WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 8, 1673-1680. Research Article ISSN 2277– 7105

EFFECT OF TRADITIONAL CANNABIS PREPARATION (TCP) ON PARACETAMOL INDUCED HEPATIC INJURY IN RATS

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Article Received on
09 June 2017,
Revised on 29 June 2017,
Accepted on 19 July 2017
DOI: 10.20959/wjpr20178-9056

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ABSTRACT

Objective: To investigate the effect of traditional cannabis preparation (TCP) on paracetamol induced hepatic injury in rats. **Methods**: Wistar rats weighing between 150-200 gm and each group contains 6 animals. Test group of animals were treated with paracetamol (2gm/kg bw po). Silymarin (100mg/kg bw po) was used as a standard reference. TCP (0.5ml/100gm po) was given alone and to the group intoxicated with paracetamol. Thereafter, the activities of alanine aminotransferase (ALT), aspartate amino transferase (AST) and alkaline phosphatase (ALP) were assayed in the serum. The levels of other biochemical markers of organ damage such as total bilirubin, pentobarbitone induced sleeping time, liver weight and liver volume were also determined. Histological examination of the liver was performed. **Result:** Group of rats received TCP showed relatively lower increase in liver enzyme parameters as compared with group of rats treated with PCM alone. However, these values were elevated when compared to normal, untreated group of rats. Also, group of rats received TCP followed by PCM showed significant increase in liver ezyme parameters as compared to PCM alone treated group of rats. However, these values were approximately 2 times higher as compared to normal, untreated group of rats. **Conclusion:** In the present study, toxic nature of TCP was dependent on the pathological state of liver as its consumption in diseased condition were further enhancing the pathologies of liver which was revealed through elevated levels of various biochemical marker, when oral feeding of TCP in paracetamol intoxicated rat.

KEYWORDS: Traditional Cannabis Preparation, paracetamol, liver injury.

INTRODUCTION

Traditional Cannabis Preparation (TCP) is a juice of cannabis pills in equal quantity of milk and water with little sugar. These cannabis pills along with TCP are easily available in licensed shop of Bhopal, Madhya Pradesh, India. The cannabis pills are nothing but the paste of freshly, dried and finely grounded leaves of *Cannabis sativa linn*. in water. For centuries preparations of Cannabis sativa L.(marijuana) have been used for both medical and recreation purposes. The therapeutic potential of this drug was documented as early as the fourth century B.C. The Chinese have used marijuana for the treatment of malaria, constipation, rheumatic pains, absent-mindedness, and female disorders. Use of marijuana spread West from early Chinese culture to India and finally to Eastern Europe. Interest in cannabis and its active constituents, Cannabinoids, as therapeutic agents is a phenomenon of interest nowadays.[1] *Cannabis sativa* contains more than 400 chemical compounds, of which about 60 are cannabinoids. The first to be isolated and the one mainly responsible for the psychoactive properties of the plant, was delta-9-tetrahydrocannabinol. Cannabis sativa and its products are the oldest psychoactive substances in use, yet the literature concerning many of its pharmacological and toxicological effects is inconclusive. One critical area has been the effect of cannabis sativa and its products on hepatic function. Some authors reported various degrees of liver dysfunction in experimental animals or humans consuming such products. Mukhta et al, 2011 suggested that canabinoids increase the (ALP) activity in both injected rats and human smokers and this will increase with the increase of dose and time but the (ALT) and the (AST) increase at the beginning of consumption then will decrease with time.[2] In a transversal study conducted by Borini et al found that chronic marijuana usage, on its own or in association with other drugs, was associated with hepatic morphologic and enzymatic alterations. This indicates that cannabinoids are possible hepatotoxic substances.[3] The objective of the present investigation is to study the effect of a traditional cannabis preparation (bhang) on normal rats as well as on the rats with paracetamol induced hepatic injury. Since the studies discussed earlier suggest that cannabinoids have variable effect on liver pathology, it would be of interest understand the effects of TCP, a widely used popular formulation on liver pathologies.

2. MATERIAL AND METHODS

2.1. Plant material: Cannabis pills were procured from licensed shop of Bhopal.

2.2. Formulation of TCP

Cannabis pills were finely sieved with muslin cloth in equal quantity of milk and water.

2.3. Drugs and chemicals

Silymarin was obtained from Micro Labs, Bangalore. The chemical kits for all biochemical parameters were obtained from Reckon Diagnosticks P. Ltd., Vadodra, GJ, India. The solvents and other chemicals used were of analytical grade.

2.4. Animals

Laboratory bred Wistar albino rats of both sexes (150- 200 g) maintained under standard laboratory conditions at (22 - 2)°C, relative humidity (50-15) % and photoperiod (12 h dark and light), were used for the experiment. Commercial pellet diet (Hindustan Lever, India) and water were provided ad libitum. In order to avoid diurnal variation all the experiments were carried out at same time of the day i.e . between 10 a.m. to 5 p.m. The study was approved by the Institutional Animal Ethical Committee (778/03/C/CPCSEA), following the guidelines of CPSCEA (approved body of Committee for the Purpose of Control and Supervision of Experiments on Animals, Chennai, India) of VNS Institute of Pharmacy, Bhopal (M.P.). Care provided to the animal was as per the 'WHO guidelines for the care and use of animals in scientific research'.

2.5 Experimental Design

The method of Kim Y W *et al*[4] was used in the study. Animals were divided into five groups of 6 animals each. The group I received saline 1 ml/kg daily for ten days (control). The group II received PCM 2gm/kg for three day followed by saline till tenth day (positive control). The group III received PCM 2gm/kg for three days followed by silymarin 100 mg/kg daily till tenth day. The IV group received TCP 0.5 ml/100 gm daily for ten days. The group V received PCM 2g/kg for three days followed by TCP 0.5ml/100gm daily till tenth day. On eleventh day, aqueous solution of thiopentone sodium (37 mg/kg, *i.p.*) in water for injection was injected to the animals and the sleeping time was noted. After complete recovery from thiopentone sodium effect, blood was withdrawn by puncturing the retro-orbital. Blood was collected, allowed to clot and serum was separated at 2500 rpm for 15 min and biochemical investigations were carried out. Animals were sacrificed with excess of light ether anesthesia. Liver was dissected out, rinsed with water and used for morphological and histopathological studies.[5]

2.6. Biochemical analysis

The collected blood samples were used for the analysis of biochemical markers AST, ALT, ALP and bilirubin levels.

2.7. Morphological studies

The morphological parameters, wet liver weight and volume were determined. The wet liver weight was determined by us*ing* an electronic balance. The wet liver volume was determined by dropping the liver in a measuring cylinder containing a fixed volume of distilled water and the volume displaced was recorded.

2.8. Functional studies

Thiopentone sodium was used to determine the functional capacity of liver. The time interval between the loss and the regaining of the righting reflex was measured as sleeping time. Onset and duration of action of sleeping time was noted.

2.9. Histopathological studies

The dissected liver was fixed in 10% formalin, dehydrated in gradual isopropyl alcohol (60-100%), cleared in xylene and embedded in paraffin. Sections were prepared and then stained with hematoxylin and eosin (H-E) dye for photomicroscopic observation.[6]

2.9. Statistical analysis

The results are expressed as mean±SEM, (N=6). Statistical significance was determined by one-way analysis of variance with $P<0.05$ considered significant. The analysis was performed by Prism software.

3. RESULT

3.1. Effect of TCP on ALT, AST, ALP, TBil level

Rats treated with TCP throughout the protocol showed relatively lower increase in liver enzyme parameters as compared with rats treated with PCM. However, the values of the biochemical parameters were elevated when compared to normal, untreated group of rats. After 7 days treatment with TCP on PCM treated (3-day) group of rats showed significant increase in liver enzyme parameters as compared to PCM alone treated group of rats. However, the values of the biochemical parameters were approximately 2 times higher as compared to normal, untreated group of rats

3.2. Effects of TCP on wet liver weight and volume

The result of the effect of TCP on morphological parameters in rats is shown in Table 3. In the PCM treated rats, wet liver weight and liver volume were increased to 4.65±0.0 g/100 g and 4.05±0.04 mL/100 g respectively whereas these values were 3.01±0.0 g/100 g and 3.0±0.05 mL/100 g in normal control rats respectively. On administration of Silymarin in group III, the levels of these morphological parameters were found retrieving towards normalcy. The values of TCP treated group of rats showed lower while TCP+PCM treated group showed higher values as compared to PCM treated group.

3.3. Effects of TCP on thiopentone sodium induced sleeping time

The result of the effect of TCP on functional parameters in rats is shown in Table 3. In the PCM treated rats, sleeping time was increased to 131.06±0.2 min., whereas these values were 57.99±0.3 min. in normal control rats. On administration of Silymarin the sleeping time were found retrieving towards normalcy. The values of TCP treated group of rats showed lower while TCP+PCM treated group showed higher values as compared to PCM treated group.

Table no. 1: Effect of Traditional cannabis preparation (TCP) on liver enjyme parameters.

Serum Biochemical parameters	Normal (Group 1)	Toxicant (Group 2)	Standard (Group 3)	TCP (Group 4)	TCP+PCM (Group 5)
ALP (IU/L)	179.95±1.4	432.78±1.9^{**a}	200.98±0.7^{**b}	279.8±0.8^{**b}	446.48±1.3^{**b}
SGOT (IU/L)	98.65±0.5	209.2±0.3^{**a}	145.98±0.3^{**b}	199.28±0.3^{**b}	215.56±0.4^{**b}
SGPT (IU/L)	24.88±0.3	43.21±0.2^{**a}	30.26±0.3^{**b}	40.6±0.4^{**b}	58.35±0.2^{**b}
T-BIL (mg/dl)	1.10±0.01	2.01±0.0^{**a}	1.62±0.0^{**b}	1.97±0.0^{**b}	2.05±0.0^{**b}

Values are expressed as Mean±S.E.M of six rat in each treatment group where, $^{*}P < 0.05$, $^{*}P < 0.01$ and $^{*}P < 0.001$ vs. control. And ns = non significant, a,b and c significantly different from normal, toxicant and std group respectively.

Table no. 2 Effect of Traditional Cannabis Preperation (TCP) on Thiopentone induced sleeping time (TST), Wet liver weight and volume

Physical Parameters	Normal (Group 1)	Toxicant (Group 2)	Standard (Group 3)	TCP (Group 4)	TCP+PCM (Group 5)
Duration of sleep (min.)	57.99±0.3	131.06±0.2^{**a}	80.49±0.2^{**b}	95.79±0.3^{**b}	136.56±1.4^{**,b}
Liver weight (g/100g)	3.01±0.0	4.65±0.0^{**a}	3.35±0.0^{**b}	3.95±0.0^{**b}	4.75±0.0^{ns,b}
Liver volume (ml/100g)	3.0±0.05	4.05±0.04^{**a}	3.1±0.05^{**b}	3.8±0.0^{**b}	4.20±0.0^{*b}

Values are expressed as Mean±S.E.M of six rat in each treatment group where, $^{*}P < 0.05$, $^{*}P < 0.01$ and $^{*}P < 0.001$ vs. control. And ns = non significant, a,b and c significantly different from normal, toxicant and std group respectively.

DISSCUSSION

These studies shown that the endocannabinoid system plays an important role in liver pathologies and one of the major concerns is their role in recovery of liver functions in chemically injured or infected livers. It would be of interest to see, what effect exogenously administered phytocannabinoids present in cannabis preparations would have on chemically injured liver. This study is important as cannabis is used by very large population in India as a part of religious culture as well as common recreational drug. Bhang, a liquid preparation intended for oral use is available legally and there are many licensed shops for it in the country. There has been no study to the best of our knowledge evaluating effect of bhang on normal or diseased liver. Borini *et al.*, have reported possible hepatotoxicity of chronic marijuana use, but it was not a controlled study and the subjects were the patients using illicit drugs.

Overdose of paracetamol causes a potentially fatal, hepatic centrilobular necrosis. The hepatotoxicity of paracetamol has been attributed to the formation of a toxic metabolite, *N-acetyl-p-benzoquinoneimine* (NAPQI) by the action of cytochrome P4502E1.[7]

In the present investigation, paracetamol administration resulted in elevated activities of ALT, AST and ALP in serum (Group II) against their respective control values (Group I). Similarly, serum bilirubin level was also found to be increased significantly as a result of paracetamol toxicity (Group II). Abnormally higher activities of serum ALT, AST and ALP after paracetamol administration as observed in the present study is an indication of the development of hepatic injury, which is responsible for leakage of cellular enzymes into the blood. When liver plasma membrane gets damaged, a variety of enzymes normally located in the cytosol are released into the circulation.[8] Oral administration of *Traditional Canabis preparation (TCP)* to paracetamol intoxicated rats resulted in elevation of the activities of ALT, AST and ALP. This evidently suggests the toxic effect of the TCP in the functional integrity of liver cells. Serum bilirubin is considered as an index for the assessment of hepatic function and any abnormal increase indicates hepatobiliary disease and severe disturbance of hepatocellular architecture.[9] Paracetamol administration resulted in increased serum bilirubin level (Group II), thereby suggesting severe hepatic injury and confirming the hepatotoxic nature of paracetamol. Treatment with TCP also increase the level of total bilirubin in serum towards the toxic level as seen in PCM group indicating its hepatotoxic nature. Hepatotoxin impairs the capacity of liver to synthesize albumin.[10]

Subsequent treatment of paracetamol intoxicated rats with TCP further increased the AST, ALT and ALP levels indicating the synergistic effect. This further signifies the toxic nature of TCP.

Histopathological examination of liver sections of the normal control group (Group I) showed normal cellular architecture with distinct hepatic cells. However, distinct hepatic necrosis was noted after paracetamol administration (Group II) with destruction of hepatic cells. TCP treatment to such paracetamol intoxicated rats showed further increase in necrosis. This also suggests that the TCP has a potentiating effect on paracetamol induced toxicity.

The toxic nature of TCP was dependent on the pathological state of liver as its consumption in diseased condition will further enhance the pathologies of liver which was revealed through elevated levels of various biochemical marker, when oral feeding of TCP in paracetamol intoxicated rat. This may, probably be through synergistic activity of oxidative enzymes and degeneration of hepatocytes that restore the structural and functional integrity of liver. Thus, the present investigation confirms the toxic nature of TCP in normal and paracetamol induced hepatotoxicity in rats.

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