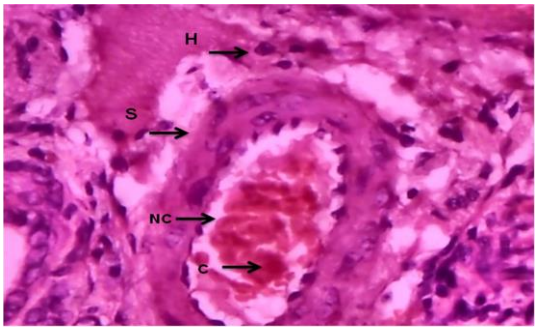
Figure I

Brain histology of control and experimental Albino rats (Fig 1 to 4). CP -Capillaries , G -Glial Cells , GL-Granular Layer, GM – Gray Matter , MDC-Mild Degenerative Changes , PC -Purkinje Cells. Fig.1: Brain of control; Fig.2: Brain effect on Tramadol LD_{50}. Fig.3 Brain of Psychobiotic therapy including Tramadol LD_{50} for Four weeks. Fig.4: Brain of Psychobiotic therapy including Tramadol LD_{50} for Eight weeks.



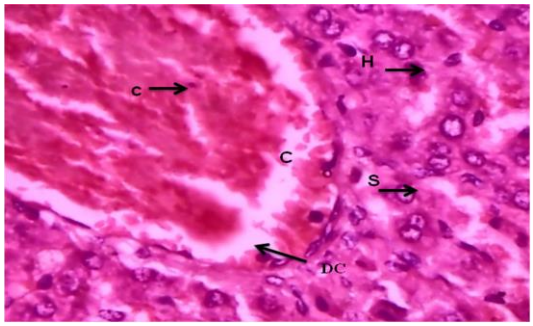
Control LIVER L 40X

Figure 5



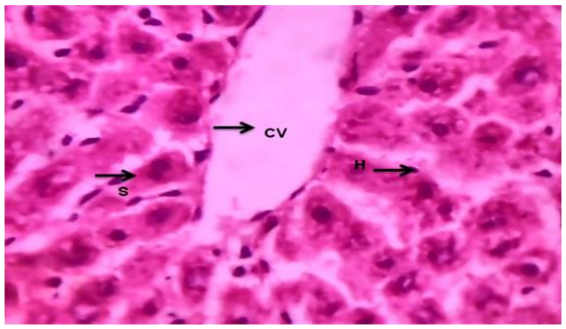
LIVER Tramadol 280 MG 40X

Figure 6



Psychobiotic *L. Helveticus Therapy with Tramadol at 4 weeks*

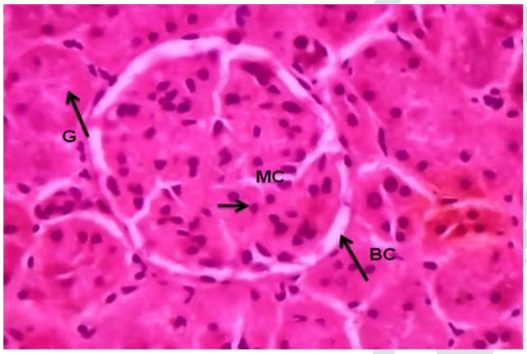
Figure 7



Psychobiotic *L. Helveticus Therapy with Tramadol at 8 weeks*

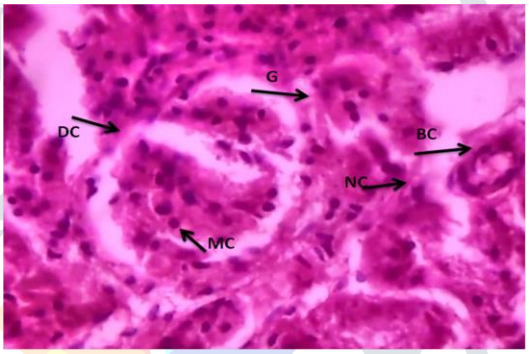
Figure 8

Liver histology of control and experimental Albino rats (Fig. 5 to 8). C-Congestion, CV-Central Vein, DC-Degenerative Changes, H –Hepatocytes, S-Sinusoidal, LD-Lipid Deposits, MDC-Mild Degenerative Changes, NC-Necrotic Changes. Fig.5: Liver of control; Fig.6: Liver effect on Tramadol LD_{50}. Fig.7 Liver of Psychobiotic therapy including Tramadol LD_{50} for Four weeks. Fig.8: Liver of Psychobiotic therapy including Tramadol LD_{50} for Eight weeks.



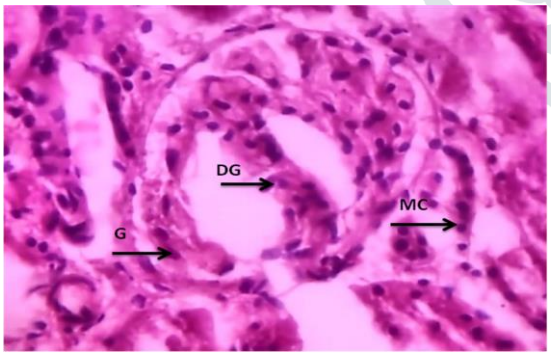
Control Kidney 40X

Figure 9



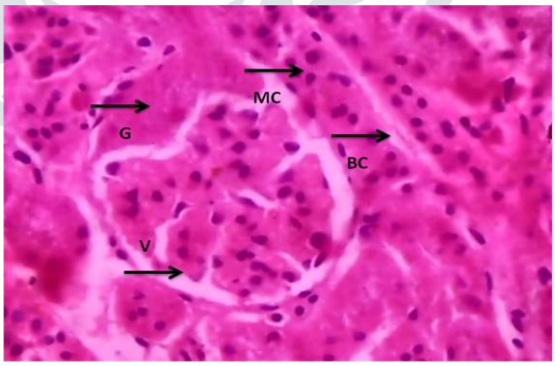
KIDNEY Tramadol 280 MG 40X

Figure 10



Psychobiotic *L. Helveticus Therapy with Tramadol at 4 weeks*

Figure 11



Psychobiotic *L. Helveticus Therapy with Tramadol at 8 weeks*

Figure 12

Kidney histology of control and experimental Albino rats (Fig.9 to 12). BC -Bowman's Capsule , D-Distal , DBC-Distractive Bowman's Capsule , DC-Degenerative Changes, DG-Degenerative Glomeruli , G-Glomeruli , MC-Mesangial Cells, NC-Necrotic Changes, V-Vacuolization. Fig.9: Kidney of control; Fig.10: Kidney effect on Tramadol LD_{50}. Fig.11 Kidney of Psychobiotic therapy including Tramadol LD_{50} for Four weeks. Fig.12: Kidney of Psychobiotic therapy including Tramadol LD_{50} for Eight weeks.

Fig.1 to12: Histological photomicrograph of cross section of Brain, Liver and renal tissue of group examination of tramadol effects on Albino Rat

IV. DISCUSSION

The opioids usage are more predominant in nowadays, among this commonly prescribed opioid named as tramadol for severe pains [42-43] , taking poly tramadol means combination with other drugs that leads increases the risk factor for inhibiting the reuptake of monoamines leads to serotonin syndrome [44] prolonging and hyper administration of tramadol leads mentally addicted, they take higher than prescribed without caring about the negative effects this allude to Seizures and losing interest in things that used to be important to them and they are desire to quits, and can even result in death. Tramadol can elicit adverse reactions as respiratory depression and slow heartbeat, Weakness, even some cases Coma [45]. American Food and Drug Administration (AFDA) was rated tramadol as under Drug Category C, and it's not preferred for kidney, liver disease, stomach disorder, mental illness, depression and mostly pregnant and also breastfeeding women's because of it cause harm the fetus, birth defects, and infect to the babies [46]. The present study leads to investigate the accurate form of tramadol Lethal Dose and Psychobiotic therapy, its histological and biochemical profiles of short and long term effects on toxicity on Brain, liver, and kidney of the albino. In liver, tramadol is metabolized by N and O demethylation in presence of glucuronic acid and sulfate conjugation, it shows higher affinity on mu-opioid receptors [47]. The Dixon's Up and down method of toxicological testing approach mostly recommended by various regulatory agencies because this method reduces the number of animals in research. This test method is relevant to both liquids and solid test substances for estimating LD_{50} dose [48]. This method not only preferable for only Lethal dose and also allows for observation signs of toxicity in an animal this could be useful in planning additional insertion or reducing toxicity testing.

The biochemical enzymes like AST, ALT, and ALP, Bilirubin, creatinine, Blood Urea were found in mainly blood, heart, skeletal muscle, liver, kidney, and brain [49]. Variation on these levels indicating the malfunctioning and damage of the above tissue parts. According to this, the tramadol treated albinos of AST and ALT leaves were raised compared with control and Psychobiotic Groups show on Graph.2 and 3, it refers to the oxidative tissue damage, indicating the malfunctioning of liver, [50-51]. Frequently elevated serum ALP levels on treated albinos were shown on the Graph.1 was associated with a variety disorder as infiltrative liver disease, extrahepatic bile obstruction, hepatitis, and hyper alkaline phosphatasemia [52-53]. Bilirubin or Liver function test recognized the pattern of liver infections, and help to assess the severity, of certain diseases like chronic liver disease, and biliary cirrhosis [54-55]. The Group.3 albinos that can be treated with tramadol and Psychobiotic therapy, the four weeks and eight weeks treated albinos groups are also shown Bilirubin levels were shows reducing to the LD_{50} dose values. This indicates the Psychobiotic is shown it's significant on enzyme activation on liver function [56-57]. The toxic kinetics process of tramadol drug is excreted through the kidneys; the

creatinine and urea are major biomarkers as to diagnosis and follow up of renal functions. Concentration levels of creatinine and urea significant variations on tramadol treated Groups were shown on Graph.5 in accordance with control [58-59]. Highly elevated creatinine may cause acute kidney injury, reduction in glomerular filtration rate [60]. The blood urea is also a major impact to find the liver and kidney functions because it produced in the liver and it transported in the blood to kidneys [61]. Kidneys are most effective on remove urea nitrogen from blood, but tramadol treated albinos levels of urea in blood were rises are shown in Graph.6, which leads kidney failure [62], and leads to causes uremia [63].

In Brain histological cross-sections of Tramadol induces Albinos group shows damage occurs on the glial cells and also necrosis on Purkinje cells and mild degenerative changes on Granular layer represent on Fig.2 and reduction of necrosis and degenerative changes are exhibited on Psychobiotic Groups on long term induce therapy Fig.4, compared to four weeks treatment Fig.3 and control Group Fig.1. The total brain mass of Glial cells is present up to 33 and 66%, [64] it maintains homeostasis, form myelin, and provide support and protection for neurons, and also play a role in neurotransmission and synaptic connections [65]. The Tramadol induces Albinos shows damage occurs on the Glial cells, it leads to apoptosis among the surrounding cellular bodies and occurs inflammation [66]. Purkinje cells have the ability to send interception projections towards the cerebellar nuclei, moreover, constitute the sole output to all motor coordination in the cerebellar cortex region. Purkinje cells can be harmed by tramadol exposure groups [67-68]. This progressive Necrosis of Purkinje cells leads to poor coordination on small dilated blood vessels refers to causes Louis–Bar syndrome [69] and Niemann Pick disease [70].

The Histological structure of the Liver was found normal in the control group and variations were on tramadol LD_{50} 280mg/dl decreasing its lethality on Psychobiotic induced groups at 4 weeks and 8weeks period were shown on Fig.5 to 8. The Lethal dose inducing albino's shows on apoptosis of hepatocytes, and membrane degeneration, hepatic arteries distraction, and damage of portal veins and sinusoids were noticed [71]. Hepatocytes are cuboidal epithelial cells that line the sinusoids perform most of the liver's functions metabolism, bile productions. Mainly Hepatocyte cell in the body that manufactures serum albumin, fibrinogen, has the ability to metabolize, detoxify, and inactivate exogenous compounds such as drugs [72]. In liver 70–80% Hepatocytes may present on the liver cells [73]. Hepatocyte damage was seen on tramadol LD_{50} results in the liver shows dysfunction can result in the activation of hepatic fibrosis, and liver cirrhosis [74]. The sinusoids are the vascular structures in the liver, which are lined by a fenestrated endothelial cell, Sinusoidal endothelial cell injury these sinusoids damages leads to manifests as the sinusoidal obstruction syndrome [75]. Hepatic apoptosis and its regulation are thought of as a pivotal step in most forms of liver injury, including liver fibrosis, cirrhosis, and the development of hepatocellular carcinoma [76-77].

Tramadol LD_{50} 280mg/dl inducing albinos shows on inflammation and degenerative of the glomeruli are may cause by Glomerulonephritis that leads to destruction in filtration on the kidney, and were clearly shown on

fig.10 [78]. The first step in the filtration of blood to form urine was performed by Bowman's capsule. It has exhibit complete distractive on lethal effects of tramadol, its leads to disruption of the filtration slits to necrotic changes the podocytes can lead to cause proteinuria [79]. L. helvitulus induced therapy on Group.3 at four and eight weeks period rats shows randomly reduction on the bowman's destruction on kidneys and also in glomeruli were seen in the histological examinations on the fig.11 and 12 that are nearer to control group Fig.9. The current above histological results pointed out the risk of increased brain, Liver and kidney damage due to acute usage of tramadol compared to L. helveticus groups and control groups. The psychobiotic L. helveticus therapy groups are shows major impact on reduction on effects causing by the tramadol , the above study shows the prolonging therapy of psychobiotic's has more effective to turn down stress and aggressiveness of albinos compared to lethal dose of tramadol.

V. CONCLUSIONS

Consumption of opiodis are regular in present days , but on repeted and higher doses leads to infections and some cases damages to the body organs . On the assumption, patients were not intrested to take medicines as regular bases, on that circumstance probiotic psychobiotic microorganims is a best alternatve. Above study stated that psychobiotic has an ability to downsizing stress and infections causing by opiodi toxicity , consequently it could be beneficial for mankind.

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