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Protective and therapeutic effects of Cannabidiol oil on Hepatocellular carcinoma (HCC) induced by dimethylnitrosamine (DENA) in male rats

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ABSTRACT **: Background: Hepatocellular carcinoma is the third most common cancer to be fatal (HCC). Binding to the transmembrane receptor Patched 1 (PTCH1) depresses the transmembrane G protein-coupled receptor Smoothened (SMO), hence initiating the hedgehog (HH) signalling pathway. This investigation looked at the potential therapeutic and preventive benefits of cannabidiol oil in adult rats that had been subjected to diethyl nitrosamine (DENA)-induced HCC.**

Materials and methodology: Of the fifty rats, five groups of ten male rats each were formed. Group I: The group for references. Group II got DENA IP injections for a duration of 12 weeks. For a duration of 12 weeks, rats in Group III received DENA injections in addition to oral CBD provided every other day at a rate of 0.5 ml/kg body weight. Group IV rats were administered oral CBD every other day for two weeks prior to getting a DENA injection for a duration of 12 weeks. After their prior DENA injection, rats in Group V received oral CBD every other day for two weeks. An examination of the histology of liver tissue was performed. In conclusion, treatment of CBD resulted in a considerable improvement in the hepatopathological liver tissues of adult male rats with DENA-induced HCC.

KEYWORDS: Hepatocellular carcinoma, cannabidiol, dimethylnitrosamine (DENA), Abbreviations:CBD: cannabidiol;HCC: Hepatocellular carcinoma; DENA: diethyl nitrosamine; ROS: reactive oxygen species; IP: intraperitoneally; MDA :malondialdehyde;

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I. INTRODUCTION

HCC is a frequently occurring primary liver cancer. It is the sixth most common neoplasm and the third most common cause of cancer-related fatalities. Furthermore, HCC is a leading cause of death for individuals with cirrhosis, and its incidence is expected to increase in the upcoming years (Hsu et al., 2020.. There is a close relationship between the development of HCC and chronic liver failure. In sub-Saharan Africa and Eastern Asia, exposure to aflatoxin B1 and chronic hepatitis B are the main risk factors for HCC patients, accounting for the majority of cases (80%). According to Baatarkhuu et al. (2018), the incidence of HCC increases in hepatitis B patients in relation to the severity of the liver disease, duration of the illness, and viral load.

Inflammation is one of the body's natural responses to infection, tissue injury, and genetic changes. It progresses through two stages: acute and chronic. If the underlying cause of the injury is left untreated, the inflammatory process progresses to chronic inflammation.Immunopathological changes such as inflammatory cell infiltration, pro-inflammatory gene overexpression, deregulation of cellular signalling, and barrier degradation are indicative of chronicity (Bruni et al., 2018)[3]. The most often used genotoxic substances in HCC models in rodents with compensatory proliferation, inflammation, fibrosis, and cirrhosis are N-nitroso compounds, such as diethyl nitrosamine (DENA) (Caviglia and Schwabe, 2015)(4). DENA is activated to reactive alkylating metabolites by hepatic cytochrome P450 enzymes such CYP2E1 complexes. This leads to the formation of

methylation macromolecules like DNA's N7- and O6-methylguanines. is mediated by reactive oxygen species (ROS), which include superoxide anions and hydrogen peroxide, and which are created by CYP2E1 activity (Schulien and Hasselblatt, 2021). As a result, the upregulation of genes involved in genotoxic stress responses and cytoprotective genes occurs. This causes hepatic damage and the release of ATP, which in turn stimulates hepatocellular purinergic receptors. Consequently, the damage to the DNA gets worse, which leads to mutations. Furthermore, the proliferation of hepatocytes promotes the transmission of mutations and increases the susceptibility of the liver to HCC (Schulien et al., 2020).

Current therapeutic approaches, including endocrine therapy, targeted therapy, radiation, and chemotherapy, have decreased cancer-specific mortality. However, these treatments have severely failed because to drug resistance developing, recurrence, distant metastases, and mortality (Tajbakhsh et al., 2017).

The plant known as cannabis (Cannabis sativa) is a member of the Cannabaceae family (Urticales, Magnoliaopsida). Since 4000 B.C., the medicinal and hallucinogenic benefits of cannabis have been understood. Every type of C. sativa plant produces active molecules, but the quantity and proportions of these substances differ and are impacted by growth conditions, climate, and genetic background, among other environmental factors (Bruni et al., 2018) .

The purpose of the current investigation was to investigate if adult rats with DENA-induced HCC could be treated or prevented with cannabidiol (CBD).

2.Material and methods

Preparation of CBD

The source of cannabidiol oil (CBD) was Zova Co. of San Diego Pharmacy in California, USA. After mixing with 1000 millilitres of distilled water, 20 grammes of CBD and 20 grammes of acacia gum were added to 100 millilitres of warm distilled water. Each millilitre of the solution includes 10 milligrammes of the aforementioned ingredients.

Experimental design

In the experiment, fifty adult male Wistar strain (Rattus norvegicus) albino rats weighed 150 ± 25 grammes. They were housed in plastic cages in the animal house with temperature control (12 hours of light and 12 hours of darkness).

Five groups of ten rats each were used to randomly distribute the experimental animals. Rats in Group I were healthy control rats. Throughout a 12-week period, the rats in group II received intraperitoneal (IP) injections of DENA (10 g/kg body weight) once a week. For a duration of 12 weeks, the rats in group III were administered an IP injection of DENA every other day in addition to an oral cannabinoid oil treatment (0.5 ml/rat body weight). Before receiving a 12-week IP injection of DENA, Group IV rats received an oral pretreatment of cannabidiol oil (0.5 ml/rat body weight) every other day for two weeks.After receiving IP DENA injections for 12 weeks, the rats in Group V were given oral cannabidiol oil (0.5 mL/kg rat body weight) every other day for a period of two weeks. Atthe end of the experimental period (14 weeks later) under Na thiopental anesthesia(40 -60 mg/kg IP . The liver tissues were gathered and was stored in formalin for histopathological examination.

Liver tissue histopathological examination

The liver tissues were fixed in 10% formalin before being stained with hematoxylin and eosin (H&E) (Bancroft and Gamble, 2008).

Statistical analysis

Version 19 of the Statistical Product and Service Solutions (SPSS) programme (IBM, Incorporation, USA) was used to analyse the collected data. The ANOVA test, which is a one-way analysis of variance, was employed to assess group differences.A P-value of less than 0.05 was deemed statistically significant..

3.Results

Histopathological findings:

Group I preserved the hepatic architecture (control group). In group II (which got DENA), the moderately differentiated HCC image was accompanied by thicker hepatic trabeculae composed of pleomorphic hepatocytes with hyperchromatic nuclei and increased mitosis. Well-differentiated HCC with numerous apoptotic entities with pyknotic fragmented nuclei was seen in Group III (CBD plus DENA). Well-differentiated HCC with necrosis and a few pyknotic apoptotic bodies was seen in Group IV (CBD used prior to DENA). There were a significant number of apoptotic bodies with fragmented nuclei and perinuclear vacuoles in I ngroup V (CBD after DENA), a moderately differentiated HCC image (Figure 1).

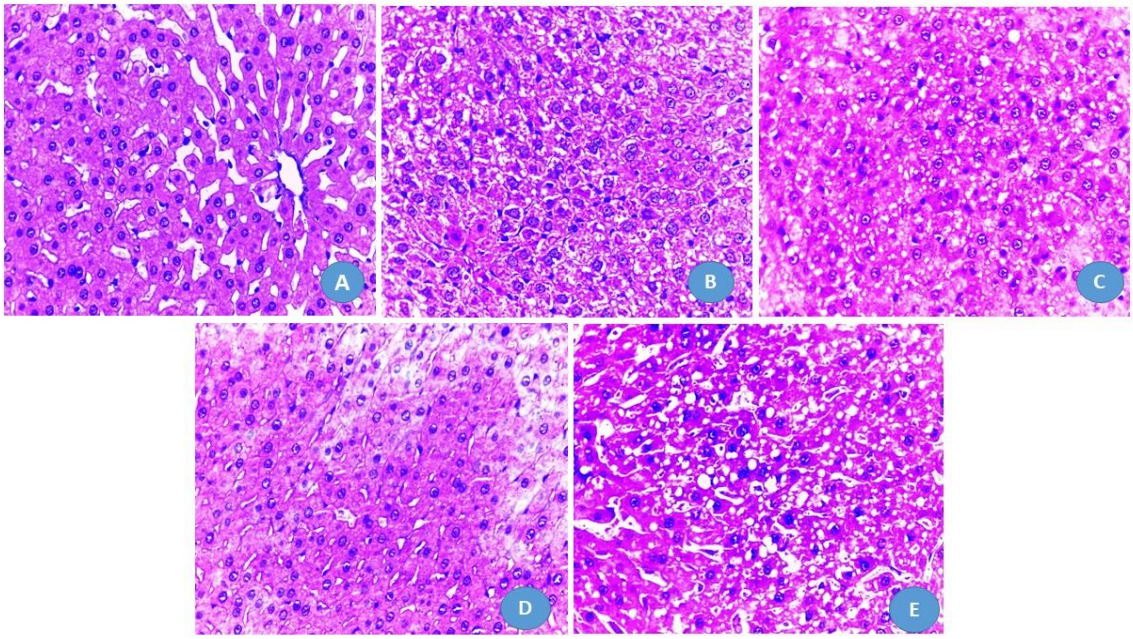

Fig.1:A: Group I (control group) displays no changes to the hepatic architecture; B: Group II (receiving DENA) displays moderately differentiated HCC with increased hepatic trabeculae thickness, which is made up of pleomorphic hepatocytes with hyperchromatic nuclei and increased mitosis; C: Group III (CBD prior to DENA) displays well-differentiated HCC with necrosis and some pyknotic apoptotic bodies (H&E X400); Group D (CBD + DENA): this group exhibits well-differentiated HCC with a lot of apoptotic bodies with pyknotic fragmented nuclei and necrosis (H&E X400); Group V (CBD after DENA): this group exhibits moderately differentiated HCC with a lot of apoptotic bodies with fragmented nuclei and perinuclear vacuoles (H&E X400) and variable areas of coagulative necrosis.

4.Discussion

Humans are susceptible to teratogenic, carcinogenic, and mutagenic effects from N-nitroso compounds, such as DENA. The main organs where DENA causes tumours include the kidney, respiratory system, upper digestive tract, liver, and haematological system. When heated to the point of disintegration, it releases toxic vapours of nitrogen oxide.It is present in industrial pollutants and is used as a stabiliser, antioxidant, and lubrication addition in plastic. According to Aldawsari et al. (2021), it is present in food, beverages, tobacco smoke, drinking water, and industrial pollution.

Type 1 (CB1) and type 2 (CB2) of the seven transmembrane G-protein-coupled receptors are members of the cannabinoid receptor family. Cannabinoid receptors, their endogenous ligands (endocannabinoids), and the enzymes that regulate their production and deactivation make up the endocannabinoid system, a key endogenous lipid signalling route. The endocannabinoid signalling system is essential for several vital physiological functions that involve the immune system, endocrine system, and central and peripheral neurological systems (Billakota et al., 2019). Furthermore, because of its analgesic, neuroprotective, anti-inflammatory, and antibacterial properties, phytocannabinoid pharmacological modulation is a newly developed therapeutic strategy (Pisanti et al., 2017) [. Persistent inflammation has a substantial impact on the development of cancer. Reducing inflammatory mediators with anti-inflammatory medications—both steroidal and non-steroidal—is one of the primary treatments for inflammatory diseases. However, there are a number of typical side effects that are usually associated with utilising synthetic anti-inflammatory medications (Pountos et al., 2011).. Preclinical and clinical data suggest that endocannabinoid agonists that target CB2 receptors may have significant anti-inflammatory benefits (Bruni et al., 2018). They are also used to maintain the homeostasis of the gut and treat inflammatory bowel conditions like Crohn's disease and ulcerative colitis (Schoch et al., 2018) [14[. Additionally, McAllister et al. (2021) reported that cannabidiol reduced the viability and growth of cancer cells.

5.Conclusion

All of these findings suggest that CBD may be useful as a drug for treating hepatocellular carcinoma.

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