1. Introduction

Cancer is a group of illnesses that may occur in many organs or tissues in the body. It is characterized by the uncontrolled growth of malignant cells, which can harm nearby tissues or spread to other parts of the body. Common malignancies in men include lung, prostate, colorectal, stomach, and liver cancer. On the other hand, breast, colorectal, lung, cervical, and thyroid cancer are the most often seen in females. The World Health Organization (WHO) predicts that there will be 20 million new cases of cancer and 9.7 million deaths from the illness in 2022. The five-year survival after diagnosed with cancer was found to be 53.5 million. One in five individuals will have cancer at some point in their lives; one in nine men and one in twelve women will pass away from this deadly disease [1].

1.1. Hepatocellular carcinoma (HCC)

HCC, a very lethal disease, accounts for a global mortality rate of 5.3 million individuals. In terms of frequency, HCC is the 5th most common kind of cancer in males and the seventh most common in women. Moreover, it is the 3rd most common cause of cancer-related death worldwide [2, 3]. According to the WHO, it is estimated that about 900,000 persons worldwide are diagnosed with HCC annually, which is recognized as the most prevalent kind of liver cancer. In general, it has been shown that 69.8% of HCC cases manifest in men, exhibiting a male-to-female ratio of 2.66. The occurrence of HCC exhibits significant regional disparities. Based on data from the Global Cancer Observatory (GCO), a division of the International Agency for Research on Cancer (IARC), it has been shown that 72.5% of newly diagnosed cases of HCC are concentrated in the Asian region. Furthermore, the standardized incidence rates in this region reach a high of 11.6 cases per 100,000 individuals per year. In the African region, HCC ranks as the fourth most prevalent malignancy, with an annual standardized incidence rate of 8.8 new cases per 100,000 inhabitants. The occurrence of HCC is comparatively lower in Oceania, Northern America, and Europe as compared to Asia or Africa. Among these regions, Europe has the lowest known incidence rate of HCC, with 5.2 new cases per 100,000 inhabitants per year. In Europe, HCC ranks thirteenth among all incident cancer types. In terms of mortality, HCC ranks as the third most prevalent cause of cancer-related deaths globally, resulting in the loss of over 830,000 lives annually. The majority of these fatalities (69.6%) transpire among the male population, exhibiting a pinnacle in mortality rates at 12.9 deaths per 100,000 individuals annually. In Africa, HCC is the third leading cause of oncological deaths. Conversely, HCC is less prevalent in Oceania, Northern

America, and Europe, where it ranks seventeenth among causes of oncological deaths. In these regions, the mortality rate for HCC is 2.4 times lower than the rate observed in Asia [4].

HCC, also known as hepatoma, is widely recognized as the primary malignant tumour affecting the liver. The liver is composed of several cellular components, including hepatocytes (the predominant cell type, accounting for 80% of the liver's biological composition), blood cells, biliary cells, Ito cells, Kupffer cells, perisinusoidal cells, and others [5]. The majority of primary liver cancer, accounting for over 90% of cases, arises from hepatocytes and is often known as HCC. During the process of hepatocarcinogenesis, initial exposure to carcinogens leads to the activation of cells derived from normal liver parenchyma or hepatocytes via genetic alterations, often involving interactions with DNA [6]. The prolonged exposure of hepatocytes to tumour promoters or carcinogens, such as phenobarbital, results in the subsequent development of tumours and the formation of hepatic altered foci. These foci represent clonally chosen expansions of started cell populations. The presence of carcinogenic substances leads to further genetic modifications, which, when aggregated to a significant extent, result in the formation of hyperplastic nodules that ultimately progress into HCC [7, 8].

1.2. Nanotechnology

Nanotechnology has been widely investigated and used in cancer therapy because nanoparticles may play an important role as a drug delivery carrier. Nanoparticle-based drug delivery provides many benefits over traditional pharmaceuticals, including greater stability and biocompatibility, enhanced permeability and retention (EPR) effect, and precision targeting [9].

1.3. Polymeric nanoparticles

Polymeric nanocarriers are well-known for their appealing properties, which include tiny size, stability during storage, extended shelf life, biodegradability, and nontoxicity. Biodegradable and biocompatible polymers, or copolymers, are the building blocks of polymeric nanoparticles. Polymeric nanocarriers are solid colloidal particles with a size range of 10–1000 nm. The drug can be encapsulated within the carrier, adsorbed physically on the surface, or chemically bound to the surface.

2. Literature review

It has been shown that different writers have varying views about the use of poly (lactic-co-glycolic acid) (PLGA) nanotechnology in drug delivery. This difference in opinion emphasizes the

intricacy and continuous discussion about the usefulness and uses of PLGA-based systems in the field. Drug delivery systems have been developed to target several biological functions via various methods in response to these differing views. These techniques often include minimizing adverse effects, improving treatment effectiveness, and focusing on certain biological processes. For example, medication delivery systems that use PLGA nanotechnology may seek to increase cellular absorption, enable controlled release, or improve drug stability. These systems aim to provide more individualized therapeutic solutions and maximize treatment results by targeting various biological targets which are the following.

- **Kumar *et al.*, (2020)** reported that PLGA-loaded nanoparticles containing betulinic acid nanoparticles may be an alternative to HCC. The molecular processes underlying the pharmacological reactions of PLGA nanoparticles to HCC were downstream modulation of Bcl-2, Bcl-xl, and upstream control of BAX proteins, i.e. activation of caspase-mediated mitochondrial death [10].
- **Nisha *et al.*, (2020)** described the creation of lactoferrin-modified PEGylated liquid crystalline nanoparticles loaded with imatinib mesylate, which inhibited tumor growth and cell proliferation, especially in cases of hepatocellular carcinoma. This occurred as a result of the inhibition of genes such as BAX, Cyt-C, BAD, e-NOS, and caspase-3 and 9, which subsequently resulted in the activation of Bcl-xl, iNOS, and Bcl-2 genes [52].
- **Mahdizade *et al.*, (2019)** indicated that dalbergin has some anticancer effects probably through inducing apoptosis in cancerous cells by changing mRNA levels of apoptosis-related proteins. Moreover, DL may cause apoptosis via p53, Bcl-2, and STAT3, polyphenols can do this by suppressing the NF-kB pathway [65].

3. Research Envisaged

Dalbergin (DL) is naturally occurring neoflavonoid with remarkable anticancer activity in hepatic cancer; however, its less bioavailability makes itself towards poor therapeutic efficacy. Recent investigations suggested that the bioavailability may be increased through the preparation of nanoparticles using PLGA. Further, grafting the nanoparticles with galactose results in targeted drug delivery towards HCC. Therefore, we aimed to prepare dalbergin-loaded PLGA nanoformulations modified with galactose [DLMF] and characterized with various analytical parameters. We explored the mechanism of anticancer potentials of those compounds in both

cellular and molecular levels. We found that the DLMF may be a better alternative for the hepatic carcinoma therapy.

4. Plan of Work

(a) Synthesis of dalbergin and its characterizations

- ✓ ^{1}H-NMR study
- ✓ ^{13}C-NMR study
- ✓ Mass spectrometry analysis
- ✓ FTIR analysis

(b) Preparation of DLF, and DLMF

(c) Characterizations of DLF, and DLMF

- ✓ Particle size analysis
- ✓ Polydispersity index (PDI)
- ✓ Zeta potential
- ✓ Entrapment efficiency and drug loading
- ✓ Drug interaction analysis by FTIR
- ✓ Shape and surface morphology using (SEM/TEM)

(d) *In vitro* cell culture experiments

- ✓ *In vitro* anti-cancer study on Hep-G2 cell lines using sulforhodamine B (SRB) assay.
- ✓ Evaluation of cellular internalization using confocal lasser scanning microscopy (CLMS)

(e) *In vivo* pharmacokinetic study

(f) *In vivo* anticancer evaluation

(g) Body weight analysis of experimental animals

(h) Biochemical investigation of blood samples

- ✓ Aspartate aminotransferase (AST)
- ✓ Alkaline phosphatase (ALP)

- ✓ Alanine aminotransferase (ALT)
- ✓ Lactate dehydrogenase (LDH)

(i) Biochemical studies on tissues

- ✓ Tissue glutathione (reduced)
- ✓ Tissue malondialdehyde / thiobarbaturic acid
- ✓ Tissue SOD (super oxide dismutase)
- ✓ Tissue catalase (CAT)
- ✓ Tissue protein carbonyl (PC)
- ✓ Bilirubin
- ✓ Biliverdin

(j) Lipid profile analysis

- ✓ Total cholesterol (TC)
- ✓ Triglyceride (TG)
- ✓ High density lipoprotein (HDL)
- ✓ Low density lipoprotein (LDL)
- ✓ Very low density lipoprotein (VLDL)

(k) Morphological changes

- ✓ Histopathology
- ✓ Tissue SEM analysis

(l) Molecular mechanistic analysis

- ✓ Enzyme Linked Immunosorbent Assay (ELISA)
- ✓ Quantitative reverse transcription polymerase chain reaction (qRT-PCR)
- ✓ Western blots analysis

(m) ^{1}H-NMR based serum metabolomics

- ✓ Stock plot
- ✓ Orthogonal projection to latent structure with discriminate analysis (OPLSDA)
- ✓ VIP Score
- ✓ Box-cum-whisker plots

(n) Statistical analysis

4. Results

The pharmacoinformatics analysis of DL is performed to predict the physiochemical properties such as, lipophilicity, water solubility, pharmacokinetic, drug-likeness, and medicinal chemistry properties of DL. The results show log P, log S, LD50, and lead likeness violations, appropriate values suggesting that DL is a drug-like molecule and not violate of Lipinski's rule, Ghose, Veber, Egan, and Muegge rule. Toxicity profiles- AMES (Ames Mutagenicity), Maximum tolerated dose (human), the hERG I inhibition, hERG II inhibition, oral rat acute toxicity (LD_{50}), oral chronic toxicity (LOAEL), hepatotoxicity, skin sensitization, *T. pyriformis* toxicity, and Minnow toxicity were predicted that DL is less toxicological. Further, DL showed the highest binding affinity for TNF-α, p53, AKT1, STAT-3, and caspase-7. The findings of this investigation showed that by activating caspases through the TNF-α/p53/AKT-1/STAT-3 pathways, this substance might cause apoptosis or programmed cell death in liver cancer cells. Therefore, we conclude that DL is a potential anticancer option for the treatment of HCC. We synthesized the DL and confirmed the structure via NMR, MS, and FTIR.

A novel formulation of DLMF was effectively prepared, and the formulation was characterized through physiological and morphological parameters. The *in vitro* anti-HCC effects of the DLMF were discovered and confirmed by the cytotoxicity and cellular uptake study against Hep G2 Cells. *In-vivo* oral pharmacokinetics as well as the biodistribution effects of DLMF confirmed the higher liver targeting. The plasma $t_{1/2}$, C_{max}, and T_{max} of DLMF were greater than that of the parent DL. DLMF was shown to have greater and more effective biodistribution in the hepatic tissue among all vital organs. As an outcome, the research may provide confirmation that DLMF improves the prevention of HCC.

We demonstrated the anti-cancer potential of targeted nanoformulation of DL in DEN induced HCC model. The specific targeting of ASGPR by DLMF contributed to the higher therapeutic potential. The normalization of antioxidant markers such as SOD, CAT, and GSH in DLMF treated animals in comparison to the CC group demonstrated the protective effect of the targeted formulation. The restoration of normal lipid profile in DLMF treated animals to normal further supports the protective potential of DLMF. Our investigation established a clear correlation between cellular, biochemical, molecular, and metabolic parameters following DL, DLF, DLMF, and 5-FU therapies. The cellular and molecular findings from our study elucidate DLMF's role in

HCC therapy, indicating its stimulation of Bcl-2 suppression and upregulation of BAX protein, leading to caspase-3 and caspase-9 mediated mitochondrial cell death. Additionally, ^{1}H-NMR-based metabolomics results revealed different metabolic profiles in DL, DLF, DLMF, and 5-FU treatments, highlighting the heightened cellular activity of DLMF in HCC.

5. Conclusion

These results clearly suggest that DLMF may serve as a potential treatment option for HCC therapy. The clinical efficacy of the DLMF may be explored by extensive clinical trials.

6. Reference:

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