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FULL PAPER

# Hepatoprotective effect of *Carica pubescens* juice and rutin through improving the Adenosine Monophosphate-activated Protein Kinase (AMPK) pathway in type 2 diabetes Mellitus Wistar Rats

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**Abstract**

Insulin resistance in type 2 diabetes mellitus (type 2 DM) results in disturbing glucose transport into the cells and increases glucose production in the liver and exacerbates hyperglycemia. It is also correlated with a decrease of adenosine monophosphate-activated protein kinase (AMPK) expression that has effects on insulin resistance aggravation, antioxidant capacity impairment, and glucose-lipid homeostasis disturbance indicates AMPK as a significant target for type 2 DM prevention and treatment. The flavonoid content of *Carica pubescens* (CP) fruit contains anti-hyperglycemic and anti-hyperlipidemic properties, however, there is no study about its effect on AMPK expression**.** This study aimed to investigate the effect of CP fruit juice and rutin on preventing liver damage in type 2 DM through the pathway of AMPK, superoxide dismutase (SOD), high-density lipoprotein (HDL), and triglyceride (TG). The experimental design was a post-test only control group. The immunohistochemical method was used to examine AMPK expression in 25 liver paraffin blocks from five groups of male Wistar rats: C1 = negative control; C2 = type 2 DM; C3 and C4 = type 2 DM given fruit juice at a dosage of 4 g CP/200 g BW/day and 8 g CP/200 g BW/day, respectively; and C5 = type 2 DM given rutin 10 mg/200 g BW/day, for 30 days treatment. AMPK expression in the liver significantly increased in the C3, C4, and C5 compared to the C2 group ($p<0.05$). There was no significant variation in AMPK expression among C3, C4, or C5, showing that C1 possesses the same capabilities as C4 and C5 groups. The increase in AMPK expression was followed by SOD and HDL levels and lower TG levels. However, a significant correlation was shown in the TG level only. This indicates CP fruit juice and rutin can act as a hepatoprotective to prevent liver damage through the AMPK pathway due to type 2 DM conditions.

## 1. Introduction

Type 2 diabetes mellitus (type 2 DM) is a metabolic disorder characterized by elevated blood glucose levels (hyperglycaemia) and impaired glucose utilization in peripheral tissues due to disturbance of carbohydrate, protein, and fat metabolism. This occurs because of the body's inability to respond to insulin (insulin resistance) or decreased insulin production, thus, glucose is unable to enter the cells (Fatimah, 2015; IDF, 2019). Low-density lipoprotein (LDL) and triglyceride (TG) levels are frequently elevated in type 2 DM, while high-density lipoprotein (HDL) levels are lowered (Josten, 2018). Due to the difficulty of glucose to enter cells, the rate of energy formation reduces, and cells suffer a lack of energy, this circumstance is noticed by the body's homeostatic system, which boosts glucose production in the liver in compensation (Guyton and Hall, 2014; Triana and Salim, 2017). The liver, as a centre for glucose and lipid homeostasis and as energy storage for the body, will meet glucose needs via the process of

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gluconeogenesis. One of the significant reasons occur hyperglycemia in type 2 DM is increased gluconeogenesis (Guyton and Hall, 2014).

Adenosine monophosphate-activated protein kinase (AMPK) is a regulator of the body's homeostatic balance in glucose and lipid metabolism (Garcia and Shaw, 2017). When the body's energy supplies decrease, AMPK acts as a sensor and attempts to improve energy formation by degrading glucose reserves in the liver. AMPK exists in isoforms α1, α2, β1, β2, γ1, γ2, and γ3, but the α2 isoform is more abundant in skeletal muscle, heart, and liver and is involved in transcription and gene expression regulation. Activating AMPK in the liver increases glucose uptake and fatty acid oxidation while suppressing gluconeogenesis, cholesterol synthesis, and TG production (Gruzman *et al.*, 2009; Toyoda *et al.*, 2012; Coughlan *et al.*, 2014). Additionally, AMPK activation contributes to mitochondrial biogenesis and dynamics by limiting the generation of reactive oxygen species (ROS). This helps avoid oxidative stress and restores mitochondrial function and the body's antioxidant capacity, including superoxide dismutase (SOD) (Dugan *et al.*, 2013).

Insulin resistance relates to a decrease in AMPK expression or activity, where glucose-deficient body cells promote glucagon secretion to stimulate the liver to create glucose via the gluconeogenesis process, hence it increases blood glucose levels in the blood and exacerbates DM problems (Oktiyani *et al.*, 2015; Jeon, 2016). AMPK regulates several hormones, including glucagon (Hasenour *et al.*, 2013). Additionally, low AMPK expression impairs antioxidant capacity and disrupts glucose and lipid homeostasis, resulting in complications. Due to AMPK's critical function in controlling energy homeostasis and DM disease pathology, AMPK has been identified as a target for DM disease therapy (Foretz *et al.*, 2005; Jeon, 2016).

Flavonoids are a type of polyphenolic compound that have been found to improve insulin resistance and increase insulin production (Soares *et al.*, 2017). *Carica pubescens* (CP) is a characteristic plant of Indonesia's Dieng Plateau. Quercetin, rutin, chlorogenic acid, and coumaric acid are bioactive compounds found in the CP fruit that have a variety of health benefits (Pinto *et al.*, 2009; Rachmawati, 2019; Kusuma *et al.*, 2020). A biochemical study showed that CP fruit has anti-hyperglycemic activity, the results of this study are in line with other studies evaluating the administration of CP fruit juice in lowering blood glucose levels and tumour necrosis factor-α (TNF-α) in type 2 DM rats (Pinto *et al.*, 2009; Kusuma *et al.*, 2020). CP fruit is also beneficial as hepatoprotective by lowering aspartate transaminase (AST) and alanine aminotransferase (ALT) levels in acetaminophen-induced rats (Sasongko *et al.*, 2018). A rutin compound was shown to decrease the expression of gluconeogenic genes involved in gluconeogenesis by reducing the activity of glucose-6-phosphate (G6Pase) and phosphorylated glycogen via increased AMPK activity in the liver at a dosage of 50 mg/kg BW (Ahmed *et al.*, 2010). Another investigation showed that a rutin compound at a dosage of up to 100 mg/kg BW could reduce G6Pase activity in the liver by 31%, G6Pase activity contributing to the increase in plasma glucose via the gluconeogenesis process (Prince and Kamalakkannan, 2006). However, research on the potential of CP fruit to ameliorate DM conditions, mainly to increase AMPK expression, is still incomplete. This study aimed to investigate the hepatoprotective effect of CP fruit juice and rutin through increased expression of AMPK in the liver and its relationship with superoxide dismutase (SOD), high-density lipoprotein (HDL), and triglyceride (TG) levels in type 2 DM.

## 2. Materials and methods

### *2.1 Material and reagent*

The CP fruit in this study grew at an altitude of 2093 masl altitude in the Dieng Plateau, Wonosobo-Indonesia, with a maturity level of 90% marked with a yellow colour on 90% of the skin (Figure 1). Nicotinamide (NA) and STZ were procured from Nacalai Tesque, Kyoto-Japan, and rutin powder was procured from Xi'an Imaherb Biotech Co., Ltd. AMPK expression measurement was carried out by immunohistochemical method using rabbit polyclonal antibody AMPK-α2 (catalogue number A7339; ABclonal). SOD levels (catalog number #K335-100; BioVision), HDL levels (catalog number 1 3540 99 90 885; DyaSis), and TG levels (catalog number 1 5760 99 10 021; DyaSis) were examined using rat serum.

Figure 1. CP fruit with a maturity level of 90%. It is characterized by a yellow colour on 90% of the fruit skin.

### *2.2 Animals and treatment*

This study used 25 paraffin blocks of the liver of Wistar rats (*Rattus norvegicus*) from previous studies (Rachmawati, 2019). Wistar rats were supplied from

 

Gajah Mada University's Central Food and Nutrition Laboratory (Yogyakarta-Indonesia) with criteria aged two months old, body weight 150-200 g, healthy condition, and active movement. Wistar rats were placed in individual cages with environmental conditions of 23±2°C, a light-dark cycle of 12-12 hrs, humidity 50±5%, and rats were given ad libitum drink. Wistar rats were acclimatized for 7 days and given standard feed comfeed II of 20 g/day containing 3-7% crude fat, 6% crude fibre, 15% crude protein, 12% water, 7% ash, phosphorus 0.6-0.9%, and calcium 0.9-1.1%.

After acclimatization, the DM rats group were given HFD feed for 14 days, as much as 20 g/rats/day. The composition of the HFD given was 90% comfeed II, 10% lard, and 1.25% pure cholesterol. On day 22, rats given HFD were induced with NA as much as 110 mg/kg BW and 15 minutes after NA induction followed by 40 mg/kg BW STZ. Sodium chloride solvent 1.5 mL/100 g BW was used for NA, and sodium citrate buffer 1.5 mL/100 g BW was used as a solvent for STZ. Three days later, the rats fasted for six to eight hours, and the blood was drawn through the retro-orbital plexus to check fasting blood glucose levels. Rats were confirmed with DM if blood glucose levels were >200 mg/dL (Ghasemi *et al.*, 2014).

Randomly, the rats were divided into two groups: 5 rats in the non-HFD as negative control rats (C1) and 20 rats receiving STZ-NA-induced HFD were divided into four groups: type 2 DM control rats (C2); type 2 DM Wistar rats and fruit juice with a dosage 4 g CP/200 g BW/day (C3); type 2 DM Wistar rats and fruit juice with a dosage 8 g CP/200 g BW/day (C4); type 2 DM Wistar rats and rutin with a dosage 10 mg/200 g BW/day (C5). For 30 days, all interventions were administered orally. Previous studies have shown that rutin with dosages of 50 and 100 mg/kg administered orally effectively lowers glucose levels, improving the condition of antioxidant capacity and lipid profiles in DM rats (Niture *et al.*, 2014).

After a fast of six to eight hours, blood was drawn through the retro-orbital plexus as much as 2 mL and centrifuged for 15 mins at 4000 rpm to obtain blood serum. Rats were terminated 30 days after receiving the intervention or on the 31$^{st}$ day. The rats were placed in a jar containing cotton treated with ether, and pain stimulation was used to determine whether the anaesthetic effect had been achieved. After the rat lost consciousness, the neck was dislocated to end the experiment. The surgical procedure was carried out on the abdominothoracic, and the liver was necropsied. The liver organs were sliced and placed overnight at room temperature in a 10% formalin buffer solution container. Using a microtome, the liver that had been fixed and processed into paraffin blocks was sliced with a thickness of 4-6 µm. The experimental animal treatment flow is illustrated in Figure 2.

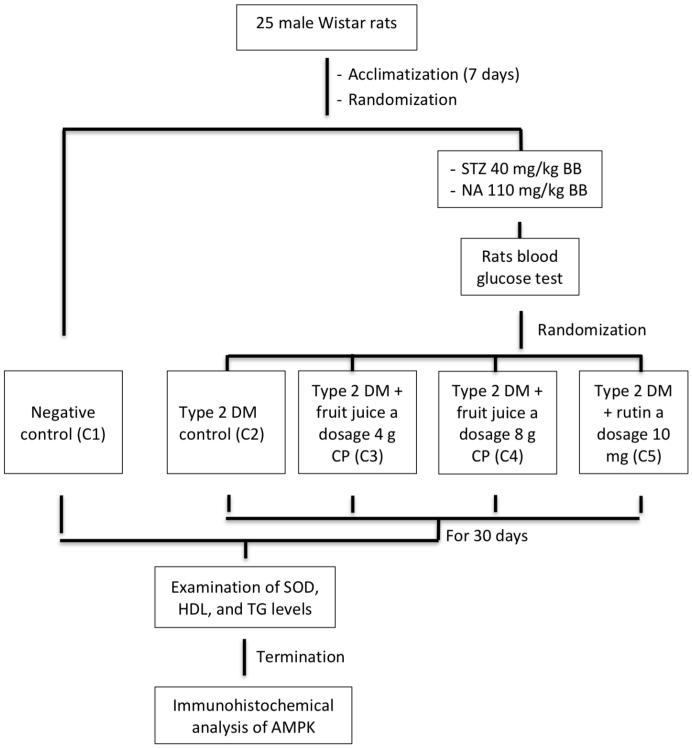


Figure 2. Experimental animal treatment flow

### *2.3 Preparation of Carica pubescens fruit juice*

CP fruit peeled and seeds removed. The fruit is cut into small pieces and boiled for 3 mins at 60°C to remove the sap and soften the texture. After that, 100 g of CP fruit was juiced using a blender and homogenizer. We prepared a daily fresh juice and gave it to the rats according to their weight. The residue of CP fruit juice that was not used (approximately 30 g per day) was diluted with water before being discarded.

### *2.4 Measurement of AMPK expression*

Slide preparation and immunohistochemical examination were carried out at the Anatomical Pathology Laboratory, Faculty of Medicine, Sebelas Maret University, Surakarta-Indonesia. Observations were made by two pathologists. AMPK expression was positive if the nucleus was stained brown and observations were made at the location of hepatocytes in the liver. AMPK expression was assessed semi-quantitatively using the modified Remmele method, the result of the immunoreactivity score was the multiplication of the proportion of cells and the intensity of staining of the stained cells. The proportion of cells is divided into 0 = no stained cells; 1 = <10% of stained cells; 2 = 10-50% of stained cells; 3 = 51-80% of stained cells; 4 = >80% of stained cells, while the colour

 

intensity is divided into 0 = negative; 1 = weak colouring; 2 = medium colouring; 3 = strong colouring. The proportion and cell intensity scores were multiplied and then divided into 4 groups, namely: 0-1 = negative (0); 2-3 = weak immunoreactivity (1); 4-8 = moderately immunoreactive (2); 9-12 = strong immunoreactive (3) (Kaemmerer *et al.*, 2012).

### *2.5 Measurement of SOD, HDL, and TG levels*

Examination of SOD levels used the enzyme-linked immunosorbent assay (ELISA) method from BioVision. The cholesterol oxidase-p-aminophenazone (CHOD-PAP) method from DyaSis and the DyaSis glycerol-3-phosphate oxidase-phenol aminophenazone (GPO-PAP) were used to determine HDL and TG levels, respectively. The detailed procedure was described in our previous study (Rachmawati, 2019).

### *2.6 Statistical analysis*

Data were analyzed using the IBM SPSS Statistics 25 computer program. Data are presented as mean ± standard deviation (SD). The normality distribution of the research data used the Shapiro-Wilk test. Then, it was analyzed using the Kruskal-Wallis test to determine the differences in AMPK expression between the intervention groups and continued with the Mann-Whitney test to determine the significant differences between the interventions. A Kendall-Tau test was used to analyze the relationship between AMPK expression in the liver with SOD, HDL, and TG levels. All of the data in the study were evaluated using a p(0.05) significance value and 95% CI.

### *2.7 Ethical clearance*

This study was approved by the Health Research Ethics Committee, Faculty of Medicine, Diponegoro University, Semarang No. 26/EC/H/FK-UNDIP/III/2021.

## 3. Results

### *3.1 Effect of CP fruit juice and rutin on AMPK expression*

Figure 3 shows AMPK expression in the hepatocytes of the liver using a light microscope with 100x magnification. AMPK expression was observed in all fields of view. AMPK expression in the positive liver was marked by brown colour in the nucleus, and the results show a significant difference in AMPK expression after intervention (p = 0.027; Kruskal-Wallis test).

Figure 4 shows that AMPK expression in the DM group is significantly lower than that in the negative control group (C2 = 1.40±0.548 vs. C1 = 2.60±0.548 immunoreactive score, p = 0.020; Mann-Whitney test). The group gives CP fruit juice and rutin is significantly effective in increasing AMPK expression compared to the untreated group of rats (C3 = 2.80±0.447, p = 0.011; C4 = 2.60±0.548, p = 0.020; C5 = 2.60±0.548, p = 0.020 vs. C2 = 1.40±0.548; Mann-Whitney test).

The fruit juice treatment group at a dosage of 4 g CP/200 g BW/day (C3) does not significantly increase AMPK expression compared to the fruit juice group at a dosage of 8 g CP/200 g BW/day (C4) and rutin at a dosage of 10 mg/200 g BB/day (C5) (C3 vs. C4 = 0.513; C3 vs. C5 = 0.513; and C4 vs. C5 = 1.000; Mann-Whitney test).

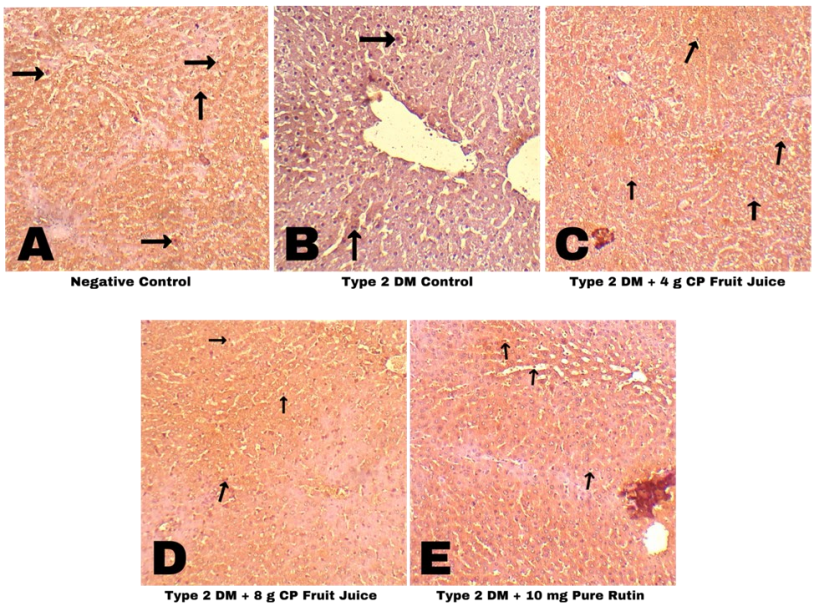


Figure 3. AMPK expression in liver hepatocytes. AMPK expression was positive when the nucleus in the hepatocyte section was stained brown. AMPK expression (black arrow) in the negative control rats (A), DM rats without intervention (B), DM rats and fruit juice at a dosage of 4 g CP/200 g BW/day (C), DM rats and fruit juice at a dosage of 8 g CP/200 g BW/day (D), as well as DM rats and rutin at a dosage of 10 mg/200 g BW/day (E). Observations using a light microscope with a magnification of 100×.

 

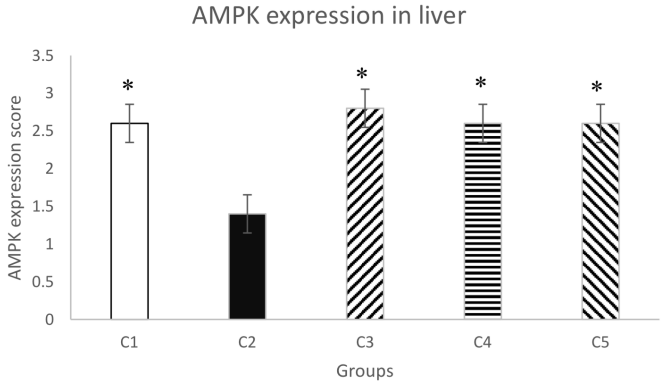


Figure 4. Mean AMPK expression scores in the liver. Comparison of AMPK expression in the liver showed a significant difference before administration of the intervention (C2) and after administration the intervention (C1) along with the addition of fruit juice at a dosage of 4 g CP/200 g BW/day (C3); fruit juice at a dosage of 8 g CP/200 g BW/day (C4), and rutin at a dosage of 10 mg/200 g BW/day (C5). Error bars show the standard error of the mean of each group. Statistical analysis was measured using the Kruskal-Wallis test and followed by the Mann-Whitney test. *compared to C2; all p<0.05; Mann-Whitney test.

### *3.2 The correlation between AMPK expression in the liver with SOD, HDL, and TG serum levels*

Figure 5 shows that mean scores of liver AMPK expression, SOD, and HDL levels have the same pattern. The negative control experimental group (C1) shows high scores and levels of AMPK expression, SOD, and HDL, while the C2 group experiences a decrease. After the intervention of CP fruit juice and rutin (C3, C4, and C5), there is an improvement in AMPK expression, SOD, and HDL levels. Differences in graphic patterns occur in the C4 group, AMPK expression decreases and are stable in the C5 group, while the levels of SOD and HDL experience an increased pattern in the C4 and C5 groups.

Figure 5 shows the difference in graphic patterns between AMPK expression and TG levels. In the C2 group, it shows high AMPK expression with low TG levels. However, in the C2 group, AMPK expression decreases and are followed by an increase in TG levels. The intervention of CP fruit juice and rutin (C3, C4, and C5) improves AMPK expression, accompanied by a decrease in TG levels in DM conditions. Differences in graphic patterns occur in group C4, AMPK expression decreases slightly and is stable in group C5, while TG levels show a decrease (C4) and slightly reduce again (C5).

Table 1 shows that there is no significant correlation between AMPK expression with SOD levels (p = 0.053; r:0.318; Kendall-Tau test), and HDL levels (p = 0.111; r: 0.260; Kendall-Tau test). However, both variables have a positive direction on AMPK expression. The activation of AMPK expression was correlated with lower TG levels (p = 0.020; r:-0.381; Kendall-Tau test).

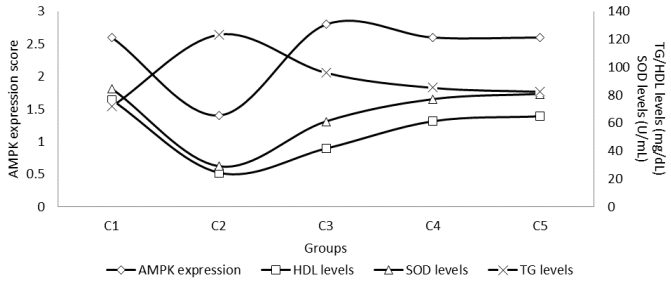


Figure 5. Mean graphic of liver AMPK expression, SOD, HDL, and TG levels. The graphic shows that the lowest AMPK expression, SOD, and HDL levels occurred in the C2 group, while TG levels increased. After being given CP fruit juice or rutin (C3, C4, and C5), it showed an increase in AMPK expression, SOD, and HDL levels accompanied by a decrease in TG levels. The correlation test reported that the graphic pattern of AMPK expression, SOD, and HDL levels had a positive (unidirectional) relationship. In contrast, AMPK expression and TG levels had a negative (opposite) relationship (showed in Table 1).

Table 1. Correlation test AMPK expression with SOD, HDL, and TG levels

| Kendall-Tau analysis | | SOD levels | HDL levels | TG levels |
|---|---|---|---|---|
| AMPK expression | r | 0.318 | 0.260 | -0.381 |
| | *p* | 0.053 | 0.111 | 0.020 |
| | n | | 25 | |

r = Correlation coefficient, p = Significant value, n = Total sample

## 4. Discussion

In DM conditions, this study demonstrated that CP fruit juice increased AMPK expression in the liver. Increased AMPK expression is expected to prevent or reduce the risk of complications associated with DM (Coughlan *et al.*, 2014). Additionally, this study examined the link between AMPK expression with SOD, HDL, and TG levels.

The results of this study indicate that the DM condition causes a decrease in AMPK expression. The low AMPK expression is an effort to stimulate glucagon secretion to increase glucose production to be used as energy through the gluconeogenesis process as compensation for insulin resistance. (Viollet *et al.*, 2010; Hasenour *et al.*, 2013; Oktiyani *et al.*, 2015; Jeon, 2016). The results of a study examining the deletion of the AMPK isoform in rats' livers revealed poor glucose tolerance and hyperglycemic conditions were associated with increased G6Pase activity, resulting in increased glucose synthesis in the liver (Andreelli *et al.*, 2006). Additionally, decreased AMPK expression might exacerbate pre-existing insulin resistance problems

 

because high levels of free fatty acids due to consequently increased gluconeogenesis result in the accumulation of diacylglycerol (DAG), a long-chain fatty acid molecule that can activate protein kinase-c (PKC). This increases insulin-receptor substrate (IRS) serine phosphorylation and inhibits insulin signalling regulation in skeletal muscle, adipose tissue, and liver (Setyawati, 2014).

The increase in AMPK expression in the intervention group is expected because of the presence of bioactive compounds in CP fruit juice, such as rutin and coumaric acid, which have been shown to inhibit gluconeogenic gene expression by increasing AMPK expression in the liver, thereby inhibiting G6Pase activity (Ahmed *et al.*, 2010; Shairibha *et al.*, 2014; Ghorbani, 2017). Additionally, the rutin and quercetin content can stimulate insulin secretion and improve insulin resistance conditions by increasing the translocation of glucose-transporter-4 (GLUT-4) in the liver, muscle, and adipose tissue to increase glucose uptake and energy needs are met, resulting in increased AMPK expression in the liver to inhibit gluconeogenesis. Rutin can also improve insulin sensitivity by raising the expression of peroxisome proliferator-activated receptor-γ (PPAR-γ), which PPAR-γ is activated by AMPK is capable of stimulating fatty acid storage in adipose tissue with the result that lowering plasma fatty acid levels and reducing lipotoxicity in muscle, pancreas, and liver (Ahmed *et al.*, 2010; Kawser *et al.*, 2016; Ghorbani, 2017).

It is well established that chlorogenic acid in CP fruit juice activates AMPK, hence it suppresses glucose synthesis in the liver by lowering G6Pase expression and activity. Additionally, chlorogenic acid can improve hyperglycemic conditions by enhancing insulin sensitivity and inhibiting α-glucosidase activity, to reduce postprandial blood glucose levels (Meng *et al.*, 2013; Ong *et al.*, 2013).

In this study, there were no significant differences between groups C3, C4, and C5, which indicated that the three treatment groups had the same ability to increase AMPK expression. The insignificant difference between groups C3 and C4 could be caused because the dosage in this study had reached the maximum therapeutic effect, as seen by the larger dosage not generating a more significant better therapeutic benefit (Katzung, 2018). The results indicated that fruit juice 4 g CP/200 g BW/day was the optimal dosage with the most therapeutic effect on boosting AMPK expression. The absence of significant differences between the C3 and C5 groups was attributed to the complex composition of bioactive compounds found in CP fruit, such as rutin, quercetin, and others, all of which have many benefits to synergize to produce effects equivalent to rutin dosages (Pinto *et al.*, 2009).

The relationship between AMPK expression and SOD and HDL levels in this study has not shown a significant value. These results indicate that an increase in AMPK expression is not always accompanied by an increase in SOD and HDL levels, presumably due to differences in the effectiveness of CP fruit juice or rutin in increasing AMPK SOD and HDL expression. Previous studies reported that a dosage of 8 g CP/200 g BW/day could increase SOD and HDL levels, while an increase in AMPK expression was obtained at a dosage of 4 g CP/200 g BW/day. These results indicate that the administration of CP fruit juice is more able to improve the factors that affect AMPK expression compared to the factor affecting SOD and HDL, such as rutin, coumaric acid, and chlorogenic acid, which can reduce the expression of gluconeogenic genes. In addition, rutin, quercetin, and chlorogenic acid can improve insulin resistance conditions and stimulate insulin secretion so that AMPK expression increases (Ahmed *et al.*, 2010; Ong *et al.*, 2013; Shairibha *et al.*, 2014; Kawser *et al.*, 2016; Ghorbani, 2017).

Although SOD levels can be activated by AMPK, a non-significant correlation is thought to occur because SOD levels can also be influenced by other pathways, such as p38 mitogen-activated protein kinase (p38MAPK), sirtuins (SIRTs), nitric oxide synthase (NOS/cGMP) that regulates the production of antioxidant enzymes through increased activation of peroxisome proliferator-activated receptor-gamma co-activator 1-α (PGC-1α) thereby enabling CP to improve SOD through this pathway. In addition, SOD levels are also influenced by the number of free radicals in the body (Ventura *et al.*, 2008; Rowe *et al.*, 2010; Leo *et al.*, 2016). Similar to SOD levels, HDL levels are not only affected by AMPK activity but can also be affected by the cholesterol ester transfer protein (CETP) pathway and the trafficking protein kinesin binding 2 (TRAK2), which contributes to HDL metabolism and function (Barter and Kastelein, 2006; Lake *et al.*, 2017).

Table 1 shows AMPK expression is significantly correlated with TG levels with a negative relationship, this indicates that the higher AMPK, the lower the TG levels. This condition is thought to occur because CP fruit contains compounds that can improve AMPK expression conditions so that it can reduce TG levels. Increased AMPK expression was able to inactivate the glycerol-3-phosphate acyltransferase (GPAT) enzyme in the liver, which is the first enzyme to initiate the TG synthesis process. When the GPAT enzyme is inactive, phosphatidate is not formed, which will later be

 

converted into TG with the help of diacylglycerol acyltransferase (DGAT). Research conducted by Linden *et al*. showed that overexpression of GPAT caused a decrease in fatty acid oxidation up to 80% and an increase in diacylglycerol resulting in a significant increase in TG (Muoio *et al.*, 1999; Ruderman *et al.*, 2003; Lindén *et al.*, 2004; Wang *et al.*, 2018).

Insulin resistance and type 2 DM are often associated with an increased risk of non-alcoholic fatty liver disease (NAFLD) disease with a prevalence of up to 70%, this is due to increased lipid production in the liver (Li *et al.*, 2020). The increase in lipid production is due to the increased expression of lipogenic genes, such as sterol regulatory element-binding proteins (SREBP-1), GPAT, acetyl-CoA carboxylase-1 (ACC1), and fatty acid synthase (FAS), where the expression of these genes is regulated by AMPK (Viollet *et al.*, 2009; Srivastava *et al.*, 2012; Jeong *et al.*, 2014; Garcia *et al.*, 2020). Besides being able to reduce TG levels, AMPK activation can reduce hepatic lipids via a variety of mechanisms, including AMPK phosphorylation of SREBP at Ser372, inhibiting the formation of mature SREBP-1c; AMPK suppresses SREBP-1 via the mammalian target of rapamycin complex (mTORC) pathway; and AMPK suppresses the expression of the SREBP-1 gene, which produces lipogenic genes such as ACC1 and FAS so that lipid synthesis will be inhibited. AMPK can also inhibit cholesterol synthesis by suppressing the activity of SREBP-2, thereby suppressing the expression of its downstream target, 3-hydroxy-3-methylglutaryl-coenzyme a reductase (HMGR). AMPK can phosphorylate HMGR, rendering it inactive and incapable of forming mevalonic acid, the raw material for cholesterol (Wang *et al.*, 2018; Garcia *et al.*, 2020).

The results showed that giving CP fruit juice or rutin could improve levels SOD, HDL, TG (Rachmawati, 2019), and expression AMPK, this indicates that giving CP fruit juice can act as hepatoprotective to prevent or reduce the risk of NAFLD in type 2 DM. These results are supported by previous studies showing that administration of ethanolic extract of *Carica pubescens* fruit can act as hepatoprotective by reducing the levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzymes in the liver (Sasongko *et al.*, 2018).

## 5. Conclusion

CP fruit juice and rutin can increase AMPK expression in the liver tissue as well as increase SOD and HDL in the serum level and reduce TG serum level in type 2 DM Wistar rats. The CP fruit juice at a dosage of 4 or 8 g/200 g BW/day has an equal effect with 10 mg/200 g BW/day of rutin in increasing AMPK expression in liver tissue. However, the effect of CP fruit in the blood biomarkers (SOD, HDL, and TG) that are equivalent to rutin is at a dosage of CP fruit juice 8 g/200 g BW/day. The increase in AMPK expression in the liver significantly correlated with decreased TG levels after CP fruit juice or rutin administration indicating that CP fruit juice or rutin can act as a hepatoprotective by improving the AMPK pathway associated with DM conditions. The results of this study suggested that CP could be a potential candidate for an alternative treatment to avert disease progression and anything related to the type 2 DM condition.

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## References

Ahmed, O.M., Moneim, A.A., Yazid, I.A. and Mahmoud, A.M. (2010). Antihyperglicemic, antihyperlipidemic and antioxidant effects and the probable mechanisms of action of *Ruta Graveolens* infusion and rutin in nicotinamide-streptozotocin-induced diabetic rats. *Diabetologia Croatica,* 39(1), 15-35.

Andreelli, F., Foretz, M., Knauf, C., Cani, P.D., Perrin, C., Iglesias, M.A., Pillot, B., Bado, A., Tronche, F., Mithieux, G., Vaulont, S., Burcelin, R. and Viollet, B. (2006). Liver adenosine monophosphate-activated kinase-α2 catalytic subunit Is a key target for the control of hepatic glucose production by adiponectin and leptin but not insulin. *Endocrinology,* 147(5), 2432-2441.

Barter, P.J. and Kastelein, J.J. (2006). Targeting cholesteryl ester transfer protein for the prevention and management of cardiovascular disease. *Journal of the American College of Cardiology,* 47(3), 492-499. https://doi.org/10.1016/j.jacc.2005.09.042

Coughlan, K.A., Valentine, R.J., Ruderman, N.B. and Saha, A.K. (2014). AMPK activation: a therapeutic target for type 2 diabetes?. *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy,* 2014, 241-253. https://doi.org/10.2147/DMSO.S43731

Dugan, L.L., You, Y.H., Ali, S.S., Diamond-Stanic, M., Miyamoto, S., DeCleves, A.E., Andreyev, A., Quach, T., Ly, S. and Shekhtman, G. (2013). AMPK dysregulation promotes diabetes-related reduction of

 

superoxide and mitochondrial function. *The Journal of Clinical Investigation,* 123(11), 4888-4899.

Fatimah, R.N. (2015). Diabetes melitus tipe 2. *Jurnal Majority,* 4(5), 93-101. https://doi.org/10.1172/JCI66218

Foretz, M., Ancellin, N., Kahn, A., Thorens, B., Andreelli, F., Saintillan, Y., Grondin, P., Vaulont, S. and Viollet, B. (2005). Short-term overexpression of a constitutively active form of AMP-activated protein kinase in the liver leads to mild hypoglycemia and fatty liver. *Diabetes,* 54(5), 1331-1339. https://doi.org/10.2337/diabetes.54.5.1331

Garcia, D., Mihaylova, M.M. and Shaw, R.J. (2020). AMPK: central regulator of glucose and lipid metabolism and target of type 2 diabetes therapeutics. In Arias, I.M., Alter, H.J., Boyer, J.L., Cohen, D.E., Shafritz, D.A., Thorgeirsson, S.S. and Wolkoff, A.W. (Eds). The Liver: Biology and Pathobiology, p. 472-484. United Kingdom: John Wiley and Sons Ltd. https://doi.org/10.1002/9781119436812.ch38

Garcia, D. and Shaw, R.J. (2017). AMPK: mechanisms of cellular energy sensing and restoration of metabolic balance. *Molecular Cell,* 66(6), 789-800. https://doi.org/10.1016/j.molcel.2017.05.032

Ghasemi, A., Khalifi, S. and Jedi, S. (2014). Streptozotocin-nicotinamide-induced rat model of type 2 diabetes. *Acta Physiologica Hungarica,* 101 (4), 408-420. https://doi.org/10.1556/APhysiol.101.2014.4.2

Ghorbani, A. (2017). Mechanisms of antidiabetic effects of flavonoid rutin. *Biomedicine and Pharmacotherapy,* 96, 305-312. https://doi.org/10.1016/j.biopha.2017.10.001

Gruzman, A., Babai, G. and Sasson, S. (2009). Adenosine monophosphate-activated protein kinase (AMPK) as a new target for antidiabetic drugs: a review on metabolic, pharmacological and chemical considerations. *The Review of Diabetic Studies,* 6(1), 13-36. https://doi.org/10.1900/RDS.2009.6.13

Guyton, A.C. and Hall, J.E. (2014). Textbook of Medical Physiology. 12th ed. United States of America: Elsevier.

Hasenour, C.M., Berglund, E.D. and Wasserman, D.H. (2013). Emerging role of AMP activated protein kinase in endocrine control of metabolism in the liver. *Molecular and Cellular Endocrinology,* 366 (2), 152-162. https://doi.org/10.1016/j.mce.2012.06.018

IDF. (2019). IDF Diabetes Atlas. 9th ed. Belgium: International Diabetes Federation.

Jeon, S.M. (2016). Regulation and Function of AMPK in Physiology and Diseases. *Experimental and Molecular Medicine,* 48(7), 245-245. https://doi.org/10.1038/emm.2016.81

Jeong, K.J., Kim, G.W. and Chung, S.H. (2014). AMP-activated protein kinase: an emerging target for ginseng. *Journal of Ginseng Research,* 38(2), 83-88. https://doi.org/10.1016/j.jgr.2013.11.014

Josten, S. (2018). Profil lipid penderita diabetes mellitus tipe 2 P. *Indonesian Journal of Clinical Pathology and Medical Laboratory,* 13(1), 20-22. https://doi.org/10.24293/ijcpml.v13i1.894

Kaemmerer, D., Peter, L., Lupp, A., Schulz, S., Sänger, J., Baum, R.P., Prasad, V. and Hommann, M. (2012). Comparing of IRS and Her2 as immunohistochemical scoring schemes in gastroenteropancreatic neuroendocrine tumors. *International Journal of Clinical and Experimental Pathology,* 5(3), 187-194.

Katzung, B.G. (2018). Basic and Clinical Pharmacology. 14th ed. United States of America: McGraw-Hill Education.

Kawser, H.M., Abdal, D.A., Han, J., Yin, Y., Kim, K., Kumar, S.S., Yang, G.M., Choi, H.Y. and Cho, S.G. (2016). Molecular mechanisms of the anti-obesity and anti-diabetic properties of flavonoids. *International Journal of Molecular Sciences,* 17(4), 569. https://doi.org/10.3390/ijms17040569

Kusuma, T.U., Rachmawati, S.N., Anjani, G. and Muniroh, M. (2020). *Carica pubescens* fruit juice reduces tumor necrosis factor-alpha (TNF-α) and fasting blood glucose (FBG) levels in type 2 diabetes mellitus wistar rats. *Food Research,* 4(Suppl. 3), 67-74. https://doi.org/10.26656/fr.2017.4(S3).S15

Lake, N.J., Taylor, R.L., Trahair, H., Harikrishnan, K., Curran, J.E., Almeida, M., Kulkarni, H., Mukhamedova, N., Hoang, A. and Low, H. (2017). TRAK2, a novel regulator of ABCA1 expression, cholesterol efflux and HDL biogenesis. *European Heart Journal,* 38(48), 3579-3587. https://doi.org/10.1093/eurheartj/ehx315

Leo, E.E.M., Fernández, J.J.A. and Campos, M.R.S. (2016). Biopeptides with antioxidant and anti-inflammatory potential in the prevention and treatment of diabesity disease. *Biomedicine and Pharmacotherapy,* 83, 816-826. https://doi.org/10.1016/j.biopha.2016.07.051

Li, S., Qian, Q., Ying, N., Lai, J., Feng, L., Zheng, S., Jiang, F., Song, Q., Chai, H. and Dou, X. (2020). Activation of the AMPK-SIRT1 pathway contributes to protective effects of Salvianolic acid A against lipotoxicity in hepatocytes and NAFLD in mice.

 

*Frontiers in Pharmacology,* 11, e560905. https://doi.org/10.3389/fphar.2020.560905

Lindén, D., William-Olsson, L., Rhedin, M., Asztély, A.K., Clapham, J.C. and Schreyer, S. (2004). Overexpression of mitochondrial GPAT in rat hepatocytes leads to decreased fatty acid oxidation and increased glycerolipid biosynthesis. *Journal of Lipid Research,* 45(7), 1279-1288. https://doi.org/10.1194/jlr.M400010-JLR200

Meng, S., Cao, J., Feng, Q., Peng, J. and Hu, Y. (2013). Roles of chlorogenic acid on regulating glucose and lipids metabolism: a review. *Evidence-Based Complementary and Alternative Medicine,* 2013, 801457. https://doi.org/10.1155/2013/801457

Muoio, D.M., Seefeld, K., Witters, L.A. and Coleman, R.A. (1999). AMP-activated kinase reciprocally regulates triacylglycerol synthesis and fatty acid oxidation in liver and muscle: evidence that sn-glycerol-3-phosphate acyltransferase is a novel target. *Biochemical Journal,* 338(3), 783-791. https://doi.org/10.1042/bj3380783

Niture, N.T., Ansari, A.A. and Naik, S.R. (2014). Anti-hyperglycemic activity of rutin in streptozotocin-induced diabetic rats: an effect mediated through cytokines, antioxidants and lipid biomarkers. *Indian Journal of Experimental Biology,* 52(7), 720-727.

Oktiyani, N., Sunarti, Prasetyastuti and Cahyono, J.A. (2015). Hubungan dosis tepung gembili (*Dioscorea esculenta*) dengan tingkat ekspresi enzim AMPK-α2 pada model tikus diabetes melitus. *Medical Laboratory Technology Journal,* 1(1), 7-13. [In Bahasa Indonesia]. https://doi.org/10.31964/mltj.v1i1.4

Ong, K.W., Hsu, A. and Tan, B.K.H. (2013). Anti-diabetic and anti-lipidemic effects of chlorogenic acid are mediated by AMPK activation. *Biochemical Pharmacology,* 85(9), 1341-1351. https://doi.org/10.1016/j.bcp.2013.02.008

Pinto, M.D.S., Ranilla, L.G., Apostolidis, E., Lajolo, F.M., Genovese, M.I. and Shetty, K. (2009). Evaluation of antihyperglycemia and antihypertension potential of native Peruvian fruits using *in vitro* models. *Journal of Medical Food,* 12 (2), 278-291. https://doi.org/10.1089/jmf.2008.0113

Prince, P.S.M. and Kamalakkannan, N. (2006). Rutin improves glucose homeostasis in streptozotocin diabetic tissues by altering glycolytic and gluconeogenic enzymes. *Journal of Biochemical and Molecular Toxicology,* 20(2), 96-102. https://doi.org/10.1002/jbt.20117

Rachmawati, S.N. (2019). Pengaruh jus buah karika (*Carica pubescens*) terhadap kadar SOD dan profil lipid (trigliserida dan high density lipoprotein) studi pada tikus wistar diabetes melitus tipe 2. Semarang, Indonesia: Diponegoro University. Thesis.

Rowe, G.C., Jiang, A. and Arany, Z. (2010). PGC-1 coactivators in cardiac development and disease. *Circulation Research,* 107(7), 825-838. https://doi.org/10.1161/CIRCRESAHA.110.223818

Ruderman, N., Park, H., Kaushik, V., Dean, D., Constant, S., Prentki, M. and Saha, A. (2003). AMPK as a metabolic switch in rat muscle, liver and adipose tissue after exercise. *Acta Physiologica Scandinavica,* 178(4), 435-442. https://doi.org/10.1046/j.1365-201X.2003.01164.x

Sasongko, H., Pratiwi, D., Amartiwi, T., Efendi, N.R. and Sugiyarto. (2018). Hepatoprotective effect of mountain papaya *(Vasconcellea pubescens A.DC.)* fruit extract against acetaminophen-induced acute liver damage, presented at the 1st Muhammadiyah International Conference on Health and Pharmaceutical Development, Jakarta, 2018. Portugal: Science and Technology Publications Lda. https://doi.org/10.5220/0008239500660070

Setyawati, T. (2014). Peningkatan HDL plasma pada diabetes melitus tipe 2 melalui terapi sinbio *Eubacterium rectale* dan pati gembili (*Dioscorea esculenta*). *Medika Tadulako: Jurnal Ilmiah Kedokteran Fakultas Kedokteran dan Ilmu Kesehatan,* 1(3), 22-34. [In Bahasa Indonesia].

Shairibha, S.R., Rajadurai, M. and Kumar, N.A. (2014). Effect of p-coumaric acid on biochemical parameters in streptozotocin-induced diabetic rats. *Journal of Academia and Industrial Research,* 3(5), 237-242.

Soares, J.M.D., Leal, A.E.B.P., Silva, J.C., Almeida, J.R. and de Oliveira, H.P. (2017). Influence of flavonoids on mechanism of modulation of insulin secretion. *Pharmacognosy Magazine,* 13(52), 639-646. https://doi.org/10.4103/pm.pm_87_17

Srivastava, R.A.K., Pinkosky, S.L., Filippov, S., Hanselman, J.C., Cramer, C.T. and Newton, R.S. (2012). AMP-activated protein kinase: an emerging drug target to regulate imbalances in lipid and carbohydrate metabolism to treat cardio-metabolic diseases. *Journal of Lipid Research,* 53(12), 2490-2514. https://doi.org/10.1194/jlr.R025882

Toyoda, T., Egawa, T. and Hayashi, T. (2012). Metabolic sensor for low intensity exercise: insights from AMPKα1 activation in skeletal muscle. *The Journal of Physical Fitness and Sports Medicine,* 1 (1), 59-64. https://doi.org/10.7600/jpfsm.1.59

Triana, L. and Salim, M. (2017). Perbedaan kadar glukosa darah 2 jam post prandial. *Jurnal*

 

*Laboratorium Khatulistiwa,* 1(1), 51-57. https://doi.org/10.30602/jlk.v1i1.97

Ventura, C.R., Garnier, A. and Veksler, V. (2008). Transcriptional control of mitochondrial biogenesis: the central role of PGC-1α. *Cardiovascular Research,* 79(2), 208-217. https://doi.org/10.1093/cvr/cvn098

Viollet, B., Guigas, B., Leclerc, J., Hébrard, S., Lantier, L., Mounier, R., Andreelli, F. and Foretz, M. (2009). AMP-activated protein kinase in the regulation of hepatic energy metabolism: from physiology to therapeutic perspectives. *Acta Physiologica,* 196(1), 81-98. https://doi.org/10.1111/j.1748-1716.2009.01970.x

Viollet, B., Horman, S., Leclerc, J., Lantier, L., Foretz, M., Billaud, M., Giri, S. and Andreelli, F. (2010). AMPK inhibition in health and disease. *Critical Reviews in Biochemistry and Molecular Biology,* 45 (4), 276-295. https://doi.org/10.3109/10409238.2010.488215

Wang, Q., Liu, S., Zhai, A., Zhang, B. and Tian, G. (2018). AMPK-mediated regulation of lipid metabolism by phosphorylation. *Biological and Pharmaceutical Bulletin*, 41(7), 985-993. https://doi.org/10.1248/bpb.b17-00724

 