Kakkatin derivatives against HCC

by Vikram Patial

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Therapeutic potential of kakkatin derivatives against hepatocellular carcinoma

Chahal S et al. Kakkatin derivatives against HCC

Sahiba Chahal, Vikram Patial

Sahiba Chahal, Vikram Patial, Dietetics & Nutrition Technology Division, CSIR-Institute of Himalayan Bioresource Technology, Palampur 176061, Himachal Pradesh, India

Sahiba Chahal, Vikram Patial, Academy of Scientific and Innovative Research (AcSIR), Headquarters, Ghaziabad 201002, UP, India

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Corresponding author: Vikram Patial, PhD, Principal Scientist, Dietetics & Nutrition Technology Division, CSIR-Institute of Himalayan Bioresource Technology, Palampur, Himachal Pradesh, India, 176061, Palampur 176061, Himachal Pradesh, India. vikrampatial@ihbt.res.in; ORCID ID: https://orcid.org/0000-0002-4912-9871

Abstract

In this editorial, we comment on the work done by Jiang et al. [12], where they synthesized a kakkatin derivative, HK, and investigated its anti-tumor activities and mechanism in gastric cancer MGC803 and Hepatocellular carcinoma (HCC) SMMC-7721 cells. HK is evaluated for its anti-tumor activity as compared to kakkatin and cisplatin. This editorial focuses on various risk factors of HCC, the mechanism of HCC progression and molecular targets of kakkatin derivative, and limitations of available treatment options. HCC is a predominant form of primary liver cancer characterized by the accumulation of multiple gene modifications, over-expression of protooncogenes, altered immune microenvironment, and infiltration by immune cells. Puerariae flos (PF) has been traditionally in practice in China, Korea, and Japan for lung clearing, spleen awakening and relieving alcohol hangovers. PF exert anti-tumor activity by inhibiting cancer cell proliferation, invasion, and migration. PF induces apoptosis in alcoholic hepatocellular cancer via the ESR1-ERK1/2 signalling pathway. Kakkatin isolated from PF is known as a hepatoprotective bioflavonoid. The kakkatin derivative, HK, exhibited anticancer activity against HCC cell lines by inhibiting cell proliferation and upregulating NF- κB1 and PDE3B. However, further preclinical and clinical studies are required to establish its therapeutic potential against HCC.

Key words: Hepatocellular carcinoma; Anti-tumor; Kakkatin; Protooncogenes; Cisplatin

Core Tip: The heterogeneity of tumour cells and their ability to migrate, invade and metastasize distant tissue make diagnosing and developing a cancer treatment difficult. HCC is one of the leading causes of cancer deaths, and

current therapies are either only effective at the early stage or have side effects. Pueraria flos-based treatment of HCC has shown a better potential to treat HCC than the known chemotherapeutic drug Cisplatin[9]. Kakkatin and its derivative HK, derived from PF, were used as treatment[13] against HCC and compared with cisplatin[9].

INTRODUCTION

As the sixth most frequently diagnosed cancer worldwide, hepatocellular carcinoma has[14] the third highest mortality rate among all cancers[1]. It is a complex multistep chronic liver disease defined by the accumulation of multiple genomic and epigenomic changes that lead to loss of growth control, suggesting the role of several parallel pathways driving oncogenesis[2]. HCC constitutes more than 75% of total liver cancers. It is estimated[15] that low- and middle-income nations, especially those in Eastern Asia and sub-Saharan Africa, account for about 85% of instances of HCC[3]. Age, sex, and geography are essential factors in the prevalence and severity of HCC. In Japan, North America, and European countries, the average age of HCC onset is 60 years and above[4]. In most parts of Asian and African countries, the age of onset is between 30 and 60 years[5].

HBV, Hepatitis B virus and HCV, Hepatitis C Virus dominate the aetiology of liver carcinoma worldwide, with 54% of cases attributing to HBV and another 31% to HCV[2]. In addition, excessive abuse of alcohol, major metabolic disorders such as obesity, diabetes, non-alcoholic fatty liver disease (NAFLD)/non-alcoholic steatohepatitis (NASH), and toxins are well-established aetiological factors of HCC[6]. NASH has become an emerging risk factor for HCC. Following the onset of hepatic steatosis, additional factors, including obesity, insulin resistance,

and genetic alterations, function as "second hits" or "multiple parallel hits" at the cellular level, ultimately resulting in hepatocarcinogenesis[7]. Conventional cancer therapies like surgery are effective at early stage only. Moreover, surgical resection increases the risk of metastases by causing cancer cells to disseminate into the bloodstream and suppressing anti-tumour immunity. It also upregulates cell adhesion molecule expression in nearby organs and recruits immune cells that can ensnare cancer cells, inducing alterations in the target tissue and cancer cells and facilitating their migration and invasion to that organ[8]. Radiations can damage healthy neighbour cells, tissues, and organs, and often, cancer cells develop resistance toward chemotherapy, making these treatment options futile[9]. A widely used HCC drug, Sorafenib is only effective in 35%-43% of cases and is known for side effects like diarrhoea and skin reactions[10]. Cancer cell metastasis and invasion of distant organs continue to be the primary causes of cancer-related deaths globally. Developing anti-metastatic therapies is hampered by difficulty in identifying the nature of tumour cells that have spread to and colonised distant sites, so it is crucial for cancer treatment to target the growth and metastasis of cancer cells [11]. For the last few decades, interest in the use of phytomedicine. Plant-based medicines are preferred over chemotherapy drugs since they are believed to have fewer side effects. One such herb, Pueraria flos, a flower-based medicine, is used to treat HCC. It is home to many metabolites and is used against alcoholism, and cures alcoholic liver disease (ALD). Kakkatin is an isoflavonoid procured from PF. Kakkatin and its derivative HK show anti-tumor and inhibitory activity on HCC and gastric cancer cell lines, targeting the cAMP (Cyclic adenosine 3,5-monophosphate) pathway[12].

PATHOPHYSIOLOGY OF HEPATOCELLULAR CARCINOMA

Transcriptomics and genomic characterization[3] of HCC reveals its complex heterogeneity and dynamic molecular and clinical features. HCC is characterized[3,21] by increasing dedifferentiation of hepatocytes, enlarged pleomorphic nuclei, increased nuclear-cytoplasm ratio, multinucleated hepatocytes, pseudoacini, irregular trabeculae, and unpaired visceral arteries[13]. <1 mm Dysplastic foci or ≥ 1 mm dysplastic nodules are precancerous stages further categorized[3] into grades, i.e., low-grade and high-grade dysplastic nodules with potential malignancy. Initial stage HCC is characterized[3,22] by the presence of[23] indistinct nodules (<2 cm) and stromal invasion. Well-differentiated HCC shows distinct nodules (<2 cm or >2cm), vascular invasion, and metastases besides stromal invasion[14]. Early-stage HCC can be effectively treated by local ablation, surgical resection, or liver transplantation[15]. Growth factor signalling, cell differentiation, angiogenic factors, tumor[24] microenvironment, and immunity disruption pathways are involved in neoplastic transformation and liver carcinogenesis. The elevated expression of immune repressing cytokines, for instance, Interleukins, IL-4, IL-5, IL-8 and IL-10, accompanied by decreased immune-inducing cytokines, namely, IL-6, tumour necrosis factor (TNF), and Interferon gamma (IFN-γ), promote tumour metastases and hinder diagnosis[16]. Regardless of diverse risk factors, one common mechanism driving HCC comprises a vicious cycle of uncontrolled liver cell death compensated with increased cell division and proliferation, eventually stacking up to genomic instability and carcinogenesis[17]. In most HCC cases, the gene promoter of telomerase reverse transcriptase (TERT), tumor[25] suppressor p53, and CTNNB1 (Catenin beta-1) are the dominantly mutated genes[18,19]. cAMP (Cyclic adenosine 3,5-monophosphate) is a second messenger in numerous signalling pathways that regulate cell proliferation. An increase in the intracellular levels of cAMP is

directly proportional to the proliferation of cells, but in transformed hepatocytes, elevated cAMP results in halted proliferation and down-regulation of liver cancer cells[20].,[27] Phosphodiesterases are intracellular enzymes that are known regulators of cAMP and cGMP. Phosphodiesterase 3 (PDE3) is an intracellular myocardial and vascular tissue enzyme. The phosphodiesterase 3 (PDE3) family is further classified[28] into two subfamilies, PDE3A and PDE3B. Similar but distinct genes encode both and have specific functions, but their expression pattern coincides in different tissues and cells[21]. Isoforms of PDE3B are comparably more expressive than PDE3A in energy homeostasis-regulating tissues such as adipose, liver, and pancreas[22].

NF-κB a key transcription factor responsible for the expression of pro-inflammatory makers such as cytokines and chemokine. The canonical and noncanonical (or alternative) signalling pathways are the two main mechanisms that activate NF-κB. While the noncanonical NF-κB pathway appears to have evolved as an additional signalling axis that collaborates with the canonical NF-κB route to regulate functions of the adaptive immune system, canonical NF-κB is functionally involved in almost every aspect of immune responses[23]. Liver cancer develops in the backdrop of inflammation, proving that HCC is not connected[29] to random probability but a series of accumulated micro-environmental factors. Thus, the research should focus on specific factors responsible for negative[30] alteration in the hepatic microenvironment, producing an adverse carcinogenic field effect and increasing the likelihood of malignancy[24]. NF-κB pathway is involved in the crosstalk between precancerous/cancerous and immune cells in this inflammation-cancer axis. NF-κB is directly and indirectly involved in the progression of liver carcinogenesis and is a potential treatment target[25]. Activation of NF-κB regulates inflammation by activating transcription of various genes such as IL-

1, IL-2, IL-8, TNF-alpha, anti-apoptotic factors such as Fas, BCL-2, Caspases, and Survivin, angiogenic factors, and cell-adhesion molecules[26] Thus, promoting cell survival, proliferation, angiogenesis, adhesion, invasion, and migration. A study on mice showed that conditional deletion of NEMO/IKKγ (IκB Kinase, IKK subunit), which usually activates NF-κB1, promotes cancer development. The inhibition of NF-κB increased the risk of hepatocarcinogenesis by sensitizing[3] hepatocytes to spontaneous apoptosis[27].

MEDICINAL POTENTIAL OF KAKKATIN SOURCE-PUERARIA LOBATE

Pueraria flos (PF), also known as 'kudzu flower', is a Traditional Chinese Medicine (TCM) extracted from dried-up flowers of Pueraria lobate, used for clearing lungs, spleen awakening and alcohol hangover. In China, PF is extensively used for the management of alcohol-driven HCC[28]. Besides, recent pharmacological studies divulge their biological significance as hepatoprotective, anti-tumorigenic, anti-diabetic, anti-inflammatory, and anti-oxidative, explaining traditional use[29]. PF is home to many metabolites, such as flavones, isoflavones, saponins, and one phenolic acid, identified in vivo[30]. According to cellular experiments, alcohol-treated HepG2 were shown[31] to be less likely to increase and migrate when exposed to serum containing PF. This[32] suggests that PF may influence alcohol-related HCC. PF induces apoptosis in alcoholic hepatocellular cancer, targeting the ESR1-ERK1/2 signalling pathway. ERK1/2 pathway regulates cell proliferation, differentiation, survival, and apoptosis. Hence, this pathway plays a significant role in cancer progression[31]. By upregulating ESR1, PF is known[33] to negatively regulate the proliferation and migration of cells in alcoholic-HCC attributing to its numerous components, including genistein, tectorigenin, Daidzein, glyccitein[34], 2-propenoic acid, 3- (3,4,5-trimethoxyphenyl)[35], butylated hydroxytoluene, 6-methoxy-4-

methylcoumarin, 1-(4-chlorophenyl)isoquinoline, 9-hydroxyoctadeca-10,12-dienoic acid, and 2-cyclopentene-1-tridecanoic acid[32]. Assessed PF metabolites against liver injury in rodents due to alcohol abuse and the hepatoprotective properties of PF may be attributed to the effective forms of phase II conjugated metabolites of isoflavonoids[33]. One such isoflavone, Kakkatin (7-methoxy-6,4'-dihydroxyisoflavone), a polyphenolic compound isolated from PF, Fusarium sp., Wisteria brachybotrys[36], fungi, and actinomycetes is a known hepatoprotective bioflavonoid. Flavonoids have various physiological processes like antioxidant, anti-inflammatory, antimicrobial, hypoglycaemic, and anti-tumor[37] properties besides hepatoprotective. Flavonoids enhance anti-inflammatory mechanisms by inhibiting the NF-κB and TNF-α activity or activating of[38] Adenosine 5'-monophosphate-activated protein kinase (AMPK). One example is kaempferol, which sustains anti-inflammatory effects by negatively regulating NF-κB and TNF-α[34]. Hence, the metabolites found in PF are known to be protective against various diseases.

ANTI-TUMOR ACTIVITY OF HK (KAKKATIN DERIVATIVE) OVER KAKKATIN

Metabolites from Puerariae flos are well known to have hepatoprotective effects. Kakkatin (7-methoxy-6,4'-dihydroxyisoflavone), isolated from dried PF, is a polyphenolic compound, and like other polyphenolic compounds, adding a different substituent on the parent nucleus may improve its functional properties. Its anti-tumor efficacy against HCC and gastric cancer remains unexplored. The author, Jiang et al. [12], likely confined the study to liver cancer and gastric cancer due to similar risk factors such as alcohol consumption is responsible for the progression of these cancers and chose these two cancer cell lines for in vitro validation of kakkatin[39] derivative HK. HK, a kakkatin[40] derivative produced by adding the competing group hept-6-yn-1-yl ethane sulphonate to the phenolic hydroxyl group of the kakkatin[41] structure, is

investigated for its anti-tumor efficacy and probable mode of action on HCC and gastric cancer cells[12]. LCMS and 1H NMR characterization is done to confirm the molecular structure of HK. Various in vitro experiments were conducted to compare HK's anti-1effects, which were studied on HCC SMMC-7721 and gastric cancer MGC803 cells. The SMMC-7221 cell line used by Jiang et al. [12] are human hepatocellular carcinoma cells known to be highly invasive when compared with other liver cancer cell lines, such as HEPG2, which is less invasive[35]. The IC50 values of HK and kakkatin on gastric cancer cells were not significantly different from one another. However, HK's IC50 value on HCC cancer cells was shown to be up to 30 times lower than kakkatin's, and less than that of the positive drug cisplatin (CDDP), a known chemotherapy drug with cancer cell inhibitory function[12,36]. Cloning experiments demonstrated that HK attenuated colony formation in HCC SMMC-7221 cells exponentially with an increase in HK dose concentration by inhibiting cell proliferation, migration, and invasion, excelling that of cisplatin in a time and dose-dependent manner. Treatment with HK resulted in an increase in apoptotic and necrotic cells in a concentration- and time-dependent manner. The count of early and late apoptotic and necrotic cells increased when HK concentration elevated over time relative to the control. The result of the examination of cell cycle stages indicated that following a 24-hour treatment, HK-induced cell cycle arrest was dose-dependently produced in SMMC-7221 cells, as evidenced by an elevation in the proportion of cells in the G2/M phase and an enhanced G0/G1 distribution of cells. The G2/M phase is a checkpoint in the cell cycle; its arrest signifies cell proliferation's downregulation and apoptosis induction [37]. To comprehend the mechanism and possible targets of HK in SMMC-7221 cells that are primarily responsible for inducing apoptosis, network pharmacology analysis was performed. This suggests a relationship

between HK and the cAMP signalling pathway and the generation of neutrophil extracellular traps (NETs). NETs show cytotoxic effects against the cancer cells by releasing various components that can kill and inhibit the growth of cancer cells[38]. NETs can destroy endothelial cells, thus hampering blood supply to tumor[56] cells. In contrast[57], studies show the metastasis-promoting nature of NETs[39]. NF-κB1, one of the major[58] targets associated with cAMP along with ADRB1, PDE3B (PDE family), and PRKACB (PKA family) downstream of the cAMP pathway, are used for further validation of inhibitory effects of HK on cancer cells. Molecular docking using AutoDock Tools software followed by RT-qPCR verification to understand the relation between HK and protein targets.[59] In silico results showed good binding activity of these four genes used as receptor molecules with the ligand molecule HK. Low binding energy indicated good binding affinity of HK with target proteins with stable configuration. RT-qPCR results showed elevated mRNA expression of NF-κB1 (p105/p50), one of the five subunits of NF-κB, and PDE3B, both responsible for the decrease in cancer migration and invasion, pointing towards tumour suppressive properties of HK[40]. Increased NF- κB1 and PDE3B inhibit carcinogenesis and metastasis, respectively.

CONCLUSION

Kakkatin derivative HK shows better anti-tumor[60] activity in SMMC-7721, a human HCC cell line, over gastric cancer MGC803 cells. HK was shown[61] to interact with key[62] targets related to the cAMP signaling[63] pathway, including PDE3B, ADRB1, NFKB1, and PRKACB. These targets are known to[64] regulate crucial cellular processes such as apoptosis, migration, and invasion, reinforcing HK's multifaceted mode of action in inhibiting HCC progression. This illustrates the specificity of HK to HCC and the considerable suppression of SMMC-7721 proliferation, migration, invasion, and metastases that it exerts by

stimulating cell cycle arrest and apoptosis. The known involvement of PF metabolites in treating liver-related anomalies might be the reason for HK's specificity against HCC. The liver and gastric tumor[66] biomarker testing may further reveal the presence of drug targets in both cancers. The tumor[67] inhibition effect of HK was seen[68] to be superior to that of kakkatin[69] and cisplatin[9]. The effect was due to the upregulated expression of PDE3B and NF-κB1 target proteins in the cAMP pathway. Further[70], RNA sequencing to compare and identify the entire gene expression profile of HCC and gastric cancer lines in response to HK can be done[71] to get a more detailed insight into which HK most influences genes, signalling pathways, and molecular markers in each cancer type. Proteome profiling of treated cells to understand protein expression and post-translational modification in response to treatment could help identify critical proteins involved in HK's mechanism of action and potential resistance mechanisms in gastric cancer cells. In the future, other liver cancer cell lines such as Huh 7, Huh 7.5, HepG2, Hep 3B, and MHCC97-H should also be explored[72] for the anti-tumour activity of kakkatin[73] and its derivative HK. Rodent models could be incorporated to further validate[74] the biomarkers identified in vitro human cell lines and improve clinical translation efficiency.

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Figure Legends

Figure 1 The intervention of 6-(hept-6-yn-1-yloxy)-3-(4-hydroxyphenyl)-7-methoxy-4H-chromen-4-one (HK), a kakkatin derivative induces the activation of apoptotic pathway, and inhibition of cell proliferation, migration, invasion, and metastasis in HCC cell line SMMC-7221. A kakkatin derivative formed by introduction of a competing group hept-6-yn-1-yl ethane sulphonate to the phenolic hydroxyl group of the kakkatin structure is explored for its anti-tumor activity in HCC SMMC-7221 cells. Binding energy (kcal/mol) of HK with PDE3B, ADRB1, PRKACB, NF- κB1 is checked by molecular docking. RT-qPCR was done to validate upregulation of NF- κB1 and PDE3B expression which in turn inhibit

cancer cell invasion, migration, and HCC progression. HCC: Hepatocellular carcinoma; PDE3B: Phosphodiesterase 3B; ADRB1: Adrenoceptor Beta 1; PRKACB: protein kinase cAMP-activated catalytic subunit beta; NF- κB1: Nuclear Factor Kappa B Subunit 1

1.	*kakkatin*	Unknown words	Correctness
2.	*Corresponding author: Vikram Patial, PhD, Principal Scientist, Dietetics & Nutrition Technology Division, CSIR-Institute of Himalayan Bioresource Technology, Palampur, Himachal Pradesh, India, 176061, Palampur 176061, Himachal Pradesh, India.*	Incomplete sentences	Delivery
3.	*synthesized; characterized; metastasize; colonised; characterization; categorized; sensitizing*	Text inconsistencies	Correctness
4.	*kakkatin*	Unknown words	Correctness
5.	~~anti-tumor~~ → anti-tumour	Mixed dialects of English	Correctness
6.	*is evaluated*	Passive voice misuse	Clarity
7.	~~anti-tumor~~ → anti-tumour	Mixed dialects of English	Correctness
8.	*kakkatin*	Unknown words	Correctness
9.	*cisplatin; Cisplatin*	Text inconsistencies	Correctness
10.	*kakkatin*	Unknown words	Correctness
11.	~~anti-tumor~~ → anti-tumour	Mixed dialects of English	Correctness
12.	*kakkatin*	Unknown words	Correctness
13.	~~used as treatment~~ → treated	Wordy sentences	Clarity
14.	~~carcinoma has~~ → carcinoma has	Improper formatting	Correctness
15.	*is estimated*	Passive voice misuse	Clarity
16.	~~stage~~ → stages	Incorrect noun number	Correctness

17.	~~and recruits~~ → . It recruits	Hard-to-read text	Clarity
18.	~~ensnare~~ → trap	Word choice	Clarity
19.	*are believed*	Passive voice misuse	Clarity
20.	~~is used to treat~~ → treats	Wordy sentences	Clarity
21.	*is characterized*	Passive voice misuse	Clarity
22.	*is characterized*	Passive voice misuse	Clarity
23.	~~the presence of~~	Wordy sentences	Clarity
24.	~~tumor~~ → tumour	Mixed dialects of English	Correctness
25.	~~tumor~~ → tumour	Mixed dialects of English	Correctness
26.	~~, but in~~ → . Still, in	Hard-to-read text	Clarity
27.	.,	Punctuation in compound/complex sentences	Correctness
28.	*is further classified*	Passive voice misuse	Clarity
29.	*is not connected*	Passive voice misuse	Clarity
30.	~~negative~~ → harmful	Word choice	Engagement
31.	*were shown*	Passive voice misuse	Clarity
32.	*This*	Intricate text	Clarity
33.	*is known*	Passive voice misuse	Clarity
34.	~~glycoitein~~ → glycinin	Misspelled words	Correctness
35.	3,4,5-tri methoxyphenyl	Misspelled words	Correctness

36.	*brachybotrys*	Unknown words	Correctness
37.	~~anti-tumor~~ → anti-tumour	Mixed dialects of English	Correctness
38.	~~of~~	Wrong or missing prepositions	Correctness
39.	*kakkatin*	Unknown words	Correctness
40.	*kakkatin*	Unknown words	Correctness
41.	*kakkatin*	Unknown words	Correctness
42.	~~anti-tumor~~ → anti-tumour	Mixed dialects of English	Correctness
43.	*is done*	Passive voice misuse	Clarity
44.	*were conducted*	Passive voice misuse	Clarity
45.	~~anti-1effects~~ → effects	Misspelled words	Correctness
46.	*were studied*	Passive voice misuse	Clarity
47.	*kakkatin*	Unknown words	Correctness
48.	~~from one another~~	Wordy sentences	Clarity
49.	~~However, HK's~~ → ¶ However, HK's	Intricate text	Clarity
50.	*HK's IC50 value on HCC cancer cells was shown*	Passive voice misuse	Clarity
51.	kakkatin's,	Punctuation in compound/complex sentences	Correctness
52.	*To comprehend the mechanism and possible targets of HK in SMMC-7221 cells that are primarily responsible for inducing apoptosis, network pharmacology analysis was performed.*	Unclear sentences	Clarity

53.	*To comprehend the mechanism and possible targets of HK in SMMC-7221 cells that are primarily responsible for inducing apoptosis*	Misplaced words or phrases	Correctness
54.	*network pharmacology analysis was performed*	Passive voice misuse	Clarity
55.	*This*	Intricate text	Clarity
56.	~~tumor~~ → tumour	Mixed dialects of English	Correctness
57.	~~In contrast~~ → ¶ In contrast	Intricate text	Clarity
58.	~~major~~ → significant	Word choice	Engagement
59.	*Molecular docking using AutoDock Tools software followed by RT-qPCR verification to understand the relation between HK and protein targets.*	Incomplete sentences	Delivery
60.	~~anti-tumor~~ → anti-tumour	Mixed dialects of English	Correctness
61.	*HK was shown*	Passive voice misuse	Clarity
62.	~~key~~ → critical, crucial	Word choice	Engagement
63.	~~signaling~~ → signalling	Mixed dialects of English	Correctness
64.	~~are known to~~	Wordy sentences	Clarity
65.	*This*	Intricate text	Clarity
66.	~~tumor~~ → tumour	Mixed dialects of English	Correctness
67.	~~tumor~~ → tumour	Mixed dialects of English	Correctness
68.	*was seen*	Passive voice misuse	Clarity
69.	*kakkatin*	Unknown words	Correctness

70.	~~Further~~ → ¶ Further	Intricate text	Clarity
71.	*be done*	Passive voice misuse	Clarity
72.	*other liver cancer cell lines such as Huh 7, Huh 7.5, HepG2, Hep 3B, and MHCC97-H should also be explored*	Passive voice misuse	Clarity
73.	*kakkatin*	Unknown words	Correctness
74.	to validate further	Inappropriate colloquialisms	Delivery
75.	~~are thankful to~~ → thank	Wordy sentences	Clarity
76.	, for	Punctuation in compound/complex sentences	Correctness
77.	~~etiology~~ → aetiology	Mixed dialects of English	Correctness
78.	~~Etiology~~ → Aetiology	Mixed dialects of English	Correctness
79.	*kakkatin*	Unknown words	Correctness
80.	*wjco*	Unknown words	Correctness
81.	~~signaling~~ → signalling	Mixed dialects of English	Correctness
82.	~~lobata~~ → Lobata	Misspelled words	Correctness
83.	~~signaling~~ → signalling	Mixed dialects of English	Correctness
84.	~~Flos~~ → Flo's	Incorrect noun number	Correctness
85.	~~4~~ → four	Improper formatting	Correctness
86.	~~signaling~~ → signalling	Mixed dialects of English	Correctness
87.	*kakkatin*	Unknown words	Correctness

88.	*Figure 1 The intervention of 6-(hept-6-yn-1-yloxy)-3-(4-hydroxyphenyl)-7-methoxy-4H-chromen-4-one (HK), a kakkatin derivative induces the activation of apoptotic pathway, and inhibition of cell proliferation, migration, invasion, and metastasis in HCC cell line SMMC-7221.*	Ungrammatical sentence	Correctness
89.	*kakkatin*	Unknown words	Correctness
90.	*kakkatin*	Unknown words	Correctness
91.	*is explored*	Passive voice misuse	Clarity
92.	~~anti-tumor~~ → anti-tumour	Mixed dialects of English	Correctness
93.	*Binding energy (kcal/mol) of HK with PDE3B, ADRB1, PRKACB, NF- κB1 is checked by molecular docking.*	Ungrammatical sentence	Correctness
94.	*is checked*	Passive voice misuse	Clarity
95.	*was done*	Passive voice misuse	Clarity
96.	*RT-qPCR was done to validate upregulation of NF- κB1 and PDE3B expression which in turn inhibit cancer cell invasion, migration, and HCC progression.*	Unclear sentences	Clarity