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Metformin and asarone inhibit HepG2 cell proliferation in a high glucose environment by regulating AMPK and Akt signaling pathway

Bhriku Kumar Das^{1,2}, Rachel M. Knott³ and Pramod C. Gadad^{1,2*}

Abstract

Background: Metabolic dysregulation is one of the hallmarks of tumor cell proliferation. Evidence indicates the potential role of the 5'adenosine monophosphate-activated protein kinase (AMPK) and protein kinase B/Akt signaling pathway in regulating cell proliferation, survival, and apoptosis. The present study explores the effect of metformin HCl and the combination of α - and β -asarone on the proliferation of HepG2 cells in the presence of high glucose levels simulating the diabetic-hepatocellular carcinoma (HCC) condition.

Results: The metformin and asarone reduced HepG2 cell viability in a dose-dependent manner and induced morphological changes as indicated by methyl thiazolyl tetrazolium (MTT) assay. The metformin and asarone arrested the cells at the G₀/G₁ phase, upregulated the expression of AMPK, and downregulated Akt expression in high glucose conditions as identified by the flow cytometry technique. Further, the upregulated AMPK led to a decrease in the expression of phosphoenolpyruvate carboxykinase-2 (PCK-2) and sterol regulatory element-binding protein-1 (SREBP-1).

Conclusion: The anti-proliferative effect of metformin and asarone in the diabetic-HCC condition is mediated via AMPK and Akt pathway.

Keywords: Asarone, Metformin HCl, HepG2 cells, High glucose, AMPK, Akt

Background

Epidemiologic evidence suggests diabetes mellitus (DM) as one of the potential risk factors in the progression of hepatocellular carcinoma (HCC) [1–3]. The complex pathophysiological relationship between HCC and DM could be due to hyperglycemia, hyperinsulinemia, insulin resistance, insulin-like growth factor (IGF)-1, obesity, or chronic inflammation with overlapping cell signaling

pathways. The various signal transduction pathways are implied in uncontrolled proliferation, mutations, invasion, migration, and survival of the tumor cells [4, 5].

There are a growing number of studies that propose the 5'adenosine monophosphate-activated protein kinase (AMPK), a highly conserved heterotrimeric serine/threonine-protein kinase, is a crucial mediator as an energy sensor in all the eukaryotic cells [6]. It plays a vital role in maintaining cellular energy homeostasis in both diabetes and cancer growth [7, 8]. Activation of the AMPK signaling pathway can influence changes in the effector's proteins involved in the regulatory process of energy metabolism, contributing to the pathogenesis of cancer [9–11]. Studies indicating AMPK activation correlates strongly with the reduction in cell proliferation in

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tumor cells as well as non-malignant cells. These effects are reported to be mediated through different mechanisms, including the modulation of cell cycle profile, autophagy, apoptosis, de novo fatty acid synthesis, and inhibition of protein synthesis [12, 13].

In addition to the AMPK signaling pathway, the Akt, a serine/threonine-protein kinase, also serves as an important pathway in multiple cellular processes that link with the increased proliferation, survival, and apoptosis of tumor cells [14, 15]. The Akt also serves as a negative regulator of AMPK, and studies have shown that the increased expression of Akt can stimulate proliferation through multiple downstream regulations affecting the cell cycle [16].

There is a body of evidence suggesting that metformin reduces tumor growth directly through the inhibition of energy metabolism and by regulation of both AMPK-dependent and independent signaling pathways [17–20]. On the other hand, alpha (α)- and beta (β)-asarone, the two major active constituents in volatile oils of *Acorus calamus*, showed anti-cancer effects in various cancer cell lines [21, 22]. Our previous study showed that asarone treatment attenuated the progression of HCC in diabetic conditions in a similar manner to that achieved by metformin [23]. The present study was aimed to understand whether the anti-proliferative effect of asarone is mediated via AMPK and Akt signaling pathway similar to the metformin, which was used as a reference drug. Further, we have also tried to address the possibility of the involvement of phosphoenolpyruvate carboxykinase-2 (PCK-2) and sterol regulatory element-binding protein-1 (SREBP-1) with cancer cell growth.

Methods

Drugs and reagents

Alpha-asarone (Lot # S18779; Purity 98% w/w; PubChem CID: 636822) and beta-asarone (Lot # STBF-1179 V; Purity 70% w/w; PubChem CID: 5281758) was purchased from Sigma-Aldrich Chemical Company, USA. Metformin HCl (Lot No: METI-1710010; PubChem CID: 14219) was a gift sample from Angels Pharma India Pvt. Ltd. Hyderabad, India. Polyclonal fluorescein isothiocyanate (FITC) goat anti-rabbit secondary antibody IgG was obtained from BD Biosciences, USA (Catalog No. 554020; Lot # 8199842). The primary rabbit polyclonal IgG antibodies viz., 5'adenosine monophosphate-activated protein kinase (AMPK α 1; Catalog no. orb10076; Lot # A1227), protein kinase B (Akt; Catalog No. orb159889; Lot # K8668), phosphoenolpyruvate carboxykinase-2 (PCK-2; Catalog no. orb5876; Lot # A4509), and sterol regulatory element-binding protein-1 (SREBP-1; Catalog no. orb11415; Lot # A1505) were purchased from Biorbyt Ltd., Cambridge, UK. Dulbecco's modified Eagle's medium (DMEM low and high

glucose), fetal bovine serum (FBS) heat-inactivated, trypsin-ethylenediaminetetraacetic acid (EDTA) digestion solution 1 \times , calcium- and magnesium-free phosphate-buffered saline (CMF-PBS), paraformaldehyde solution, methyl thiazolyl tetrazolium (MTT), and Triton X-100 were purchased from HiMedia Laboratories Pvt. Ltd., Mumbai, India. All other reagents used in this study were of cell culture grade obtained from commercial suppliers.

Cell culture conditions

The HCC cell line HepG2 (ATCC[®] HB-8065[™]) was obtained and grown as recommended by the American Type Culture Collection (ATCC), USA. HepG2 cells were cultured in DMEM either in normoglycemic (5.5 mM) or in hyperglycemic (25 mM) glucose conditions depending upon the experiments that contained 10% FBS supplemented with 100 U/ml penicillin and 100 μ g/ml streptomycin (Sigma-Aldrich Chemical Company, USA) maintained at 37 °C in an incubator (Labwit Scientific Pty Ltd., Australia).

Cytotoxicity by MTT assay

The cytotoxic effect of metformin HCl, α -asarone, and β -asarone on the HepG2 cell line was measured separately by the MTT assay [24]. Briefly, the cells were seeded in a 96-well flat-bottom plate (Nunc[™] MicroWell[™], Thermo Fisher Scientific Inc., USA) with 200 μ l of cell suspension containing approximately 10,000 cells/well and allowed to adhere by incubating for 24 h at 37 °C. After 24 h, the medium was discarded and replaced with a fresh medium (200 μ l) containing different concentrations of metformin HCl (1.6, 3.2, 6.4, 12.8, and 25.6 mM) and α - and β -asarone (0.12, 0.24, 0.48, 0.96, and 1.92 mM) followed by incubation for another 48 h. Untreated cells were used as control. All the media was discarded, and 200 μ l of methyl thiazolyl tetrazolium (MTT) solution (0.5 mg/ml in phosphate-buffered saline) was added to each well and incubated for 3 h. Further, the MTT culture medium was removed without disturbing the crystals formed and 100 μ l of dimethyl sulfoxide (DMSO) was added to each well to solubilize formed formazan crystals. Absorbance was recorded at 570 nm using a microplate reader (Biobase[®] EL-10A, China) to calculate the percent cell viability using the following formula:

$$\text{Percent cell viability} = \frac{(At - Ab)}{(Ac - Ab)} \times 100$$

where At is the absorbance value of the test compounds (metformin and asarone), Ab is the absorbance value of the blank, and Ac is the absorbance value of the control (untreated cells).

Table 1 The IC₅₀ values of metformin HCl and α - and β -asarone based on the MTT assay and dose selected for further studies

Test compounds	IC ₅₀ values in mM (MTT assay)	One half of the IC ₅₀ in mM used for detailed studies
Metformin HCl	25.74	12.87
α -Asarone	1.22	0.61
β -Asarone	1.46	0.73

Further, the percent of the cell viability against the concentration of each test compound was plotted and the inhibitory concentration 50 (IC₅₀) value was generated from the dose-response curve.

Experimental study design

The IC₅₀ values of metformin HCl and α - and β -asarone were 12.87, 0.61, and 0.73 mM, respectively, and this represents one half of the IC₅₀ value generated from the MTT assay (Table 1). Further, the HepG2 cells were divided into four groups: the normoglycemic (NG) cultured in normal glucose of 5.5 mM, the hyperglycemic (HG) cultured in high glucose of 25 mM, the HG group treated with metformin HCl (12.87 mM), and the HG group treated with a combined dose of asarone (0.61 mM (α)-asarone; 0.73 mM (β)-asarone).

Cell cycle analysis

The phases of the cell cycle were analyzed by measuring the amount of propidium iodide (PI)-labeled DNA staining in ethanol-fixed cells, as described elsewhere [25]. In

brief, HepG2 cells (5×10^5 cells/well) were seeded in a 6-well plate (Nunc™, Thermo Fisher Scientific Inc., USA) and allowed to adhere for 24 h at 37 °C. Thereafter, fresh DMEM medium containing 10% FBS was cultured with NG, HG, HG + Metformin, and HG + Asarone for 16 h. Subsequently, the trypsinized cells were fixed (70% chilled ethanol at 4 °C for 30 min), stained with PI-RNase solution (BD Biosciences, USA), and incubated at room temperature in the dark for 30 min. Further, the stained cells were collected and mixed well for analysis. A cytomics FC500 flow cytometer (Beckman-Coulter, USA) equipped with CXP software was used to collect the data. The analysis of results was made by measuring the forward scatter (FS) and side scatter (SS) for the identification of the single cells. Further, pulse shape processing was enabled to exclude all the clumps and doublet cells for the analysis by using pulse area vs. pulse width depending upon the experiment. Finally, the data were evaluated by using an algorithm (fit Gaussian curves) available in the FlowJo v10.0.7 software, and the PI-stained histogram plots represent the percentage of

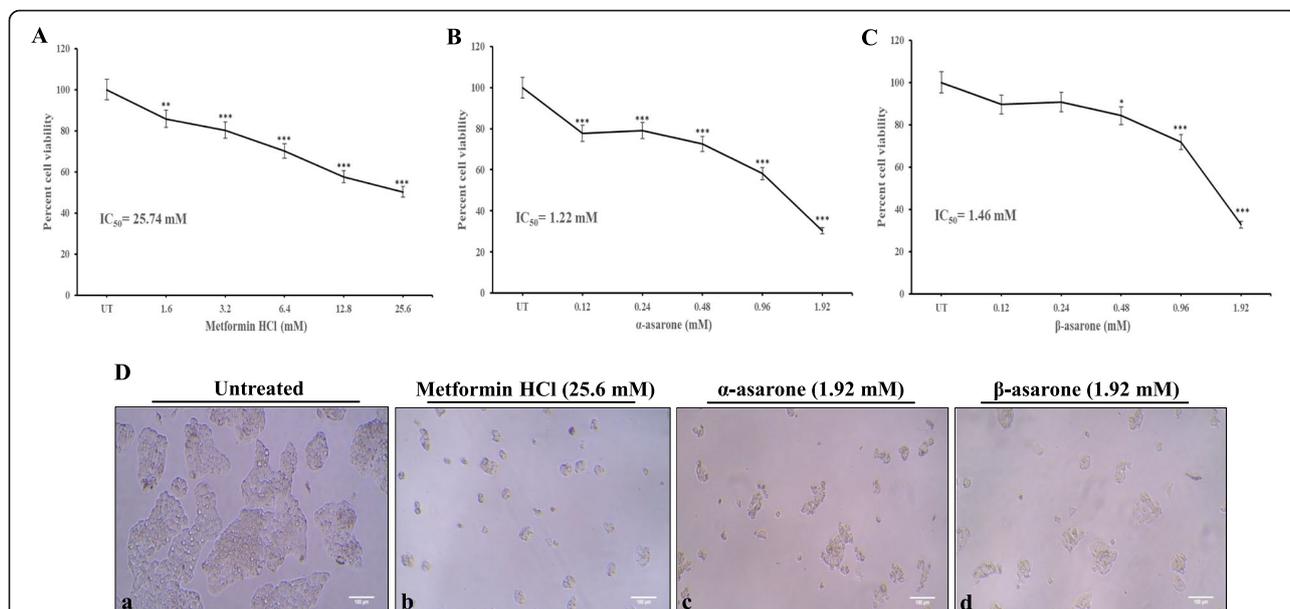
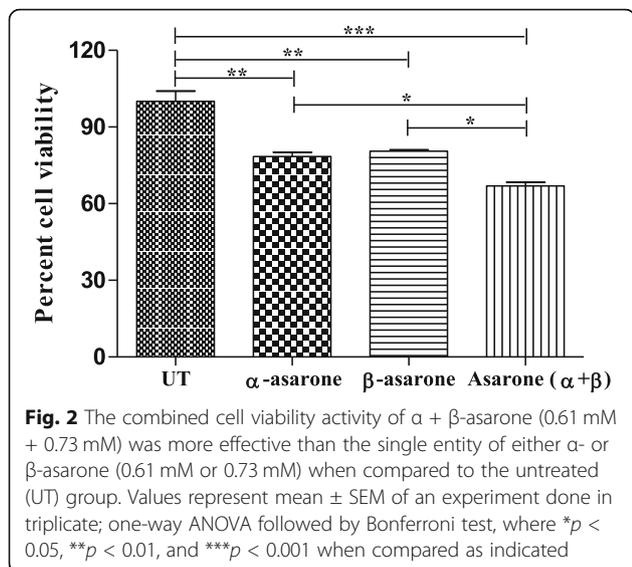


Fig. 1 Metformin and asarone inhibit the growth and modify the morphology of HepG2 cells. **A–C** Cells were treated with metformin HCl, α -asarone, and β -asarone as indicated for 48 h and the cell viability was determined by MTT assay. **D** Representative images to show the morphology of HepG2 cells under an inverted microscope. The scale of the bar is 100 μ m. **a** Untreated group; the morphology of HepG2 cells had a high density with adherent cell colonies, whereas, in the treatment group, metformin HCl (**b**) and asarone (**c**, **d**) at higher concentration showed lower density with a small round body. Values represent mean \pm SEM of an experiment done in triplicate; one-way ANOVA followed by Bonferroni test, where * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ compared to the untreated (UT) group

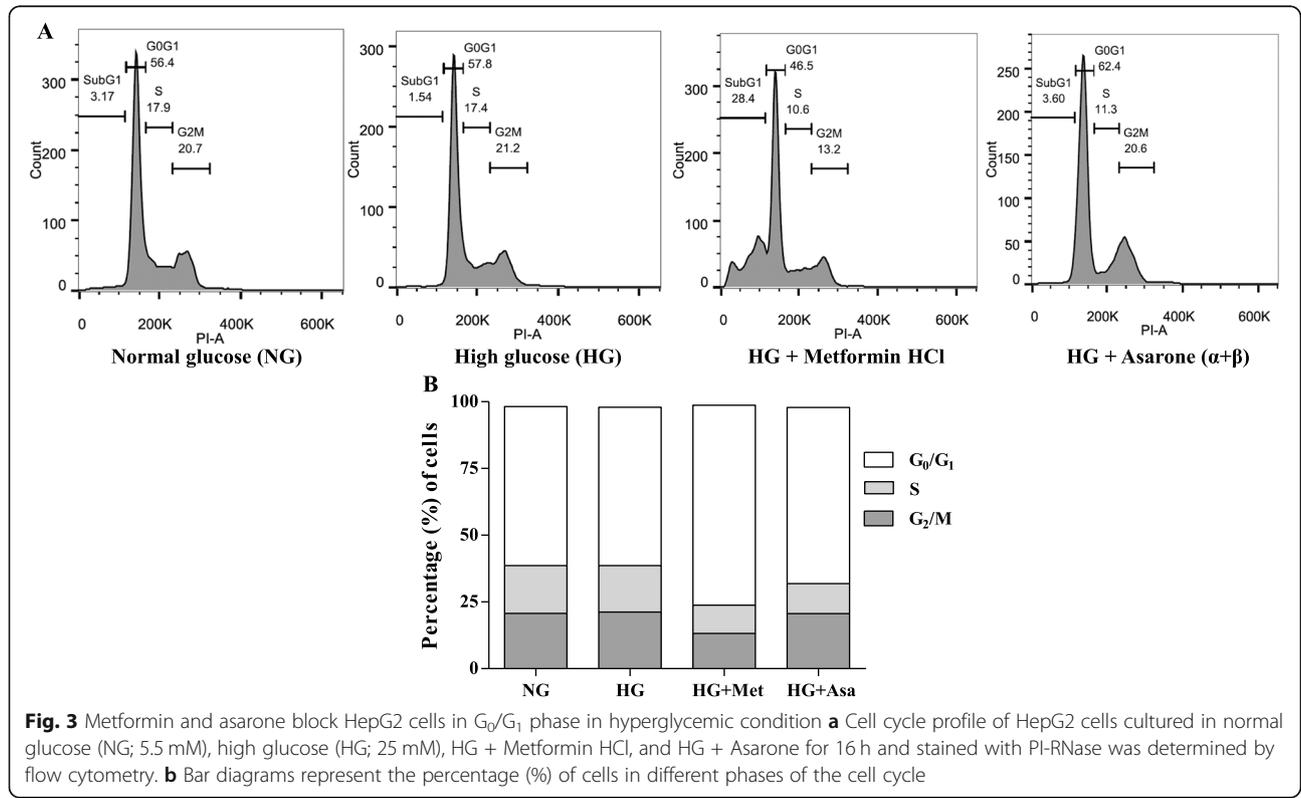


cells in different phases of the cell cycle of an experiment.

Expression of AMPKα1, Akt, PCK-2, and SREBP-1

The quantitative analysis of the expression of the intracellular markers was performed by flow cytometry using a 6-well plate. The cells were cultured at a density of 3 × 10⁵ cells/2 ml and allowed to adhere for 24 h at 37 °C.

The next day, the medium was discarded and washed with 1000 μl of 1× phosphate-buffered saline (PBS) and the cells were treated with metformin and asarone; if not, they are served as a control. The drug-treated media were removed from all the wells, transferred into a 5-ml centrifuge tube, and washed with 500 μl PBS. The PBS was then removed and transferred to the same centrifuge tube. Further, 200 μl of the trypsin-EDTA solution was added to all the wells and incubated for 4 min at 37 °C. Finally, the cells were harvested directly into the centrifuge tube after transferring back the medium from the respective wells. Thereafter, the cells containing the media were centrifuged at 300×g at 25 °C for 5 min. The supernatant was decanted carefully, treated with 500 μl of 2% paraformaldehyde (PFA) solution, and incubated for 20 min at room temperature. Cells were further mixed with 1000 μl of 1× PBS to dilute PFA solution and centrifuged at 300×g at 25 °C for 5 min. After decanting the supernatant, cells were permeabilized by treatment with 500 μl of 0.1% Triton X-100, mixed well, and incubated for another 10 min. Afterwards, cells were mixed with 1000 μl of 0.5% BSA solution for settlement and centrifuged at 200×g at 25 °C for 5 min and the supernatant was decanted. Further, 0.5% BSA solution in 1× PBS was added with 6 μl of primary antibody (AMPKα1, Akt, PCK-2, and SREBP-1) not exceeding the final volume more than 100 μl, mixed well, and



incubated at 25 °C for 1 h. After incubation, it was washed with 0.5% BSA solution, resuspended with 500 µl of 0.5% BSA in 1× PBS, and added with 6 µl of the secondary antibody and incubated. After 1 h of incubation in a dark room at 25 °C, cells were centrifuged and decanted. Finally, cells were resuspended in 500 µl of 0.5% BSA, mixed well, and analyzed. A cytomics FC500 flow cytometer (Beckman-Coulter, USA) equipped with CXP software was used to collect the data. The analysis of the results was carried out, as explained previously. The higher the value of geometric mean fluorescence intensity (GMFI), the higher the expression of individual markers of the cells as a population.

Statistical analysis

Statistical analysis was performed using statistical software GraphPad Prism version 6.0 (GraphPad Software, San Diego, CA, USA). Data from the individual experiments are represented as mean ± SEM and were conducted in triplicate. The results obtained were analyzed using one-way analysis of variance (ANOVA) followed by Bonferroni multiple comparison test, and *p* < 0.05 were considered as significant.

Results

Metformin and asarone inhibit the growth and modify the morphology of HepG2 cells

Metformin HCl (1.6 to 25.6 mM), α-asarone (0.12 to 1.92 mM), and β-asarone (0.48 to 1.92 mM) inhibited the cell viability of HepG2 cells in a concentration-dependent manner compared to untreated cells (Fig. 1A-C). The original morphology of the cells in the untreated group had a high density with adherent cell colonies, wherein few cells piled up, with a multilayered appearance. Following treatment with metformin HCl, α-asarone, and β-asarone, the cell morphology was characterized by low density, small round bodies due to shrinkage and the detachment of non-viable cells in a dose-dependent manner (Fig. 1D). The results are representative of experiments performed on three different occasions.

The combined effect of α- and β-asarone inhibits the cell proliferation

The corresponding MTT results showed that the cell proliferation of α- or β-asarone was inhibited compared to the untreated cells (*p* < 0.01) and that the combined effect of α- and β-asarone (0.61 mM + 0.73 mM) was

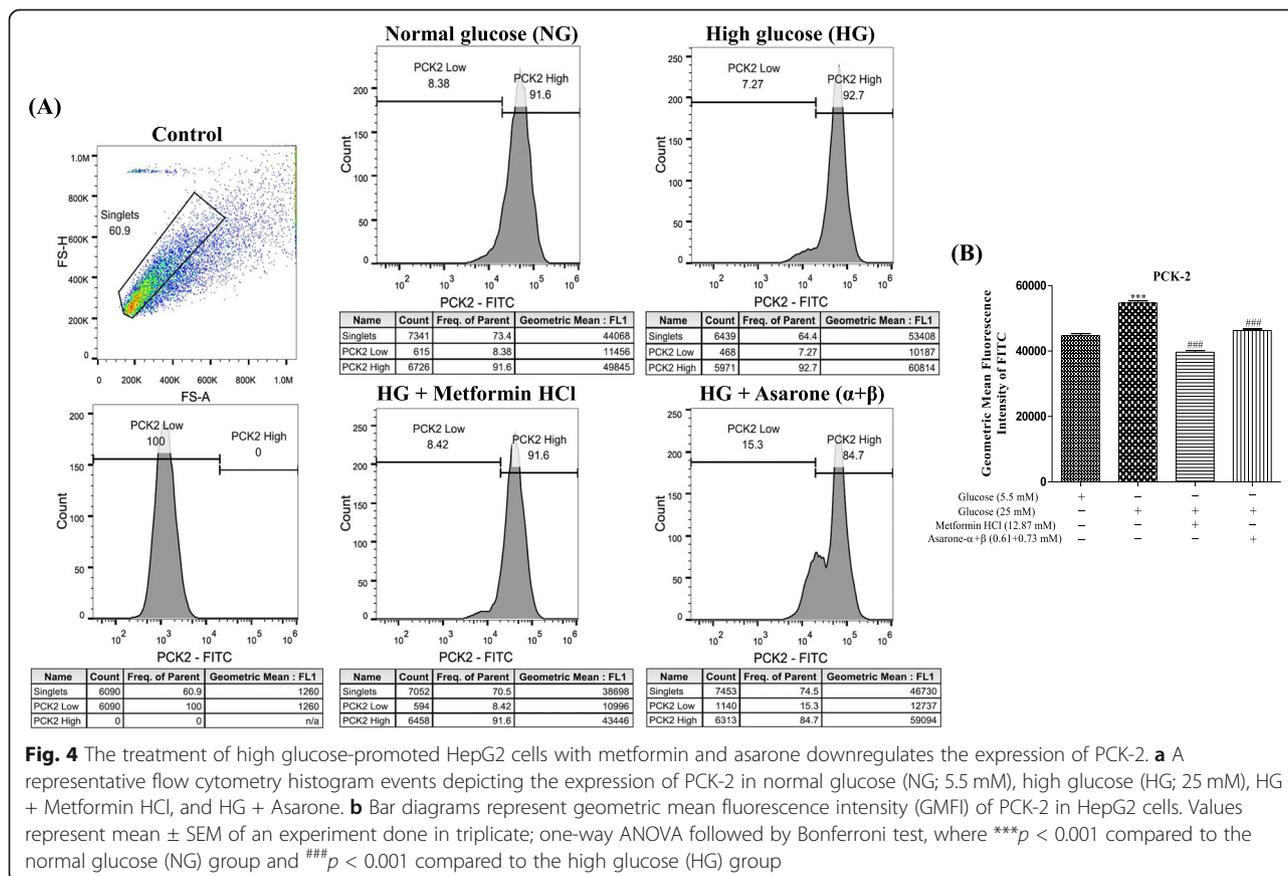


Fig. 4 The treatment of high glucose-promoted HepG2 cells with metformin and asarone downregulates the expression of PCK-2. **a** A representative flow cytometry histogram events depicting the expression of PCK-2 in normal glucose (NG; 5.5 mM), high glucose (HG; 25 mM), HG + Metformin HCl, and HG + Asarone. **b** Bar diagrams represent geometric mean fluorescence intensity (GMFI) of PCK-2 in HepG2 cells. Values represent mean ± SEM of an experiment done in triplicate; one-way ANOVA followed by Bonferroni test, where ****p* < 0.001 compared to the normal glucose (NG) group and ###*p* < 0.001 compared to the high glucose (HG) group

better ($p < 0.05$) than the single effect of α - or β -asarone (0.61 mM or 0.73 mM) (Fig. 2).

Metformin and asarone block HepG2 cells in the G₀/G₁ phase in hyperglycemic condition

We observed that in HepG2 cells, the percentage of the cells in the sub G₁ phase was less in high glucose than in normal glucose. Metformin and asarone treatment arrested the cells in the G₀/G₁ phase and reduced the cell count in S-phase cultured in high glucose concentration. Further, metformin retained the cells in the sub G₁ population stage (Fig. 3a, b). These results indicate that metformin and asarone inhibit cell proliferation at the G₀/G₁ phase of the cell cycle.

Metformin and asarone inhibit the expression of PCK-2 and SREBP-1 via the AMPK α 1 pathway

Based on the observation that metformin and asarone treatment induces the cytotoxicity of HepG2 cells in high glucose conditions, we measured whether this is a consequence of altered cellular energy homeostasis. The PCK-2 and SREBP-1 are the downstream regulators in the AMPK pathway, which regulate gluconeogenesis, glucose uptake, and de novo lipogenesis. Flow cytometry

analysis revealed that AMPK α 1 expression was enhanced by both metformin and asarone. Figures 4, 5, and 6 indicate the flow cytometry histogram events and geometric mean fluorescence intensity (GMFI) depicting the expression of respective markers in HepG2 cells. A significant increase in the expression of AMPK α 1 was observed indicated by the increase in the GMFI for metformin ($p < 0.01$) and asarone ($p < 0.001$) compared with the negative control (Fig. 6). Further, it was observed that the expression of PCK-2 ($p < 0.001$; $p < 0.001$) and SREBP-1 ($p < 0.01$; $p < 0.001$) decreased in the metformin- and asarone-treated groups (Figs. 4 and 5).

Metformin and asarone decrease the expression of Akt

The phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt), a prototypic signaling pathway, is increasingly implicated in carcinogenesis and plays a key role in the regulation of the growth and proliferation of cells. The change in the glucose concentration did not have any effect on the expression of Akt in HepG2 cells. However, a significant decrease in the expression levels of Akt was observed in metformin ($p < 0.01$) and asarone ($p < 0.001$) groups compared to untreated cells as indicated by GMFI (Fig. 7).

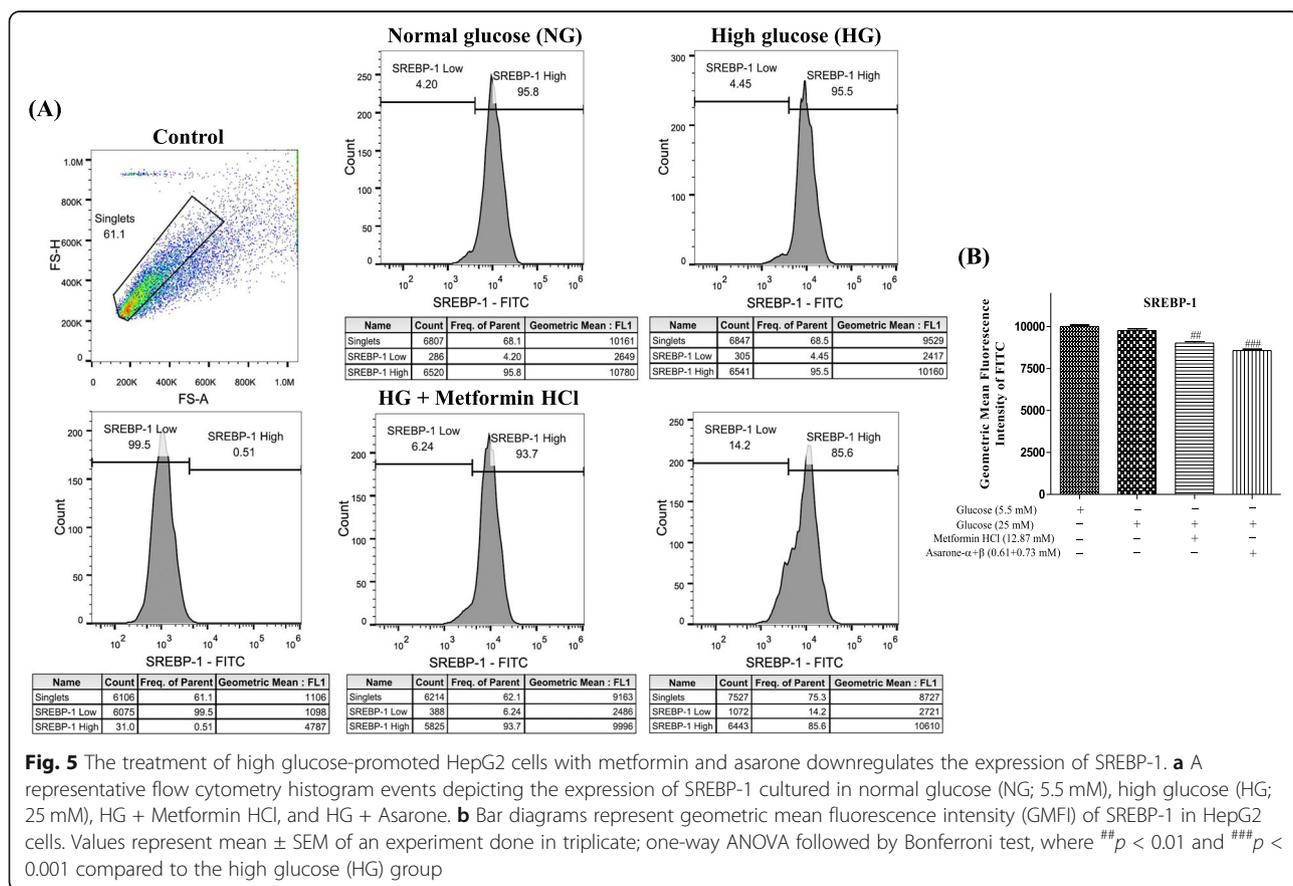


Fig. 5 The treatment of high glucose-promoted HepG2 cells with metformin and asarone downregulates the expression of SREBP-1. **a** A representative flow cytometry histogram events depicting the expression of SREBP-1 cultured in normal glucose (NG; 5.5 mM), high glucose (HG; 25 mM), HG + Metformin HCl, and HG + Asarone. **b** Bar diagrams represent geometric mean fluorescence intensity (GMFI) of SREBP-1 in HepG2 cells. Values represent mean \pm SEM of an experiment done in triplicate; one-way ANOVA followed by Bonferroni test, where $^{##}p < 0.01$ and $^{###}p < 0.001$ compared to the high glucose (HG) group

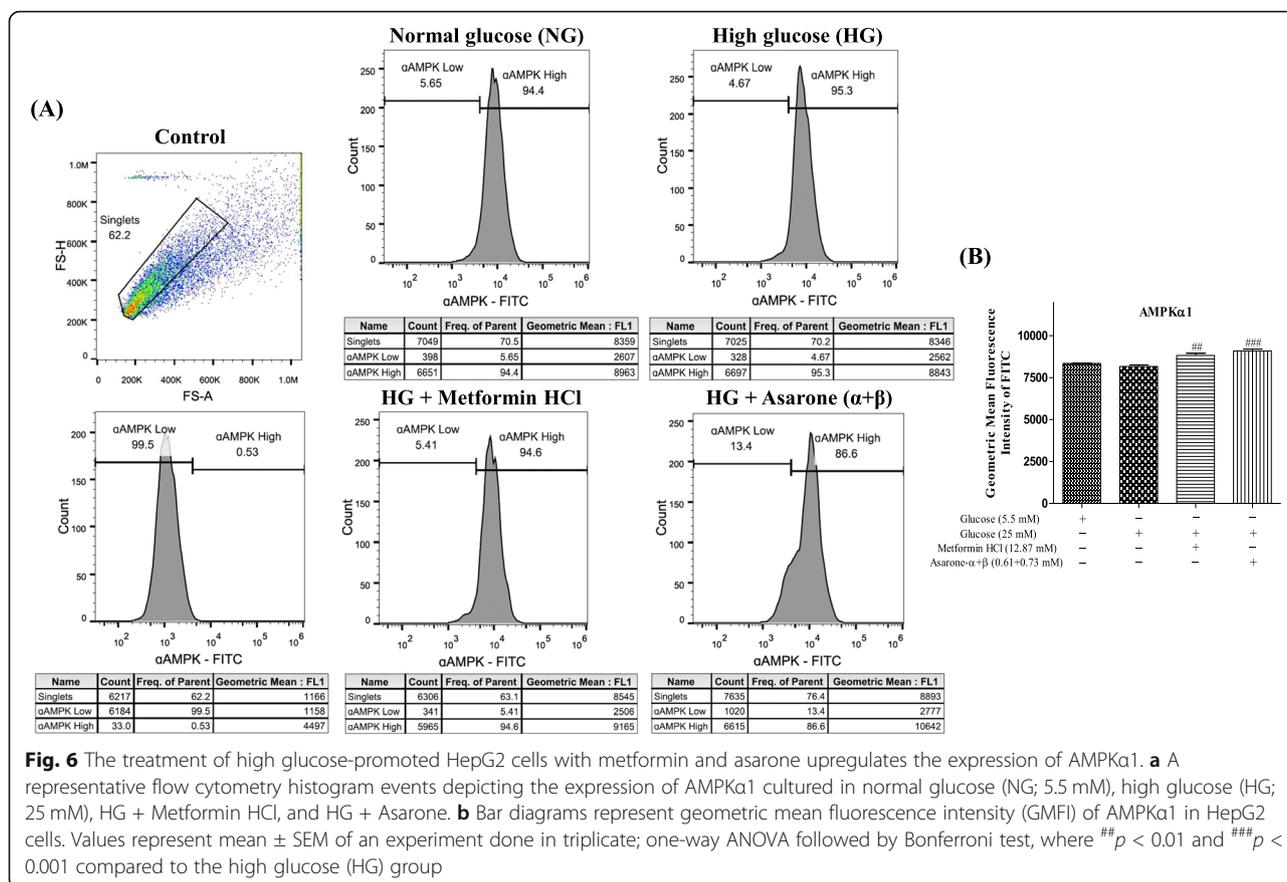


Fig. 6 The treatment of high glucose-promoted HepG2 cells with metformin and asarone upregulates the expression of AMPKα1. **a** A representative flow cytometry histogram events depicting the expression of AMPKα1 cultured in normal glucose (NG; 5.5 mM), high glucose (HG; 25 mM), HG + Metformin HCl, and HG + Asarone. **b** Bar diagrams represent geometric mean fluorescence intensity (GMFI) of AMPKα1 in HepG2 cells. Values represent mean ± SEM of an experiment done in triplicate; one-way ANOVA followed by Bonferroni test, where ## *p* < 0.01 and ### *p* < 0.001 compared to the high glucose (HG) group

Discussion

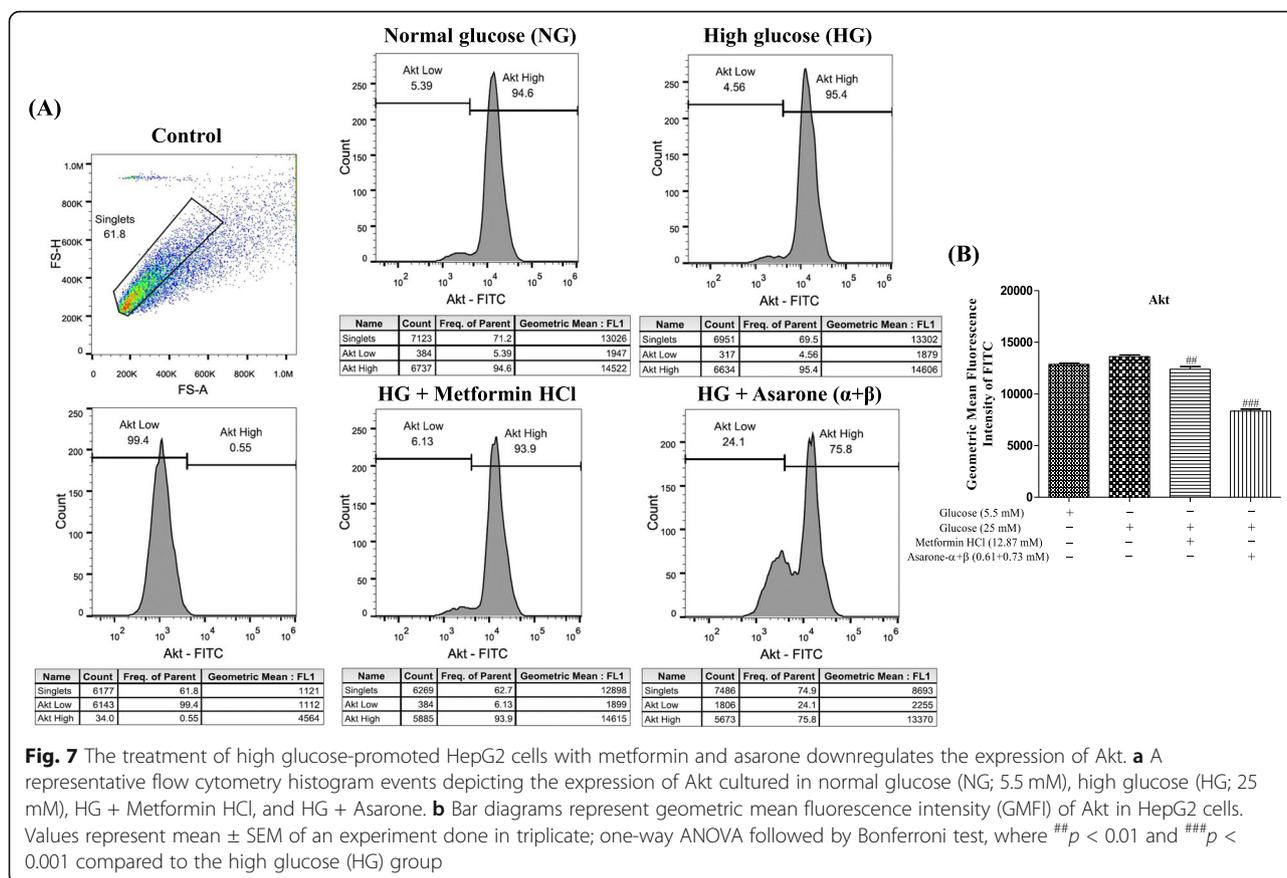
Many experimental as well as epidemiological studies suggest that the higher plasma glucose level might play a crucial role in cancer development and progression [26–29]. However, the involvement of regulatory pathways between the effect of hyperglycemia (HG) and hepatic cancer remains largely unexplored. In the present study, we attempted to explore the effect of metformin and asarone in abating the proliferation of HepG2 cells in high glucose conditions. The results of this study suggest that metformin and asarone suppress the hepatic cancer cell viability and arrested the cell cycle at the G₀/G₁ phase by AMPK activation and Akt inhibition.

Studies, including our own, suggest that higher blood glucose concentration may be associated with the increased risk of cancers [23, 30–33]. The increased proliferation of HepG2 cells at elevated glucose levels may be due to glucose toxicity resulting from increased production of reactive oxygen species (ROS) and alteration in the expression of genes [4, 34]. Other work reported that this could be due to a favorable environment for the cancer-signaling pathway leading to proliferation, mutations, invasion, migration, and survival [4]. The AMPK and Akt signaling pathways, along with their downstream regulators, are indicated to play an important

role in the regulation of cancer as well as glucose metabolism [35].

Classically, the 5' adenosine monophosphate-activated protein kinase (AMPK) has been identified as a central metabolic sensor playing a significant role in energy homeostasis. However, the emerging evidence suggests AMPK is a possible metabolic tumor suppressor and has been implicated in preventing and treating tumor growth [36–38]. The AMPK activation can regulate various tissue- and cell-specific downstream markers responsible for proliferation, autophagy, and apoptosis [12, 13, 39]. The observed anti-proliferative actions of metformin and asarone in a high glucose environment and changes of AMPKα1 expression are in accordance with many other studies that correlate with AMPK activation and inhibition of tumor growth [9–11]. These findings suggest that the HepG2 cell line proliferation is inversely correlated to AMPK activity in high glucose conditions.

The evidence indicates the altered expression of gluconeogenic enzymes during HCC growth and differentiation, in particular, the phosphoenolpyruvate carboxykinase (PCK or PEPCK) genes. The metformin and asarone treatment decreased the expression of PCK-2 (a downstream regulator of AMPK), a gluconeogenic factor in the liver that plays a role in the regulation of

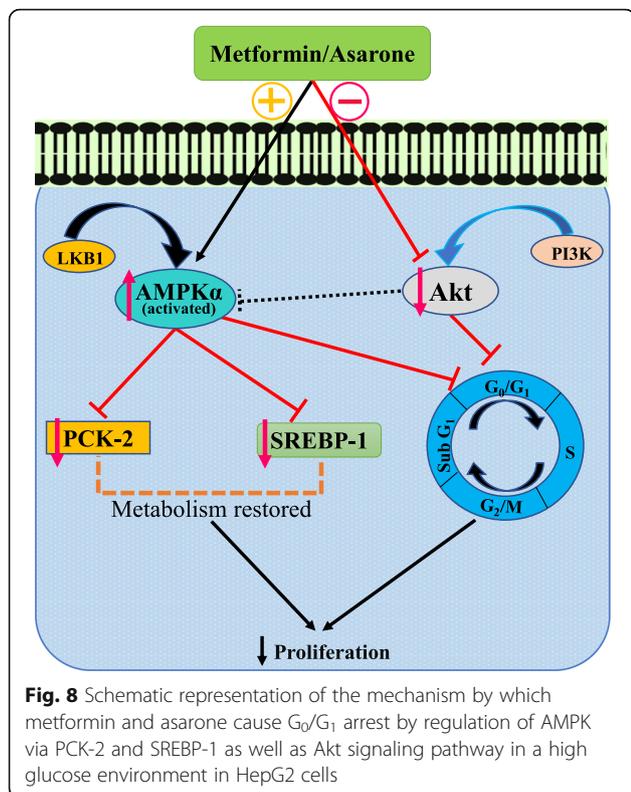


the gluconeogenesis process by catalyzing the conversion of oxaloacetate (OAA) to phosphoenolpyruvate (PEP) [40, 41]. Elevated expression of the PCK-2 gene is found in different cancers and is linked to increased anabolic metabolism, thereby playing a vital role in cancer cell proliferation [42, 43]. The observed upregulation of PCK-2 expression in our study could be due to an increase in glucose uptake and utilization, supporting the anabolic metabolism and promoting cell proliferation. In contrast, the expression of PCK-2 in the metformin and asarone treatment was downregulated, indicating the involvement of the glucose metabolism pathway in suppressing the growth of cancer cells. The earlier work suggested the role of PCK or PEPCK in increasing the glutamine and glucose concentration, thus linking anabolic pathways to cancer cell proliferation both in vivo and in vitro [44]. Furthermore, Akt also regulates the PCK or PEPCK expression mediated through phosphorylation of cyclic AMP response element-binding/Forkhead box protein O1 (CREB/FOXO1) transcriptional activity [45].

Furthermore, both AMPK and Akt are the upstream regulator of sterol regulatory element-binding protein-1 (SREBP-1) and can directly phosphorylate SREBP-1. Recently, SREBP-1, which gets upregulated in different types of cancer, has been linked to play a role in the

oncogenic signaling-mediated glucose uptake and de novo lipogenesis [46–48]. The pharmacological targeting of SREBP-1 has revealed to significantly inhibit the glioblastoma cell growth, suggesting it as a novel molecular target in cancer [49]. In addition to regulation by AMPK, SREBP-1 has shown to be activated by the Akt/PI3K prototypic survival signaling pathway in different types of cancer. Collectively, it demonstrated the role of the Akt/PI3K signaling pathway via SREBP-1 integrating lipogenesis and glucose metabolism for rapid cancer progression [49–51]. In our study too, metformin and asarone decreased SREBP-1 expression in a high glucose-induced proliferation of HepG2 cells, indicating the involvement of the AMPK/SREBP-1 or Akt/SREBP-1 signaling pathway.

The deregulation of the Akt/PI3K prototypic survival signaling pathway also plays an important role in cell proliferation. Evidence indicates that abnormal activation of the Akt/PI3K signaling pathway frequently occurs in HCC patients [14–16]. In this study, metformin and asarone treatment significantly decreased the expression level of Akt, thereby suppressing HepG2 cell proliferation. These findings are supported by other workers who suggest that metformin inhibited the growth of various cancer cells through inhibition of Akt [52–54].



The observed antagonistic relationship between AMPK and Akt and their impact on the proliferation of cancer cells is in concurrence with earlier studies by other workers. The Akt/PI3K mediated carcinogenesis and tumor progression gets upregulated due to the mammalian target of rapamycin (mTOR) signaling pathway, which in turn is activated due to deregulation of the AMPK [35, 55, 56]. Besides this, the deregulation of the AMPK disturbs the insulin signaling pathway and results in elevated levels of insulin-like growth factor-1/insulin, a common pathway for both HCC and diabetic conditions [57].

Conclusion

The metformin and asarone inhibit the HepG2 cancer cell proliferation, specifically at the G₀/G₁ phase of the cell cycle in a high glucose environment, which is attributable to the regulation of AMPK and Akt signaling pathways (Fig. 8). Further, the inhibition of PCK-2 and SREBP-1 indicates the link between the glucose metabolic pathway and cancer cell growth. However, the potential limitations of the study include the possibility of adopting other molecular biological methods to verify our findings, along with the role of specific activators or inhibitors in high glucose-induced HepG2 cell proliferation through modulation of both the AMPK and Akt signaling pathways. Further, the investigation about the possibility of involvement of multiple pathways is essential to elucidate the molecular mechanism/s of metformin and asarone in controlling the progression of cancer.

Abbreviations

AMPK: 5'Adenosine monophosphate-activated protein kinase; BSA: Bovine serum albumin; DM: Diabetes mellitus; DMEM: Dulbecco's modified Eagle's medium; FBS: Fetal bovine serum; GMFI: Geometric mean fluorescence intensity; HCC: Hepatocellular carcinoma; HG: Hyperglycemic/high glucose; MTT: Methyl thiazolyl tetrazolium; NG: Normoglycemic/normal glucose; PCK-2: Phosphoenolpyruvate carboxykinase-2; PBS: Phosphate-buffered saline; PFA: Paraformaldehyde solution; PI3K: Phosphatidylinositol 3-kinase; SREBP-1: Sterol regulatory element-binding protein-1

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Authors' contributions

BKD and PCG designed all the experiments and wrote the manuscript. BKD has investigated the experiments and analyzed the data. RMK has reviewed and edited the manuscript. PCG was associated with supervising, advising, reviewing, and editing the final version of the manuscript. All the authors have read and approved the final manuscript.

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Availability of data and materials

All data and materials are available upon request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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