Pharmacogn. Mag.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
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ORIGINAL ARTICLE

Pentamethylquercetin Inhibited the Growth of Hepatic Ascitic Tumor Cell H22 by Improving Metabolic Environment and Aerobic Glycolysis in Monosodium Glutamate-Induced Obese Mice

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Submitted: 28-11-2018 Revised: 17-12-2018 Published: 16-05-2019

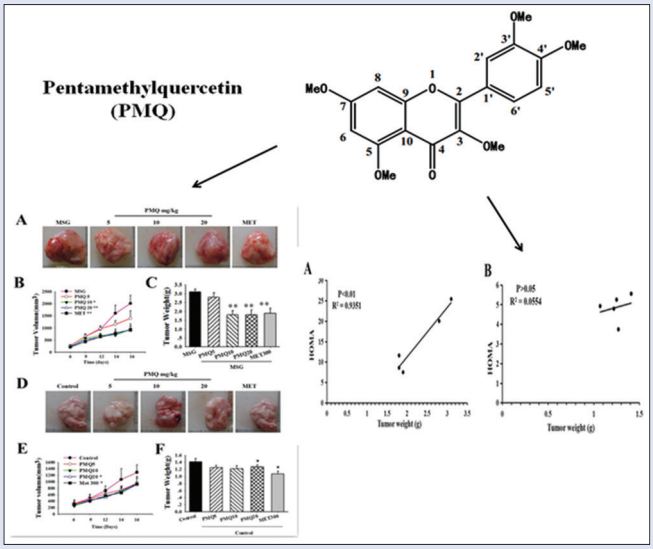
ABSTRACT

Aim: We investigated the effects of pentamethylquercetin (PMQ) on the tumor growth in monosodium glutamate (MSG)-induced obese mice. **Materials and Methods:** At the age of 5 weeks, control and MSG mice were, respectively, divided into five groups (n = 10): Vehicle group; PMQ 5, 10, 20 mg/kg; and metformin (MET) 300 mg/kg groups. All mice were administrated PMQ and MET by gastric gavage from 5- to 24-week age. 22-week-old mice were injection with H22 hepatic ascitic tumor cells. After 2 weeks, animals were anesthetized and blood, tumor, and liver tissues were harvested. **Results:** Compared with control mice, MSG mice showed obviously metabolic disorders and larger tumor weight and volume than those of control mice. PMQ and MET administration reduced body weight, improved glucose and lipid metabolism, and insulin resistance and inhibited tumor growth in MSG mice. However, PMQ and MET had a litter effect on the tumor growth and metabolic indexes in the control mice. Furthermore, there is significant positive correlation between improved insulin resistance and inhibited tumor growth by chronic PMQ and MET treatment. Further experiments showed PMQ and MET treatment upregulated mRNA expressions of sirtuin 6 (sirt6) both in tumor and liver tissues. **Conclusion:** Our results demonstrated PMQ decreased tumor growth in the MSG mice and the potential mechanisms might be attributed to upregulated mRNA expressions of sirt6.

Key words: Metabolic syndrome, monosodium glutamate mice, pentamethylquercetin, sirtuin 6, tumor

SUMMARY

- Pentamethylquercetin (PMQ) administration reduced body weight, improved glucose and lipid metabolism and insulin resistance, and inhibited tumor growth in monosodium glutamate mice
- The antitumor effect of PMQ might include indirect effect – improving insulin resistance and direct effect – correcting Warburg effects attributed to upregulation sirtuin 6.



Abbreviations used: MSG: Monosodium glutamate; Mets: Metabolic syndrome; PMQ: Pentamethylquercetin; Sirt6: Sirtuin 6; MET: Metformin.

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DOI: 10.4103/pm.pm_605_18

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INTRODUCTION

Metabolic syndrome (MetS) is a cluster of cardiovascular risk factors that include hypertension, diabetes mellitus, obesity, hypertriglyceridemia, and low-high-density lipoprotein cholesterol.[1] Recently, increasing evidence suggest that MetS is functioned as an independent etiologic factor involving in the development and progression of certain types of cancer, including breast cancer,[1] endometrial cancer,[2] colorectal cancer,[3] pancreatic cancer,[4] and prostate cancer.[5] Insulin resistance, the underlying hallmark feature and pathological basis of MetS,[6] plays an important role in multiple cancers.[7-10] Insulin resistance is external metabolic disorder effect on tumor progress. In addition to this, Warburg effect is internal metabolic disorder effect on tumor progress.[11]

Sirtuin 6 (Sirt6) is a member of sirtuins family and plays an important role in improving insulin resistance, keeps balance in glucose and lipid metabolism, and inhibits inflammation and tumor.[12-16] Sirt6 expression

Cite this article as: Gao W, Xiao X. Pentamethylquercetin inhibited the growth of hepatic ascitic tumor cell H22 by improving metabolic environment and aerobic glycolysis in monosodium glutamate-induced obese mice. Phcog Mag 2019;15:416-22.

level is related with glycolysis, lipid synthesis, and β-fatty acid oxidation process.[17] Activation of Sirt6 may therefore be therapeutically useful for treating insulin-resistant diabetes.[18] Furthermore, Sirt6 is functioned as a tumor suppressor that regulates aerobic glycolysis (Warburg effect) in cancer cells as well.[19]

Pentamethylquercetin (PMQ), a member of polymethoxylated flavonoids, is presented in sea buckthorn (*Hippophae rhamnoides*)[20] and the rhizome of *Kaempferia parviflora*.[21] PMQ in our laboratory is a methylation product of quercetin. It has significant pharmacokinetic and pharmacodynamic advantages.[22-24] Prior research showed that PMQ possessed multiple pharmacological activities, including anti-MetS,[25] antidiabetes mellitus,[26] and antitumor.[27] To better understand the protective effects of PMQ on tumor growth in the background of MetS, monosodium glutamate (MSG)-induced obese mice model was used. Our previous research showed that MSG mice exhibited multiple metabolic disorders and therefore served as an appropriate animal model mimicking human MetS.[25] In the present research, we determined the antineoplastic effect and potential mechanisms of PMQ on MSG mice in the context of MetS.

MATERIALS AND METHODS

Animals

Metabolic disorder mice model was established by prior research of our laboratory.[25] Food and water were given *ad libitum*. All experiments were approved by the Ethics Committee of Animal Use for Teaching and Research of Tongji Medical College at HuaZhong University of Science and Technology. At the age of 5 weeks, control and MSG mice were, respectively, divided into five groups (n = 10): vehicle group; PMQ 5, 10, 20 mg/kg; and metformin (MET) 300 mg/kg groups. All mice were administrated PMQ and MET by gastric gavage from

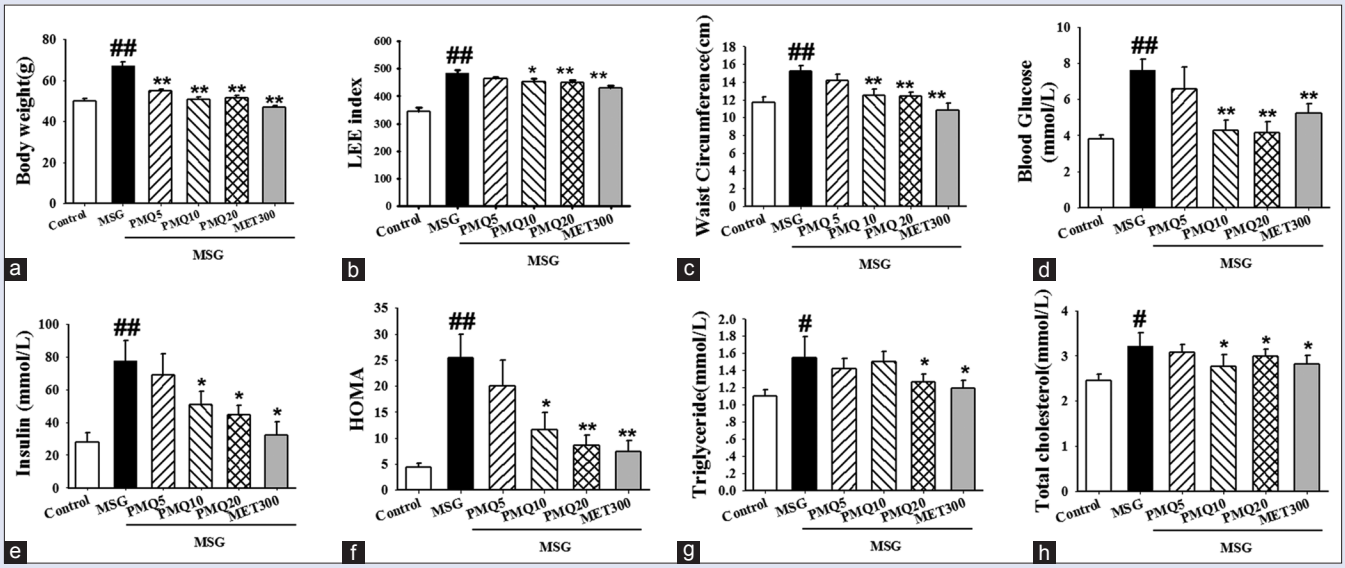

Figure 1: Levels of body weight, LEE index, waist circumference, fasting glucose, triglyceride, total cholesterol, insulin, and homeostasis model assessment of insulin resistance of monosodium glutamate mice (a-h). Data expressed as mean ± standard error (n = 10). $^{**}P < 0.01$, $^{*}P < 0.05$ versus monosodium glutamate. $^{\#\#}P < 0.01$ versus control group

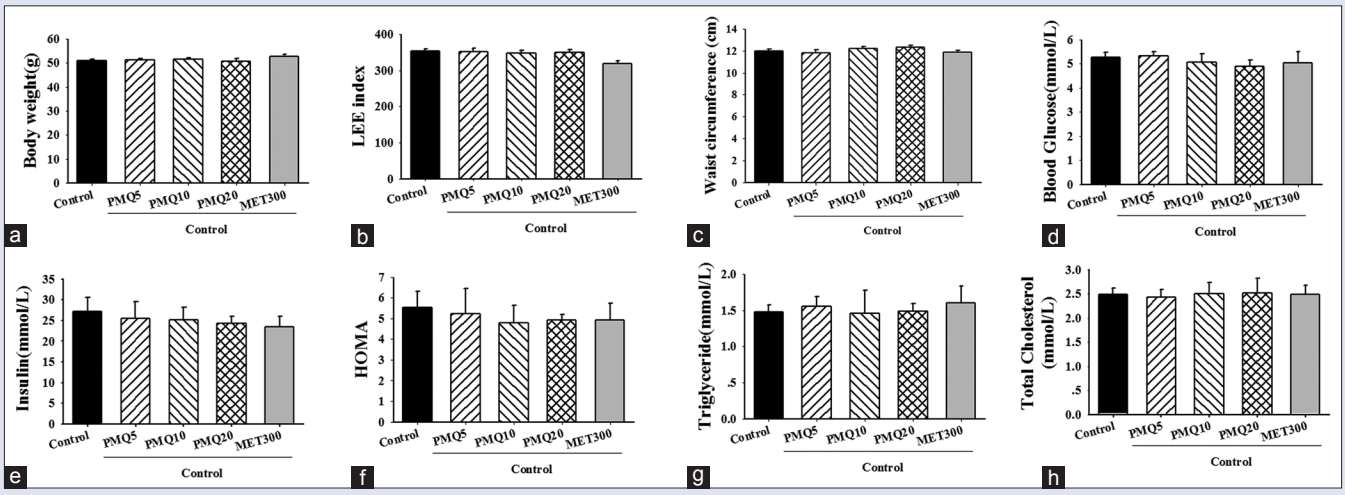

Figure 2: Levels of body weight, LEE index, waist circumference, fasting glucose, triglyceride, total cholesterol, insulin, and homeostasis model assessment of insulin resistance of control mice (a-h). Data expressed as mean ± standard error (n = 10)

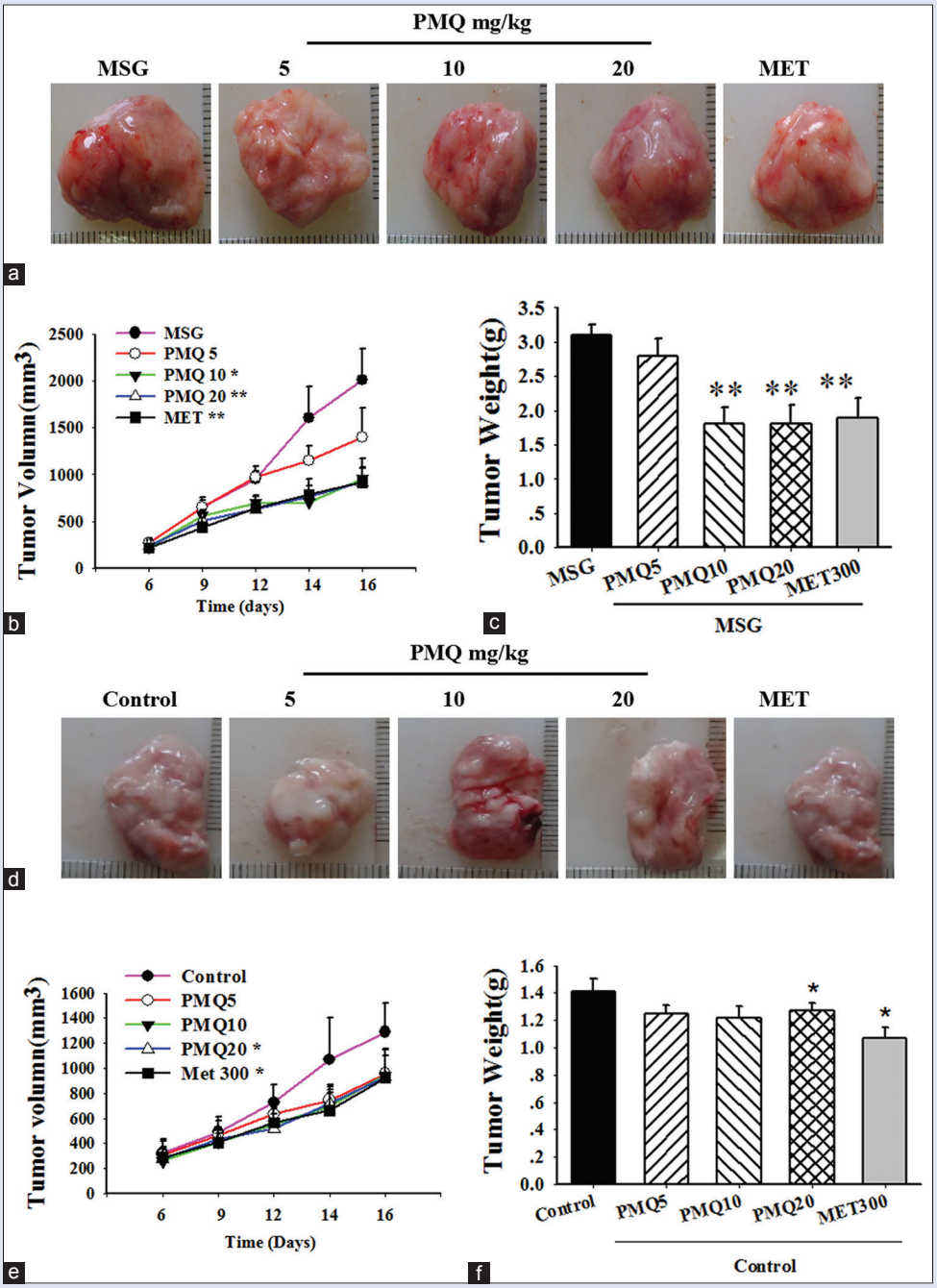

Figure 3: Tumor volume, tumor weight, and tumor image of monosodium glutamate mice (a-c) and control mice (d-f). Data expressed as mean ± standard error ($n = 10$). $^{**}P < 0.01$, $^{*}P < 0.05$ versus vehicle

5- to 24-week age. Vehicle groups were administered an equipotent volume of vehicle. At the age of 22 weeks, all mice were injected subcutaneously (sc) with 1×10^6 H22 cells. Tumors were measured daily by calipers. At 24-week age, after a 12 h fast, blood samples were collected for separating serum. Then, all mice were sacrificed by CO_2 after 12 h fast; tumor and liver were weighed, frozen, and prepared for test.

Serum analysis

Fasting serum levels of glucose, triacylglycerol, and total cholesterol in each group were detected using commercial kits. Fasting serum insulin levels were measured by commercial radioimmunoassay kit (Beijing North, Beijing, China) performed in duplicate. Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated by the following equations: HOMA-IR = fasting glucose (mmol/l) × fasting insulin (μ IU/ml)/22.5.[28]

Syngeneic tumor model

At the age of 22-week age, all mice were injected sc with 1×10^6 H22 cells. Tumors were measured daily by calipers.

RNA preparation and reverse transcription polymerase chain reaction

Reverse transcription-polymerase chain reaction (PCR) was performed according to a previously described procedure.[29] The primer sequences

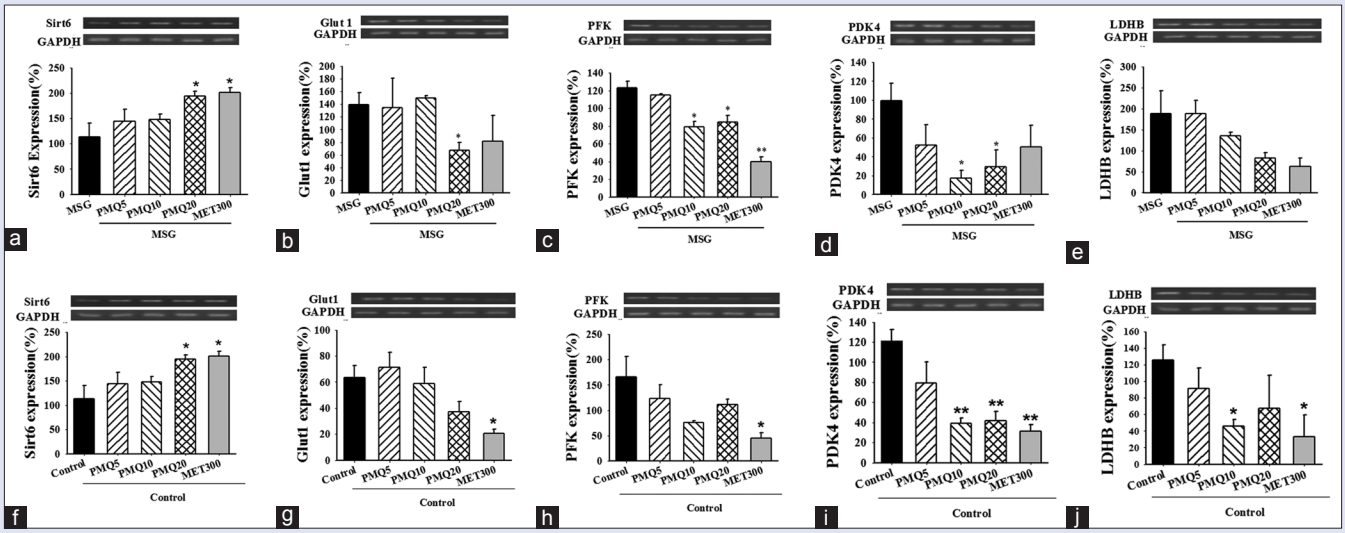

Figure 4: Sirtuin 6, Glut 1, PFK, PDK4, and LDHB mRNA expressions in tumor tissue of monosodium glutamate mice (a-e) and control mice (f-j). Data expressed as mean ± standard error ($n = 3$). $^{**}P < 0.01$, $^{*}P < 0.05$ versus monosodium glutamate or control

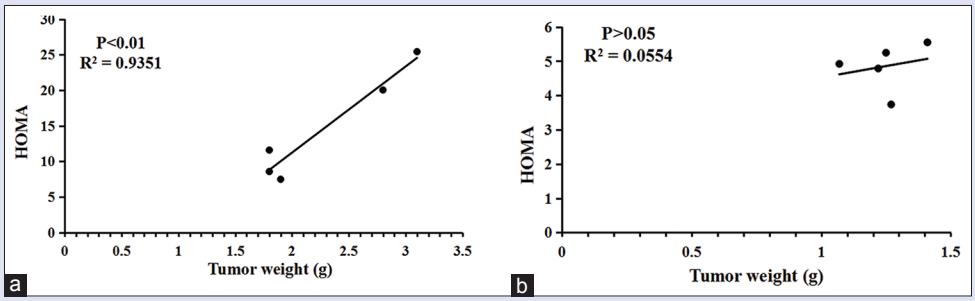

Figure 5: The correlation of insulin resistance level with tumor weight in monosodium glutamate mice (a) and control mice (b)

were designed according to the corresponding mice genes. A reverse transcription kit (Takara, Japan) was used to synthesize the first strand of cDNA by the template RNA and quantitative PCR instrument (Agilent Technologies Co., Ltd) was performed for gene amplification. The forward and reverse primer sequences were as below:

GAPDH:

Forward: 5'-GACAAAATGGTGAAGGTCGGTG-3'

Reverse: 5'-TGATGTTAGTGGGGTCTCGCTC-3'

Glut 1:

Forward: 5'-GCAGTTCGGCTATAACACTGG-3'

Reverse: 5'-GAGACCAAAGCGTGGTGAGT-3'

PDK4:

Forward: 5'-GATTGACATCCTGCCTGACC-3'

Reverse: 5'-CTTCTGGGCTCTTCTCATGG-3'

PFK:

Forward: 5'-GCCGTGTTGACCTCTGGT-3'

Reverse: 5'-GCCCGTGAAGATACCAACTC-3'

LDHB:

Forward: 5'-TGGTGGACAGTGCCTATGAA-3'

Reverse: 5'-GAATCCGGGAGAGGTTTTTC-3'

G-6-Pase:

Forward: 5'-GACAACGTCAGATGGTGTCAT-3'

Reverse: 5'-TGCTTGTGTCAACAAAACAATGT-3'

PEPCK:

Forward: 5'-ATCAAGGGACGGCTGAACAG-3'

Reverse: 5'-ACTGCTGGATGTACCTTTTTCC-3'

Sirt6:

Forward: 5'-GGAGAATGTGTCAGAGGACGAGAT-3'

Reverse: 5'-GTGACAGTGAAAATGGACCGTAGAA-3'

Statistical analysis

The data were shown as means ± standard error. The comparisons between groups were performed using one-way analysis of variance (ANOVA) followed by *post hoc* least significant difference test or two-way ANOVA followed by *post hoc* Bonferroni's test. $P < 0.05$ was considered as statistically significant.

RESULTS

Pentamethylquercetin improved abdominal obesity, glucose and lipid metabolism, and insulin resistance of monosodium glutamate mice

The effectiveness of PMQ in alleviating MetS was assessed in MSG mice. Our data showed that body weight, LEE index, and waist circumference were increased in MSG mice. However, PMQ treatment significantly improved abdominal obesity [Figure 1]. PMQ has no obvious effect on

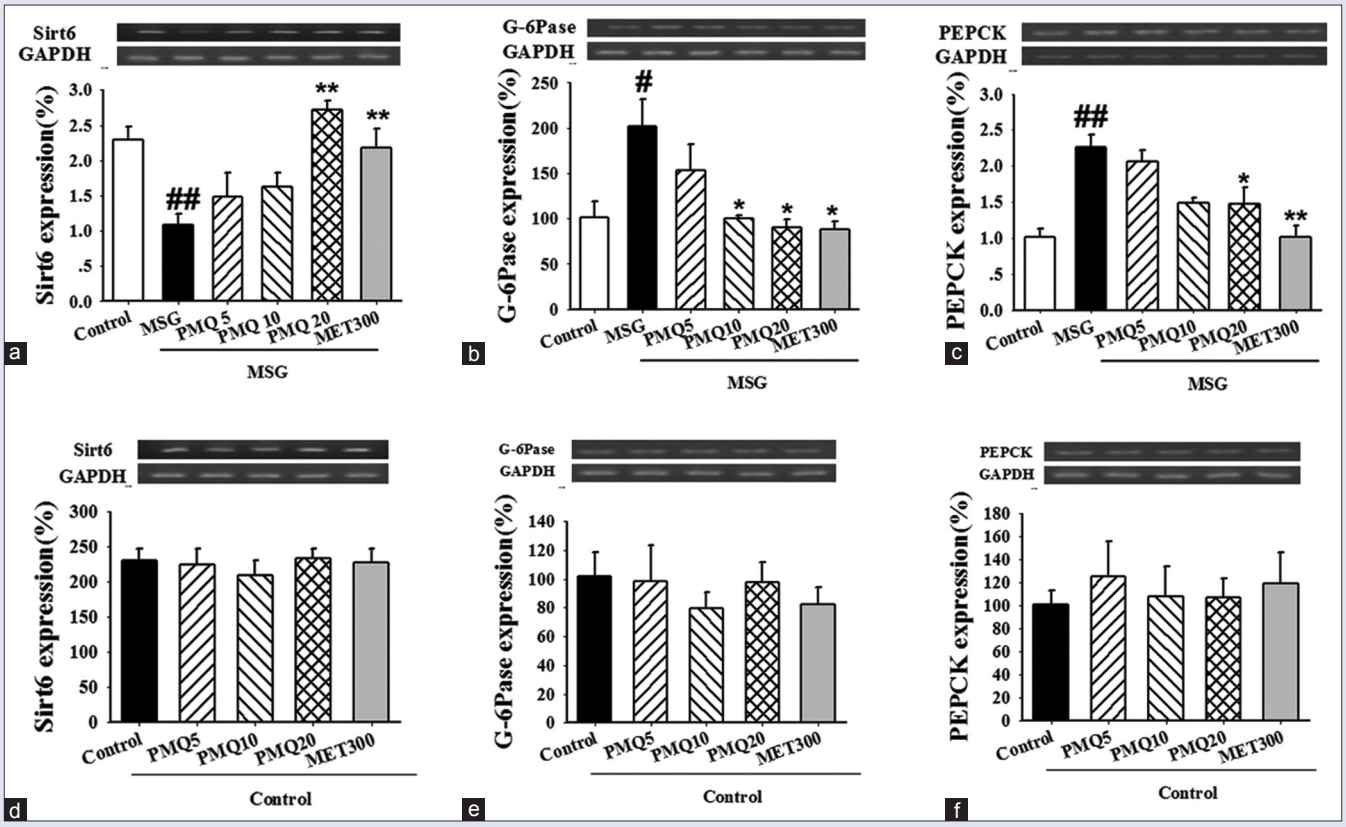

Figure 6: Sirtuin 6, G-6-Pase, and PEPCK mRNA expressions in the liver tissue of monosodium glutamate mice (a-c) and control mice (d-f). Data expressed as mean ± standard error ($n = 3$). Data expressed as mean ± standard error ($n = 3$). *$P < 0.05$ versus monosodium glutamate

control mice.

In addition, blood glucose, triglyceride, cholesterol, insulin level, and especially HOMA index, were increased in MSG mice as well. However, PMQ improved the above metabolic indexes in the MSG mice. In all, PMQ treatment significantly improved abdominal obesity, hyperglycemia, dyslipidemia, and insulin resistance in MSG mice and had no effect on the normal mice [Figure 2].

Inhibition effect of pentamethylquercetin on tumor growth

As shown in Figure 3, tumor size and weight in MSG mice were much larger than those of control mice. However, PMQ intervention significantly reduced tumor growth rate and tumor size in MSG mice and had a little suppressive effect on tumor growth in the control mice. Taken together, PMQ significantly decreased tumor growth rate in MSG mice.

Pentamethylquercetin inhibits tumor growth by enhancing Sirtuin 6 expression and reducing aerobic glycolysis

Sirt6, a member of sirtuins family, serves as a tumor suppressor that regulates aerobic glycolysis (Warburg effect) in cancer cells. To determine whether PMQ decreased tumor growth by inhibition of aerobic glycolysis (Warburg effect), we investigated mRNA expressions of Sirt6 and important enzymes in aerobic glycolysis pathway in tumor tissue, including GLUT1, PDK4, PFK, and LDHB. Our data showed that mRNA expressions of Sirt6 were significantly upregulated in all PMQ-treated mice tumor tissue [Figure 4]. Moreover, after PMQ intervention, the mRNA expressions of GLUT1, PDK4, PFK, and LDHB were all downregulated [Figure 4]. Taken together, PMQ possessed a suppressive effect on aerobic glycolysis of tumor tissue both in control and MSG mice.

Pentamethylquercetin improving insulin resistance and thereby reducing monosodium glutamate mice tumors

Since we observed no significant effect of PMQ on tumor growth in control mice, we hypothesized that PMQ attenuated tumor growth in the MSG mice through improving metabolic disorders, in addition to aerobic glycolysis. To evaluate a possible association between metabolic changes and tumor growth, the correlation of insulin resistance level with tumor weight was assessed. We found that tumor growth varied in proportion to the insulin resistance, suggesting that tumor growth inhibition might rely on lower insulin resistance index after PMQ administration [Figure 5].

Specifically, elevation hepatic Sirt6 expression might be useful in suppressing the chronically active hepatic gluconeogenesis commonly found in insulin-resistant diabetes. To explore whether PMQ improving insulin resistance by enhancing Sirt6 expression in liver, we investigated mRNA expressions of Sirt6 and important enzymes in hepatic gluconeogenesis in liver tissue, including G-6-Pase and PEPCK. Our results demonstrated that PMQ significantly improved mRNA expressions of Sirt6, G-6-Pase, and PEPCK in the liver of MSG mice compared with those of control mice [Figure 6]. In all, the effects of PMQ on the insulin resistance might be attributed to elevation Sirt6 expression in liver.

DISCUSSION

In our research, we found that MSG mice had obvious central obesity, metabolic disorders, and insulin resistance. Tumor size and weight in MSG mice were much larger than those of control mice. Correlation analysis showed that tumor growth varied in proportion to the insulin resistance of MSG mice. These results indicated that metabolic disorders lead to excessive tumor growth.

Using MSG mice model to assess the antitumor effect PMQ in the metabolic syndrome mice model. We showed that PMQ treatment significantly improved hyperglycemia, dyslipidemia, and insulin resistance in MSG mice. PMQ intervention significantly reduced much more tumor growth rate and tumor size in MSG mice than those of control mice. Moreover, correlation analysis indicated that tumor growth inhibition in MSG mice might be partly attributed to insulin resistance correction after PMQ administration. We speculated that the antitumor effect of PMQ might include indirect effect – improving insulin resistance and direct effect – correcting Warburg effect. All of the above results showed that PMQ might be a promising agent for preventing tumor in the context of MetS.

Sirt6, a member in sirtuins family, serves as a tumor suppressor. Sirt6 regulates cancer development by interaction with oncogenes along with inhibiting the metabolic shift toward anaerobic glycolysis – correcting Warburg effect. Human pancreatic and colorectal tumors are highly glycolytic with downregulation of Sirt6 expression in these tumors.[19] Sirt6 expression is also downregulation in human HCC compared with normal liver.[30] In addition to influence cancer development, Sirt6 also plays important role in liver cancer initiation through binding and deacetylate H3K9-Ac target on the promoter of oncogene survivin. In the present research, all dose PMQ could increase Sirt6 mRNA expressions and decrease Glut 1, PDK4, PFK, and LDHB mRNA level both in tumor tissue of control and MSG mice. This result showed that the direct effect of PMQ in inhibition tumor growth might be attributed to correct Warburg effect in the tumor tissue.

Sirt6 also plays a significant role in metabolic disorders. Livers of diabetic db/db mice are found to contain reduced levels of hepatic sirt6. Re-expression of Sirt6 in the db/db mice can suppress expression of gluconeogetic genes, reduce circulating glucose level, and improve insulin resistance.[18] Overexpression of Sirt6 in mice protects them from various pathologies caused by high-fat-diet-induced obesity.[12] The present study showed that PMQ treatment enhanced Sirt6 mRNA levels and decreased G-6-pase, PEPCK mRNA expressions in the liver of MSG mice. However, PMQ had no significant effect on the control mice. These results indicated that indirect antitumor effect of PMQ might be attributed to improving insulin resistance by upregulation Sirt6 in liver and inhibition gluconeogenic genes mRNA expressions.

In conclusion, the present study suggested that PMQ exerted its beneficial effects on tumor growth in MSG mice, which might be attributed to improving insulin resistance and correct Warburg effect. Therefore, PMQ could be a recommended and possible candidate for preventing tumor growth in people with MetS. However, further studies are needed to clarify the exact mechanisms of PMQ in inhibition tumor in the context of MetS.

CONCLUSION

MQ decreased tumor growth in the MSG mice and the potential mechanisms might attribute to upregulated mRNA expressions of sirt6. PMQ could be a recommended and possible candidate for preventing tumor growth in people with MetS. However, further studies are needed to clarify the exact mechanisms of PMQ in inhibition tumor in the context of MetS.

Financial support and sponsorship

We gratefully acknowledge the Yichang Key Laboratory of Ischemic Cardiovascular and Cerebrovascular Disease Translational Medicine Foundation (2017KXN09) for generous financial support.

Conflicts of interest

There are no conflicts of interest.

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