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HISTOPATHOLOGICAL, ANTIOXIDANTS, BIOCHEMICAL EXAMINATION AND HEPATOPROTECTIVE EFFECTS OF CANNABIS SATIVA AGAINST CCL4 INDUCED TOXICITY IN RATS

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Abstract

Cannabis's historical shift from natural medicine to illegal substance and back to therapeutic product upon legalization open a new age application as anti-inflammatory during COVID-19. This motivated to explore the anti-inflammatory, biochemical and hepatoprotective profiling of cannabis sativa. Carbon tetra chloride is a well-known xenobiotic hepatotoxic chemical that attacks cytochromes P450 enzymes of the liver. That produces free radicals, which starts lipid peroxidation, leading to loss of Ca^{2+} and causing liver injury. To determine the hepato-toxic effect of Carbon Tetra Chloride and Hepato-protective effects of Cannabis Sativa and their correlation between CCl_4, Cannabis Sativa and antioxidant activity in albino rats. Ethanolic extract of *C. Sativa* (50 mg/kg + CCl_4) and (100 mg/kg + CCl_4) was used thrice a week for five weeks. Hepatotoxicity was induced in albino rats by using CCl_4. Normal Group, CCl_4 induced Hepato-toxicity (0.5ml / kg), Ethanolic extract of *C. Sativa* treated (50mg / kg + CCl_4) and Ethanolic extract of *C. sativa* (100mg/kg + CCl_4). 5.0 ml Blood samples of all groups (Group A, B, C and D) were taken in gel clotted vial and centrifuged at 4000 rpm for 10 minutes and serum was separated. Serum samples were further processed for the estimation of Reduce Glutathione (GSH), Catalyze (CAT), Superoxide Dismutase (SOD), Malondialdehyde (MDA) Estimation of Nitric oxide (NO), Estimation of micronutrients (Vitamin A, Vitamin C and Vitamin E), and Electrolytes concentration by a flame photometer (Na^+ and K^+), estimation of AGE's and AOPP, Liver Function Test (LFTs), Lipid Profile, Serum Albumin and Histopathological examination. Induction of CCl_4 significantly increased Malondialdehyde (MDA) Catalyze (CAT) Nitric Oxide (NO) Advance Glycation end product (AGE's) and Vitamin –A (VIT-A) while decreasing the Super Oxide Dismutase, LFTs and RFTs in serum. Remedy of *C. sativa* 50mg/kg and 100mg/kg body weight noticeably defended the rats from CCl_4 administered liver damage. Overall 50mg/kg + CCl_4 dose is more valid and promising to cure the hepatic damage induced by CCl_4. The findings show that C. sativa has the capacity to protect against CCl_4-mediated liver damage. These activities may be related to the synergistic impact of the identified substances and probable interactions with the hepatic system. It can be recognized as a food appendage against liver reperfusion.

Keywords: *CCl4, SOD, CAT, GSH, MDA, Cannabis Sativa, Hepatoprotectivity*

Introduction

The liver comprises about 2% of body weight in humans. It receives 20% blood from hepatic artery and 80% from hepatic portal veins. The merging of superior and splanchnic mesenteric veins forms a portal vein. Celiac, splanchnic and left gastric arteries collectively form hepatic artery. Liver is distributed into two different lobes. It is connected with the abdominal wall through falciform ligament with a base surrounding ligamentoum teres. The umbilical vein recanalization is responsible for cirrhosis (Hiatt *et al*. 1994). The liver comprises Kupffer cells, stellate cells, Cholangiocytes, Parenchymal Hepatocytes and Non Parenchymal Sinusoidal endothelial cells Hepatocytes are involved in the synthesis, storage and filtration of blood. Liver performs immunological and phagocytic roles with the help of specialized cells called Kupffer cells. Stellate cells are essential for liver pathology. Bile transportation and pH are regulated by the bile duct (Juza *et al.* 2014). Excess dosage of drugs and xenobiotics leads to hepatotoxicity. Hepatotoxins are involved in hepatotoxicity. Hepatotoxins include a high dose of medicinal drugs, herbal medicines, industrial substances and dietary enhancements (Nathwani *et al.* 2005). Liver damage is due to reactive primary metabolites and autoimmune responses affecting liver cells. Hepatotoxicity depends on the number of toxic chemicals, expression level of enzymes and difference in the number of cofactors in blood. Different symptoms related to liver injury are jaundice, yellowish skin and eyes because of an increased level of bilirubin, lethargy, bleeding, unusual weight gain and dark-colored urine (Wright *et al*. 2007). Carbon tetrachloride has been studied to develop hepatotoxicity in mammals, including rats and rabbits. It is converted into trichloromethyl free radical (CCl_3^-) which results in peroxidative degradation in the adipose cells causing fatty infiltration of liver cells. In the presence of oxygen, these free radicals from mitochondria stimulate lipid per membrane oxidation (Muriel *et al.* 2008). *Cannabis sativa* is a flowering plant native to Central Asia (Appendino *et al.* 2008). There are 489 natural substances discovered in this plant. About 70 are classified as cannabinoids. These cannabinoids are further divided into 11 types which are Cannabigerol, Cannabichromene, Cannabicyclol, Cannabielsoin, Cannabinol, Cannabinodiol, Cannabitriol, and Cannabidiol, Δ9-Trans-Tetrahydrocannabinol, Δ8-Trans-Tetrahydrocannabinol and Miscellaneous types (Di Marzo V. 2008). The *Cannabis sativa* leaf, shoot and seed are used for various medicinal purposes. It is used in different forms (Lotersztajn *et al.* 2008). The expression of cannabinoid receptors in liver has been unclear because of low levels of CB_2 receptors in Kupffer cells and CB_1 receptors in hepatic endothelial cells. However, considerable levels of 2-AG and anandamide are present. The expression of monoacylglycerol lipase shows the liver breakdown of endo cannabinoids. In Kupffer cells, CB_2 receptors show considerable upregulation while CB_1 receptors in hepatocytes and endothelial cells. The increased concentration of endocannabinoids in the liver depends on type of liver injury. Kupffer cells and endothelial cells produced AEA, but increased level of 2-AG in hepatic stellate cells has been studied during hepatic injury (Wisse *et al.* 1996).

Oxidative stress (OS) plays a key role in the induction of hepatotoxicity and is well reported in both human and animal models (Valente *et al.* 2012.). Oxidative stress is caused by the elevation of reactive oxygen species (ROS) and/or reactive nitrogen species (RNS) or a reduction in antioxidant defense mechanism which can change this ratio. (Burton and Jauniaux 2011) (Cindrova-Davies *et al.* 2007)

Furthermore, several lines of evidence indicate that excessive OS with the increased free radical generation, impairment of mitochondrial respiration, and intense inflammatory reaction plays a crucial role in CCl_4-induced hepatotoxicity. OS is associated with the dysregulation of signaling pathways modulating cell death and survival. It leads to necrosis and apoptosis of hepatocytes (Vitcheva 2012). Although previous studies have yielded mechanistic information on CCl_4 induced hepatotoxicity, the current understanding on this toxic event remains insufficient (Shi *et al.* 2012). Besides its toxicity for the cardiovascular and central nervous systems, CCl_4 causes liver injury in

human and animal models (Cunha-Oliveira *et al.* 2013) (Pateria *et al.* 2013). This may lead to severe acute toxicity due to cellular necrosis, which can be life threatening. Indeed, as diminished liver function contributes to various adverse health effects, hepatotoxicity has been linked to mortality (Guollo *et al.* 2015).

All the living organisms including human beings uses anti-oxidative defense system which protect them against oxidant damage. Human body cells utilize the antioxidant compounds involved in the reaction with oxidizing agents and deactivate them. Catalase (CAT), superoxide dismutase (SOD), Malondialdehyde (MDA) and Glutathione peroxidases (GSH) are main antioxidants parameters Vitamin C and Vitamin E are both considered as major membrane bounded and aqueous-phase antioxidants respectively. (Badrul *et al.* 2012). The purpose of present study was to determine the Hepato-toxic effect of Carbon Tetra Chloride, Hepato-protective effects of Cannabis Sativa and antioxidant activity in Albino rats.

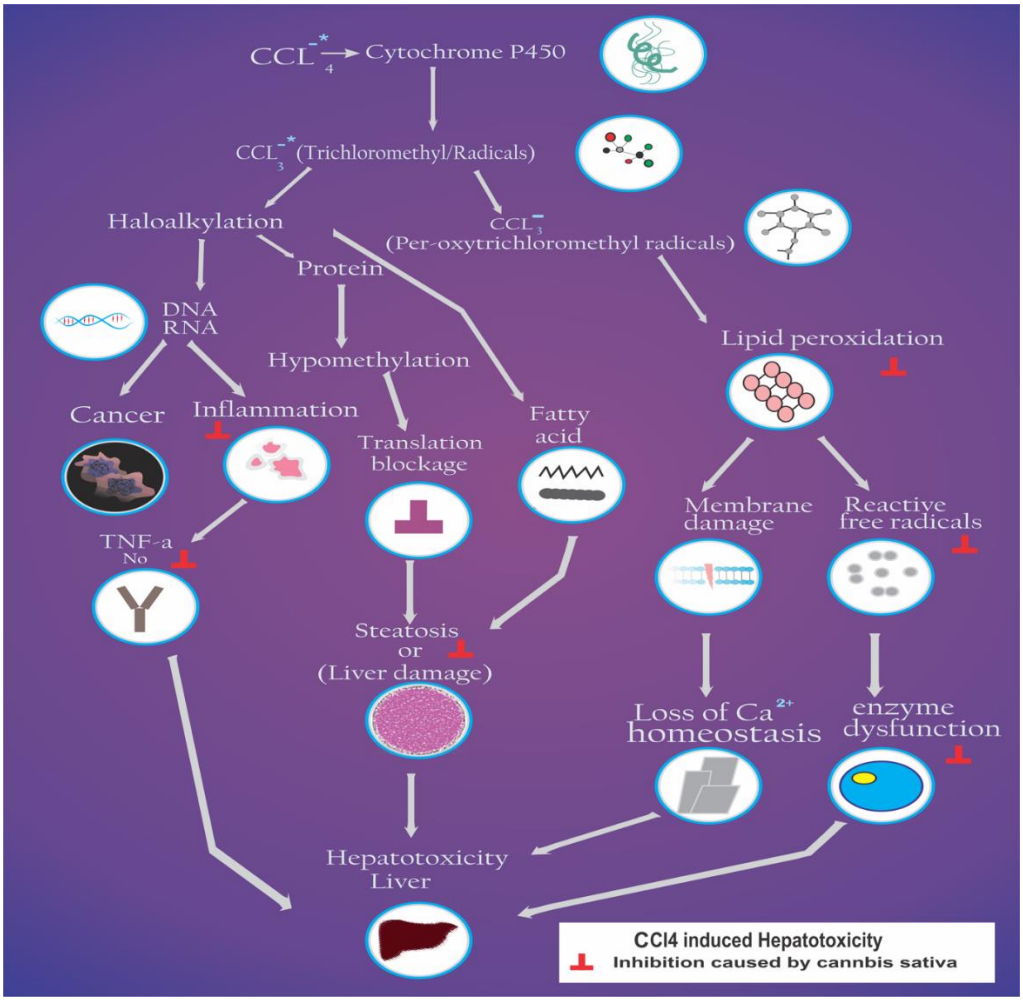

Figure 1: CCl_4 induced hepatotoxicity and inhibition mechanism of *C. Sativa*

METHODOLOGY

Place of work

The whole experimental work was performed in School of Biochemistry Minhaj University Lahore, Pakistan after the ethical and research committee approval per "Principles of Laboratory Animal Care".

Plant pro curement

About 20 kg *C. Sativa* plant were collected from a local road side from Sialkot, Punjab Pakistan in December, 2019. Its confirmatory identification and recognition was done at Seed center,

Department of Botany University of Punjab, Lahore and Department of Taxonomy Government College University Lahore, Pakistan.

Extract preparation

Meshed leaves of C. Sativa were extracted with 280ml concentrated ethanol and 120ml dH2O ethanol using Soxhelt extractor. Leaves of plant were separated thoroughly washed with water at ambient temperature, subjected to air drying in shadow, pulverized, sieved through 80 mesh sieves and stored in the sterile amber glass bottle. The ethanolic extract thus obtained was covered with aluminum foil and kept in electric thermostatic drying oven for whole night at temperature of 90ºC (Rožanc *et al*. 2021). After the whole night drying the pure extract of *C. sativa* was collected in powdered form now its measured weigh was 148.05grm.

Experimental design

Male Albino rats were purchased and bred in university of veterinary and animal sciences, Lahore Pakistan weighing (230-250g) mean age of 3 months were used for this research work. Rats were indiscriminately divided into 4 groups, each have sample number (n=5) group named as Group 1 Control group, 2nd group CCl_4 (0.5ml / kg) induced group, 3rd group received *C. Sativa* 50mg/kg + CCl_4 (0.5ml / kg) and 4th group received *C. sativa* 100mg/kg + CCl_4 (0.5ml / kg). The normal group was fed on a regular diet without any alteration and this group was used as a control group.2nd was treated with CCl_4 (0.5ml / kg) (Dolatabad and Khaki 2014). subcutaneously twice a week for five weeks. 3rd group was treated with *C. sativa* (50mg/kg) thrice a week orally +CCl_4 (0.5ml / kg) subcutaneously twice a week for five weeks.4th group was treated with *C. sativa* (100mg/kg) thrice a week orally + CCl_4 (0.5ml / kg) subcutaneously twice a week for five weeks. (Vilela *et al.* 2015)

Inclusion Criteria

1. Male healthy rats were used for this research work.
2. The average weight of rats was not less than 200 grm.

Exclusion Criteria

1. Unhealthy/female rats were excluded from the study.
2. Rats less than 200grm were omitted from the study.

Sr. No	Group	Sample Size (n)
A	Normal	5
B	CCl_4 induced	5
C	*C. Sativa* 50mg/kg + CCl_4	5
D	*C. Sativa* 100mg/kg + CCl_4	5

Blood sampling

Blood was directly collected from rats' hearts using a 5ml syringe and stored in gel clotted vials for further processing.

Biochemical assays

Already reported protocols were followed for the identification of Biochemical parameters. Blood was further processed for the estimation of Reduce Glutathione (GSH) (Moron *et al.* 1979), Catalase (CAT) (Aebi 1984).), Superoxide Dismutase (SOD) (Kakkar *et al.* 1984).), Malondialdehyde (MDA) (Ohkawa *et al*. 1979).), Estimation of Nitric oxide (NO) (Moshage *et al.* 1995), Estimation of micronutrients (Vitamin A, Vitamin C and Vitamin E) (Roe and Keuther 1943), Rosenberg *et al.* 1992) , Estimation of AOPPs, Estimation of AGEs, Liver Functions tests (LFTs), Lipid Profile, Estimation of urea, creatinine and Electrolytes concentration by flame photometer (Na^+ , K^+ & Cl^-) by kit method.

Blood/sample analysis
Blood was centrifuged at 4000 rpm for 10 minutes and serum was separated. Blood sample was collected into EDTA tubes or gel clotted vials.

Isolation /separation of organs
The rats were anesthetized in Vacuum desecrator containing cotton soaked in Chloroform. When rats became unconscious they were brought out of the jar, their abdominal region was dissected linearly to cut alba and diaphragm with surgical/scalpel blade to expose the organs. Liver and heart removed softly and carefully preserved in 10% Formalin for histopathological examination (Fouad *et al.* 2011).

Histopathological analysis
After Fixation of organs in 10% formalin the Histo-pathological examination procedure was further sub-divided into following steps (Marques *et al.* 2015).

Dehydration
Fixed organs were dehydrated with ascending grades of Alcohol (50%, 70% and 90%) after that 2 time with absolute alcohol each after one hour to remove the impurities and other contaminations from the organs (Chung *et al.* 2008).

Clearing
After dehydration, organs were soaked in pure Xylene to remove the alcohol from the organs (Iranloye *et al.* 2009).

Embedding
Xylene cleared organs were placed in hot Paraffin wax at 70ºC constant temperature for two hours for better absorption of Xylene (Ezhilarasan *et al.* 2016). Blocks were formed carefully after the removal of organs from paraffin wax. After block formation 4µm thicker slice was cut with blade and placed on slide. Hematoxylin and Eosin stainers were used (Henriques 1981). After the staining process the cover slips were placed on slide and placed for 24 hours to dry off the slides (Kaushal *et al.* 2020)

Statistical analysis
Results of biochemical estimations were expressed as mean ± S.D and were analyzed by Statistical Package for the Social Sciences (SPSS-23) and $p<0.05$ was considered as significant.

Results

Table 1: Comparison of Antioxidant biomarkers in different groups of Albino rats model.

Parameters	Normal (n=5) Mean ±S.D	CCl_4 (n=5) Mean ±S.D	50mg/kg (n=5) Mean ±S.D	100mg/kg (n=5) Mean ±S.D	P-Value (0.05)
MDA (nmol/Ml)	0.313±0.035	1.014±0.128	0.945±0.104	0.999±0.131	0.000
GSH (nmol/mg)	1.484±0.013	0.055±0.007	1.550±0.074	1.474±0.013	0.000
SOD (U/mg)	1.479±0.106	0.228±0.052	1.465±0.043	1.604±0.059	0.01
CAT mmol/mg)	3.076±0.029	3.232±0.038	3.052±0.049	3.047±0.036	0.00
NO (nmol/mg)	0.322±0.238	0.326±0.060	0.103±0.009	0.224±0.075	0.042
AOPP (µmol/L)	0.282±0.233	0.191±0.047	0.176±0.001	0.185±0.005	0.055
AGESs (mU/ml)	0.098±0.004	0.282±0.046	0.112±0.009	0.089±0.009	0.000

Data existing in table 1 clearly shows the activity of antioxidant bio-markers evaluated in dissimilar groups of albino rat's serum MDA level in control group is (0.313±0.035) while in CCl_4 induced group is (1.014±0.128) Results clearly shows that the MDA level in group 3 (*C. Sativa* 50mg/kg

+CCl_4) is (0.945±0.104) whereas in group 4 (*C. Sativa* 100mg/kg +CCl_4) is (0.999±0.131). Data also predicted that MDA has significant statistically (P = 0.000<0.05).
GSH concentration in control group is (1.484±0.013) while in CCl_4 induced group is (0.055±0.007) Results apparently visualize that the GSH level in group 3 (*C. Sativa* 50mg/kg + CCl_4) is (1.550±0.074) conversely in group 4 (*C. Sativa* 100mg/kg + CCl_4) is (1.474±0.013). Data also foresee that GSH has significant statistically (P = 0.000<0.05).
SOD level in normal group is (1.479±0.106) while in CCl_4 induced group is (0.055±0.052) outcomes visibly shows that the SOD level in group 3 (*C. Sativa* 50mg/kg + CCl_4) is (1.465±0.043) while in group 4 (*C. Sativa* 100mg/kg + CCl_4) is (1.604±0.059). Data also foretell that SOD has significant statistically (P = 0.01<0.05).
CAT level in control group is (3.076±0.029) while in CCl_4 induced group is (3.232±0.038) consequences noticeably shows that the CAT level in group 3 (*C. Sativa* 50mg/kg + CCl_4) is (3.052±0.049) while in group 4(*C. Sativa* 100mg/kg + CCl_4) is (3.047±0.036). Data also guess that CAT has significant statistically (P = 0.000<0.05).
NO level in control group is (0.322±0.238) while in CCl_4 induced group is (0.326±0.060) Results evidently shows that the NO level in group 3 (*C. Sativa* 50mg/kg + CCl_4) is (0.103±0.009) while in group 4(*C. Sativa* 100mg/kg + CCl_4) is (0.224±0.075). Data also predicted that NO has in-significant statistically (P = 0.42>.05).
AOPP level in control group is (0.282±0.233) while in CCl_4 induced group is (0.191±0.047) Results noticeably shows that the AOPP level in group 3 (*C. Sativa* 50mg/kg + CCl_4) is (0.176±0.001) while in group 4(*C. Sativa* 100mg/kg + CCl_4) is (0.185±0.005). Data also predicted that AOPP has in-significant statistically (P = 0.055>0.05).
AGESs level in control group is (0.098±0.004) while in CCl_4 induced group is (0.282±0.046) Results obviously shows that the AGEs level in group 3 (*C. Sativa* 50mg/kg + CCl_4) is (0.112±0.009) although in group 4(*C. Sativa* 100mg/kg + CCl_4) is (0.089±0.009). Data also forecast that AGESs has significant statistically (P = 0.000<0.05).

Table 2: Comparison of Micro-Nutrients level in different groups of Albino rats model.

Parameters	Normal (n=5) Mean ±S.D	CCl_4 (n=5) Mean ±S.D	50mg/kg (n=5) Mean ±S.D	100mg/kg (n=5) Mean ±S.D	P-Value (0.05)
Vitamin-A (nmol/dL)	2.312±0.195	2.663±0.220	2.000±0.295	2.549±0.045	0.000
Vitamin-C (nmol/dL)	0.447±0.116	0.180±0.031	0.462±0.144	0.377±0.030	0.000
Vitamin-E (nmol/dL)	0.338±0.031	0.108±0.073	0.291±0.058	0.320±0.026	0.000

Data offered in table 2 gives the clear view of micro-Nutrients level evaluated in different groups of albino rat's serum Vitamin-A level in control group is (2.312±0.195) while in CCl_4 induced group is (0.2.663±0.220) Results openly shows that the Vitamin-A level in group 3 (*C. Sativa* 50mg/kg + CCl_4) is (2.000±0.295) while in group 4 (*C. Sativa* 100mg/kg + CCl_4) is (2.549±0.045). Data also predicted that Vitamin-A has significant statistically (P = 0.000<0.05).
Vitamin-C level in control group is (0.447±0.116) while in CCl_4 induced group is (0.180±0.031) Results visibly shows that the Vitamin-C level in group 3 (*C. Sativa* 50mg/kg + CCl_4) is (0.0.462±0.144) while in group 4(*C. Sativa* 100mg/kg + CCl_4) is (0.377±0.030). Data also predicted that VIT-C has significant statistically (P = 0.000<0.05).
Vitamin-E level in control group is (0.338±0.031) while in CCl_4 induced group is (0.108±0.073) Results clearly shows that the Vitamin-E level in group 3 (*C. Sativa* 50mg/kg + CCl_4) is (0.291±0.058) while in group 4(*C. Sativa* 100mg/kg + CCl_4) is (0.320±0.026). Data also predicted that VIT-E has significant statistically (P = 0.000<0.05).

Table 3: Comparison of parameters of Renal function tests in different groups of Albino rats' model.

Parameters	Normal (n=5) Mean ±S.D	CCl_4 (n=5) Mean ±S.D	50mg/kg (n=5) Mean ±S.D	100mg/kg (n=5) Mean ±S.D	P-Value (0.05)
Urea (mg/dL)	30.800±12.111	25.200±3.563	24.800±7.463	55.400±13.464	0.001
Creatinine (mg/dL)	1.4000±0.418	0.634±0.149	0.800±0.122	1.398±0.230	0.001

Data exist in table 3 shows the clear image of parameters of Renal function tests level evaluated in different groups of albino rat's serum Urea level in control group is (30.800±12.111) while in CCl_4 induced group is (25.200±3.563) Results shows that the Urea level in group 3 (*C. Sativa* 50mg/kg + CCl_4) is (24.800±7.463) while in group 4 (*C. Sativa* 100mg/kg + CCl_4) is (55.400±13.464). Data also predicted that Urea has significant statistically ($P = 0.000<0.05$).
Creatinine level in control group is (1.400±0.418) while in CCl_4 induced group is (0.634±0.149) Results clearly shows that the Creatinine level in group 3 (*C. Sativa* 50mg/kg + CCl_4) is (0.800±0.122) while in group 4(*C. Sativa* 100mg/kg + CCl_4) is (1.398±0.230). Data also predicted that Creatinine has significant statistically ($P = 0.000<0.05$).

Table 4: Comparison of bio-markers of Liver function tests in different groups of Albino rats model.

Parameters	Normal (n=5) Mean ±S.D	CCl_4 (n=5) Mean ±S.D	50mg/kg (n=5) Mean ±S.D	100mg/kg (n=5) Mean ±S.D	P-Value (0.05)
ALT (U/L)	24.200±10.109	44.400±12.116	27.200±6.833	83.600±30.286	0.002
AST (U/L)	23.200±5.263	138.00±48.00	88.800±32.06	141.200±79.490	0.06
T.B (mg/dL)	0.760±0.054	0.720±0.083	0.740±0.054	0.640±0.114	0.000

Data presented in table 4 shows the clear image of parameters of Liver function tests level evaluated in different groups of albino rat's serum ALT level in control group is (24.200±10.109) while in CCl_4 induced group is (44.400±12.116) Results clearly shows that the ALT level in group 3 (*C. Sativa* 50mg/kg + CCl_4) is (27.200±6.833) while in group 4 (*C. Sativa* 100mg/kg + CCl_4) is (83.600±30.286). Data also predicted that ALT has significant statistically ($P = 0.000<0.05$).
AST level in control group is (23.200±5.236) while in CCl_4 induced group is (138.00±48.00) Results clearly shows that the AST level in group 3 (*C. Sativa* 50mg/kg + CCl_4) is (88.800±32.026) while in group 4 (*C. Sativa* 100mg/kg + CCl_4) is (141.200±79.490). Data also predicted that ALT has significant statistically ($P = 0.006<0.05$).
Total Bilirubin level in control group is (0.760±0.054) while in CCl_4 induced group is (0.720±0.083) Results clearly shows that the Total Bilirubin level in group 3 (*C. Sativa* 50mg/kg + CCl_4) is (0.740±0.054) while in group 4 (*C. Sativa* 100mg/kg + CCl_4) is (0.640±0.114). Data also predicted that Total Bilirubin has significant statistically ($P = 0.000<0.05$).

Table 5: Comparison of Lipid profiles in different groups of Albino rats model.

Parameters	Normal (n=5) Mean ±S.D	CCl_4 (n=5) Mean ±S.D	50mg/kg (n=5) Mean ±S.D	100mg/kg (n=5) Mean ±S.D	P-Value (0.05)
Total Cholesterol (mg/dL)	195.200±1752	160.40±11.01	186.200±1623	176.00±24.02	0.000
Triglycerides (mg/dL)	196.60±12.16	163.00±35.07	182.80±17.23	176.40±8.080	0.000

Data presented in table 5 shows the clear image of parameters of Lipid profiles level evaluated in different groups of albino rat's serum Total Cholesterol level in control group is (195.200±17.852) while in CCl_4 induced group is (160.40±11.01) Results clearly shows that the Total Cholesterol level in group 3 (*C. Sativa* 50mg/kg + CCl_4) is (186.200±16.223) while in group 4 (*C. Sativa* 100mg/kg +

CCl_4) is (176.00±24.062). Data also predicted that Total Cholesterol has significant statistically (P = 0.000<0.05).
Triglycerides level in control group is (196.60±12.116) while in CCl_4 induced group is (163.00±35.007) Results shows that the Triglycerides level in group 3 (*C. Sativa* 50mg/kg + CCl_4) is (182.80±17.23) while in group 4 (*C. Sativa* 100mg/kg + CCl_4) is (176.00±8.080). Data also predicted that Triglycerides has significant statistically (P = 0.000<0.05).

Table 6: Comparison between bio-markers of Serum Electrolytes in different groups of Albino rats model.

Parameters	Normal (n=5) Mean ±S.D	CCl_4 (n=5) Mean ±S.D	50mg/Kg (n=5) Mean ±S.D	100mg/kg (n=5) Mean ±S.D	P-Value (0.05)
Sodium (mmol/L)	114.0±12.083	118.2±14.923	104.2±15.77	110.8±10.52	0.000
Potassium (mmol/L)	3.84±0.541	3.40±0.316	3.76±0.482	4.0±0.353	0.000
Chloride (mmol/L)	93.80±8.04	97.2±2.94	93.40±10.23	98.8±6.340	0.000

Data presented in table 6 shows the clear image of serum electrolytes bio-markers evaluated in different groups of albino rat's serum Sodium level in control group is (114.0±12.083) while in CCl_4 induced group is (118.2±14.923) Results clearly shows that the Sodium level in group 3 (*C. Sativa* 50mg/kg + CCl_4) is (104.2±15.77) while in group 4 (*C. Sativa* 100mg/kg + CCl_4) is (110.8±10.52). Data also predicted that Sodium has significant statistically (P = 0.000<0.05).
Potassium level in control group is (3.84±0.541) while in CCl_4 induced group is (3.40±0.316) Results clearly shows that the Triglycerides level in group 3 (*C. Sativa* 50mg/kg + CCl_4) is (3.76±0.482) while in group 4 (*C. Sativa* 100mg/kg + CCl_4) is (4.0±0.353). Data also predicted that Potassium has significant statistically (P = 0.000<0.05).
Chloride level in control group is (93.80±8.04) while in CCl_4 induced group is (97.2±2.94) Results shows that the Triglycerides level in group 3 (*C. Sativa* 50mg/kg + CCl_4) is (93.40±10.23) while in group 4 (*C. Sativa* 100mg/kg + CCl_4) is (98.8±6.340). Data also predicted that Chloride has significant statistically (P = 0.000<0.05).

Histopathological Findings
GROUP 1 (LIVER) CONTROL/HEALTHY
Gross Description
Received liver, measuring 6.2x4.9x1.3cm. Light brown in appearance. Representative sections are submitted in a single block. Histologic examination of the sections shows a liver tissue with intact architecture. The portal area shows no inflammatory infiltrate. No altered architecture is seen. No fatty involution is seen. No sign of fibrosis is seen (Fig.2).

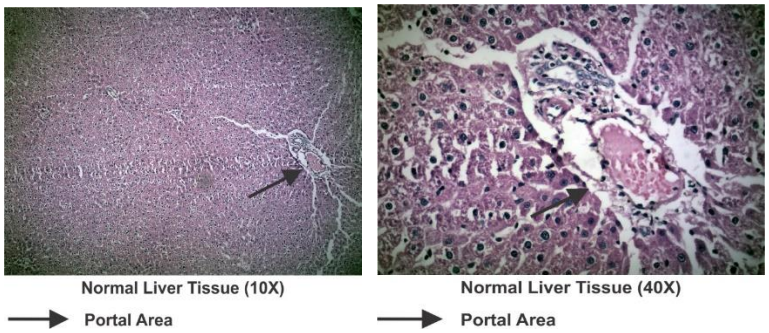

Figure 2: Histopathological examination of Normal Liver of Albino rats at 40X.

GROUP 2 (LIVER) CCL_4 TREATED

Gross Description

Received liver, measuring 5.6x4.9x2.2cm. Light brown in appearance. Representative sections are submitted in a single block. Histologic examination of the sections shows a liver tissue with altered architecture. The portal area shows moderate chronic inflammatory infiltrate. No fatty involution is seen. No sign of fibrosis is seen (Fig. 3).

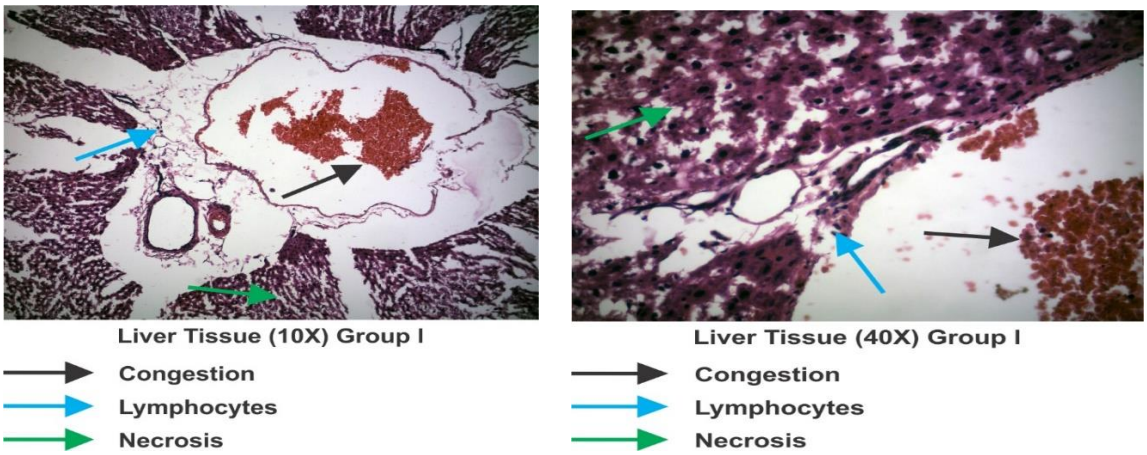

Figure 3: Histopathological examination of Liver treated with CCl_4 of Albino rats at 40X.

GROUP 3 (LIVER) 50mg/Kg *C. SATIVA* + CCl_4

Gross Description

Received liver, measuring 5.2x5.0x1.8cm. Light brown in appearance. Representative sections are submitted in a single block. Histologic examination of the sections shows a liver tissue with intact architecture. The portal area shows mild chronic inflammatory infiltrate. No fatty involution is seen. No sign of fibrosis is seen (Fig. 4).

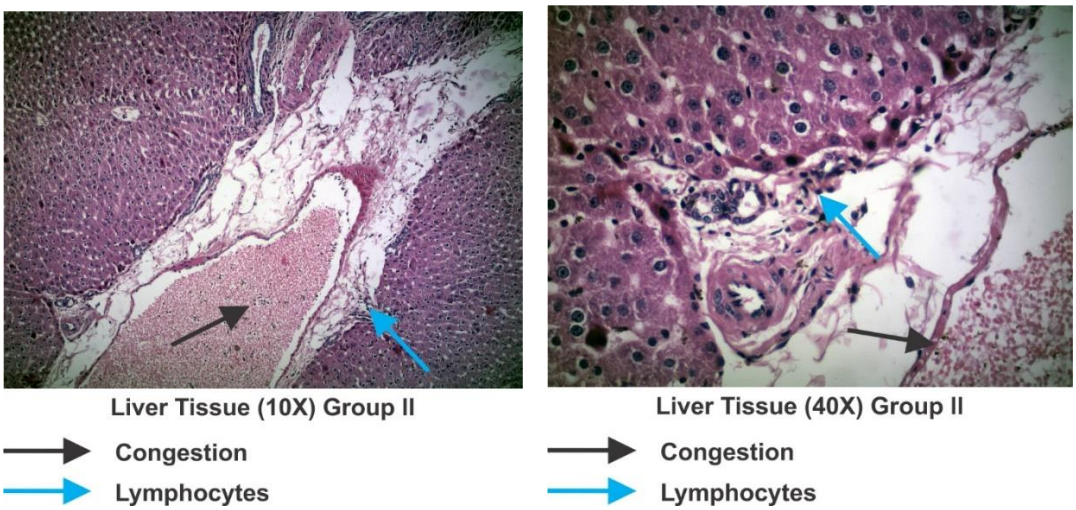

Figure 4: Histopathological examination of Liver treated with 50mg/kg *C. Sativa* + CCl_4 of Albino rats at 40X.

GROUP 4 (LIVER) 100mg/Kg *C. SATIVA* + CCl_4

Gross Description

Received liver, measuring 5.9x5.2x1.3cm. Light brown in appearance. Representative sections are submitted in a single block. Histologic examination of the sections shows a liver tissue with altered architecture. The portal area shows mild chronic inflammatory infiltrate. The architecture is altered. No fatty involution is seen. No sign of fibrosis is seen (Fig. 5).

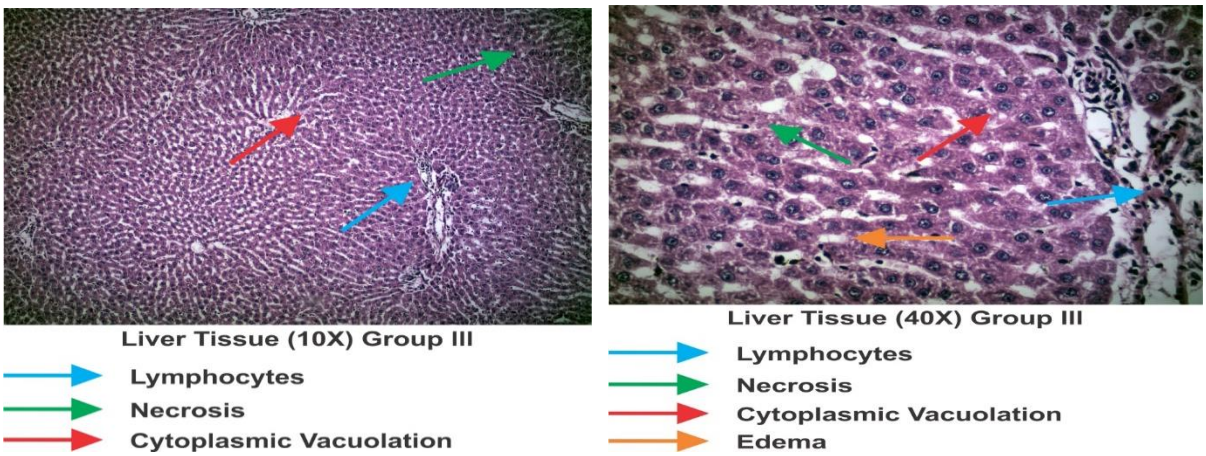

Figure 5: Histopathological examination of Liver treated with 100mg/kg *C. Sativa* + CCl_4 of Albino rats at 40X.

GROUP 1 (HEART) CONTROL/HEALTHY

Gross and Microscopic Description

Received heart, measuring 0.7cm. Light brown in appearance. Two sections are submitted in a single block. Histologic examination of the sections shows heart with intact architecture. No muscular atrophy or distortion is seen. No vascular congestion is seen. No inflammatory infiltrate is seen (Fig. 6).

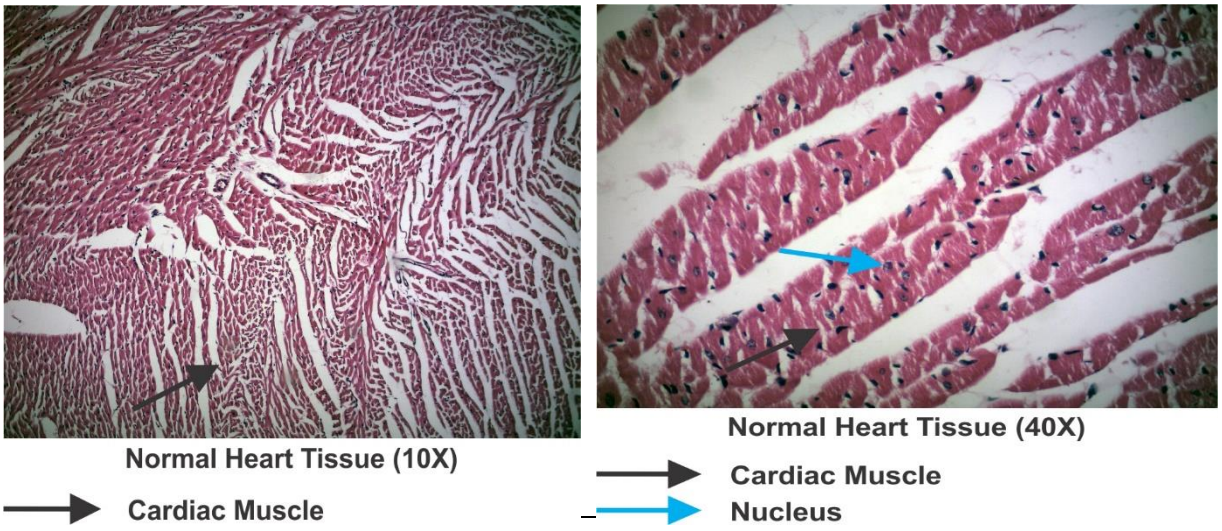

Figure 6: Histopathological examination of Normal Heart of Albino rats at 40X.

GROUP 2 (HEART) CCl_4

Gross and Microscopic Description

Receive heart, measuring 0.7cm. Two sections are submitted in a single block. Histologic examination of the sections shows heart with intact architecture. Light brown in appearance. No muscular atrophy or distortion is seen. Mild vascular congestion is seen. No inflammatory infiltrate is seen (Fig. 7).

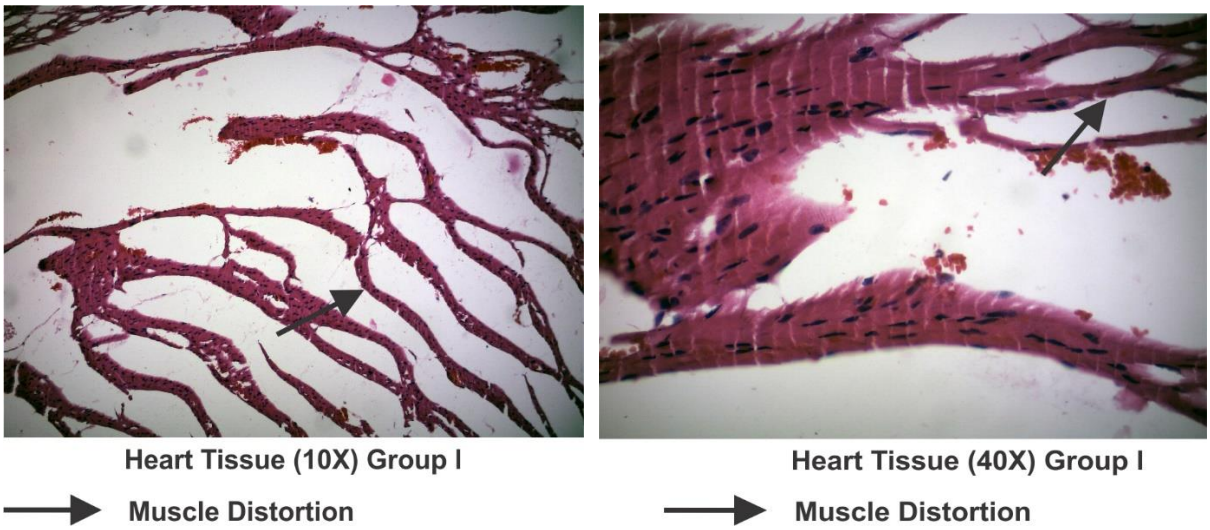

Figure 7: Histopathological examination of Heart treated with CCl_4 of Albino rats at 40X.

Group 3 (Heart) 50mg/kg *C. Sativa* + CCl_4

GROSS DESCRIPTION

Receive heart, measuring 0.7cm. Two sections are submitted in a single block. Histologic examination of the sections shows heart with intact architecture. Light brown in appearance. No muscular atrophy or distortion is seen. Mild vascular congestion is seen. No inflammatory infiltrate is seen (Fig. 8).

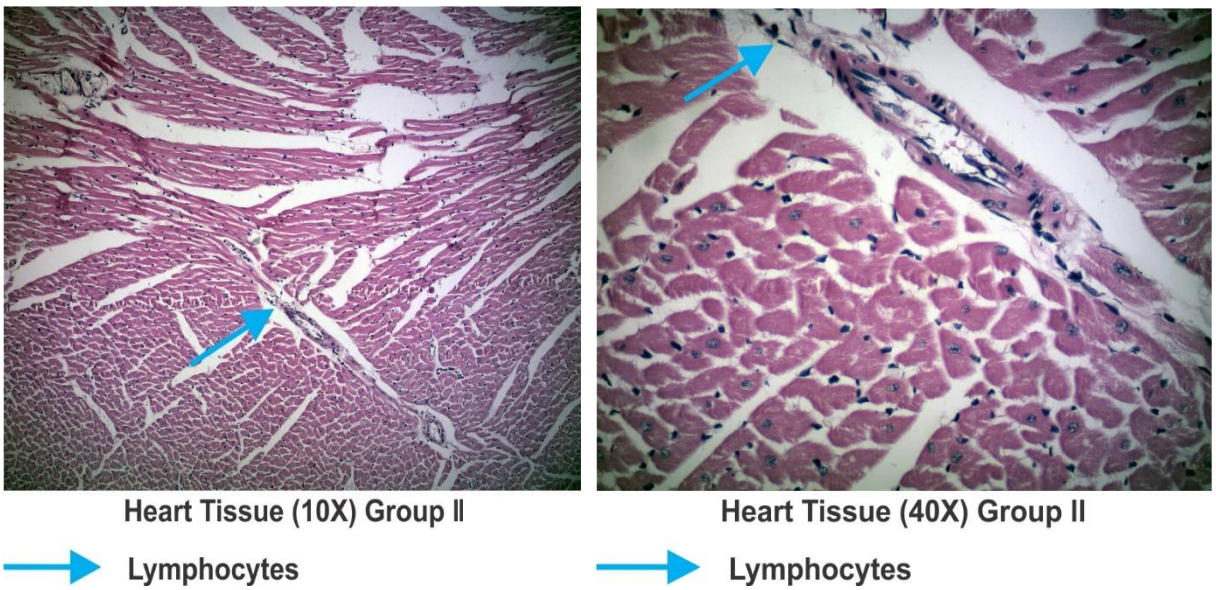

Figure 8: Histopathological examination of Heart treated with 50mg/kg *C. Sativa* +CCl_4 of Albino rats at 40X.

Group 4 (Heart) 100mg/kg *C. SATIVA* + CCl_4

GROSS DESCRIPTION

Receive heart, measuring 0.7cm. Two sections are submitted in a single block. Histologic examination of the sections shows heart with altered architecture. Light brown in appearance. Mild muscular atrophy or distortion is seen. Mild vascular congestion is seen. No inflammatory infiltrate is seen (Fig. 9).

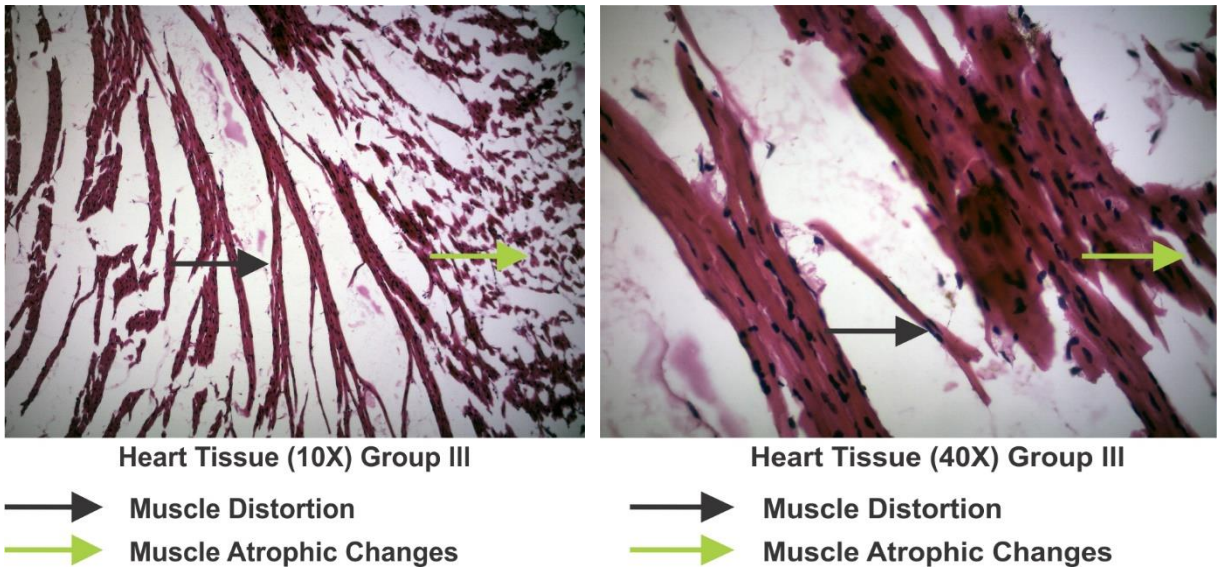

Figure 9: Histopathological examination of Heart treated with 100mg/kg *C. Sativa* + CCl_4 of Albino rats at 10X.

HEART					LIVER				
Parameters	Control	CCl_4	50mg/kg + CCl_4	100mg/kg + CCl_4	Parameters	Control	CCl_4	50mg/kg + CCl_4	100mg/kg + CCl_4
Architecture	Intact	Intact	Intact	Intact	Periportal Necro inflammatory changes	0	+	0	+
Edema	No	No	Yes	Yes	Bridging and Confluent Necrosis:	0	0	0	0
Vascular congestion	No	Yes	No	Yes	Focal spotty lobular necrosis & Hepatocellular Apoptosis:	0	0	0	0
Muscular distortion	No	Mild (+)	Not Seen	Seen	Portal Inflammation:	0	+	+	+
Muscular thickness	Within Normal Limits	Minimize	Normal	Normal	Fibrosis:	0	0	0	0
Inflammation	Not Seen	Not Seen	Not Seen	Not Seen	Edema	0	+	+	+
Mild--- (+), Moderate--- (++), Severe--- (+++) None… (0)					Congestion:	+	+	0	0

Discussion

Hepato-Toxicity induced by CCl_4 caused liver injury in a variety of laboratory animals is considered analogous to liver damage caused by various hepato-toxins in humans (Mohi-Ud-Din *et al.* 2019). Outcome of hepatic injury, the distorted permeability of the membrane allows enzymes from the cells to be set to release into the bloodstream, causing hepatic cell damage, as evidenced by the abnormally elevated serum level hepato specific enzymes (Rai *et al.* 2016). Oxidative stress triggered by an increase in free radicals or decline in the antioxidant defense (Ritesh *et al.* 2015). The hepato-toxicity of CCl_4 depends on its metabolism in hepatocytes by Cytochromes P450 2EI (CYP2EI) which generates highly reactive trichloromethyl (CCl_3^-) free radicals, and reactive oxygen species (ROS) leading to lipid per-oxidation and membrane damage (Fernández-Rodriguez *et al.* 2004). These free radicals not only targets liver but it can also cause free radical generation in other tissues like kidneys, heart, lungs testis brain and blood (Unsa *et al.* 2020). CCl_4 induced fibrosis shares several characteristics within human fibrosis of different etiologies (Zhang *et al.* 2016). The most valuable pathway of liver injury progression is cells proliferation although in previous years many clinical trials have tested the intended results of agents that selectively target important routes involved in control of this process (Nada *et al.* 2017). The possible anti-fibrotic and

antioxidant effects of low or high doses of Cannabis sativa, a new ATI receptor antagonist, in a chronic model of liver fibrosis experimentally induced by CCl_4 (Saber *et al.* 2019). Liver has inter-convertible effects on numerous functions of many organs in body. It is suffering to xenobiotics induced injury due to its central role in xenobiotics metabolism and its portal location within the system (Hinton *et al.* 2017). Hepato-Toxicity induced by CCl_4 is the most commonly used model system for screening hepato-protective activity of C. *Sativa* extracts/ drugs. Administering CCl_4 to rats markedly increases serum AST, ALT, ALP, and LDH levels, reflecting the severity of liver injury (Idoh *et al.* 2018). Kupffer cells (KCs) are activated by free radicals and produce pro-inflammatory mediators, resulting in the triggering of inflammatory cascade (Bleibel *et al.* 2007). In addition, increased nitric oxide production in the liver tissue was reported to be involved in the pathogenesis of liver injury. Therefore, an alteration in the biomarkers of liver function indices might be used to monitor the level of injury or damage (Jan *et al.* 2015). The endo-cannabanoids 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 phyto-cannabinoids present in cannabis preparations would have on chemically injured liver (Chourasia *et al.* 2017). CB2 receptors are expressed in cultured hepatic myo-fibroblasts and activated HSCs, and CB2 receptor stimulation resulted in growth inhibition and apoptosis (Dai *et al.* 2017). Endo-cannabinoids are unregulated during liver fibro genesis, and these might act as an anti-fibrogenic agent in the liver by selectively inducing cell death in activated HSCs, but not in hepatocytes (Brunati *et al.*2010). Cannabidiol is the major non-psychoactive cannabinoid component derived from the plant Cannabis sativa it possesses powerful antioxidant and anti-inflammatory activities. However, the exact mechanisms of action of Cannabidiol remain obscure. In contrast to the other cannabinoids, Cannabidiol has a very low affinity for the cannabinoid CB1 and CB2 receptors. The antioxidant, anti-inflammatory and hepatoproytective effects of Cannabidiol may be due to its direct action or mediated through a new abnormal cannabinoid, non-CB1 and non-CB2, receptor (Fouad *et al.* 2013). Cannabidiol may also exert its beneficial effects by inhibiting adenosine uptake and activating transient receptor potential vanillooid-1 (De Almeida and Devi 2020). CBD reduced cocaine lethality and inhibited cocaine-induced seizures and liver injury in mice. A FAAH inhibitor, URB597, suppressed seizures but did not affect the hepatic inflammatory process. We also discovered that APAP-induced hepatic disorder enhanced cocaine-induced seizures and toxicities. (Vilela *et al.* 2015). Cannabis sativa ("marijuana," "hemp") has been known for centuries for its abuse-related effects and potential medical interventions, such as the treatment of inflammatory diseases, diabetes, cancer, affective or neurodegenerative diseases, and epilepsy-related dysfunction. more than 60 chemical compounds identified in this herb, the two most studied are Δ^9-tetrahydrocannabinol (Δ^9-THC) and Cannabidiol (CBD) (Prospéro-García *et al.* 2019). Δ^9-THC, which accounts for the majority of classic cannabis actions, may have therapeutic effects against a variety of diseases, though its use is restricted due to its psychoactive effects as a result, focusing on cannabis-based therapies could be an option on CBD, the main non-psychotomimetic phyto-cannabinoid (Hussein *et al.* 2014). This chemical acts on a variety of therapeutical objectives, such as the assistance of the endo-cannabinoid structure, initiation of transient receptor potential vaniloid type- 1 (TRPV1) network, the peroxisome proliferators-activated receptor ɣ (PPARɣ), GPR55, 5-hydroxy-tryptamine receptor subclass 1A (5-HT_{1A}), the adenosine membrane carrier phospholipase A_2, lip-oxygenase (LOX) and cyclo-oxygenase- 2 (COX-2) enzymes, and (Ca^{2+} homeostasis) (Izzo *et al.* 2009). The enrich pharmacology, associated with the evidence that it does not share the psychotomimetic, amnesic, and sedative impacts of Δ^9-THC, has a wide variety of potential therapeutic uses, such as the cure of epilepsies and similar symptoms. (Pertwee 2005, and Zuardi 2008). As a result, a recent clinical study found that CBD-enriched cannabis extracts greatly lower seizure rates in children with treatment-resistant epilepsy, implying that it could be used as a backup strategy if other pharmacological treatments flop. (Porter and Jacoboson 2013).

(Geetha *et al.* 2008) Their studies explained that the elevation in the levels of final products of lipid per-oxidation in the liver of rats treated with CCl_4 was observed. The rise in MDA levels in liver suggested improved lipid peroxidation leading to tissue destruction and failure of antioxidant defense system to prevent development of excess free radicals. Glutathione content in the liver plays a primary role in protection against tri-chloromethyl radical induced liver damage It has been suggested that the lipid peroxides generated after CCl_4 intoxication are eliminated by GPx in the presence of glutathione, thus curbing the propagation of lipid peroxidation. The present study found a significant decrease in hepatic glutathione level, GPx, SOD, ALT and AST activity following CCl_4 exposure. This observation confirms earlier results. Administration of C. *Sativa* extract significantly decreased MDA levels and also maintained higher level of antioxidants such as GSH, CAT, NO, SOD, ALT and AST. These results suggest that the Biochemical, hepatoprotective and anti-inflammatory action of C. *Sativa* might be due to the presence of various antioxidant substances.

Conclusion

Carbon tetra chloride causes hepato-toxicity in human beings. We use halogenated hydrocarbons in our daily routine. CCl_4 elevate the serum NO level and MDA level and initiate cell proliferation, which decrease the GSH level of the body and trigger the free radical's production, increasing the lipid per-oxidation and finally destroying the hepatocytes.

C. Sativa is a dieous plant and is prominent about its anti-inflammatory, bio-chemical and hepato-protective activity. The present study concluded that ethanolic extract of *C. Sativa* maintain the liver enzymes in their normal working state, inhibiting the production of free radicals. *C. Sativa* 50mg/kg + CCl_4 dose is very reasonable, beneficial dose significantly control the lipid per-oxidation, increase the antioxidant activity and to cure the hepato-toxicity caused by CCl_4. Hepatic damage is an alarming situation for human being's exposure to high concentrations of carbon tetra chloride can affect the central nervous system and degenerate the liver, heart and kidneys. Prolonged exposure can be fatal.

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