Research Article

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The Formula Composed of Chlorogenic Acid and Total Lignans From *Fructus Arctii* has Stronger Hypoglycemic Effect on Kkay Mice Than When They are Used Alone

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ABSTRACT

Fructus Arctii, the dried ripe fruit of *Arctium lappa L.* has been used to treat diabetes since ancient times in China. Total lignans from *Fructus Arctii* (TLFA) and chlorogenic acid (3-O caffeoylquinic acid, CA) are the main components of it, which have been reported to have hypoglycemic activity respectively. In this study, they were combined according to several content ratios that different from the ratio they exist in *Fructus Arctii,* for obtaining stronger hypoglycemic effect. KKAy mice, a spontaneous Type 2 diabetic model were gavaged once daily with the experimental drugs for 6 weeks. The result indicates that **w**hen CA and TLFA were used alone, their hypoglycemic effect were lower than that of metformin. However, when they are used in combination, their hypoglycemic effect has been greatly strengthened, and the effect of the formula is not a simple addition of the activities of the two substances.

Keywords: *Frutus Arctii*; Total lignans from *Fructus Arctii*; Chlorogenic acid; KKAy mice; Hypoglycemic effect

INTRODUCTION

In 2019, the International Diabetes Federation (IDF) estimated that approximately 463 million adults (20-79 years) were living with diabetes [1]; by 2045 this will rise to 700 million. Among them, type 2 diabetes (T2DM) patients account for about 90%. Controlling of blood glucose is the key to preventing or treating T2DM and its complications. Metformin is the most commonly prescribed and guideline recommended initial glucose-lowering drug for people with type 2 diabetes mellitus, especially overweight patients [2]. Nevertheless, metformin-associated adverse drug reactions (ADRs) are common, the most common ADRs of metformin include gastrointestinal symptoms like nausea, vomiting, diarrhoea, abdominal pain and loss of appetite. Other less frequent ADRs include dysgeusia, skin reactions, abnormal liver functions, hepatitis and vitamin B12 deficiency [3] Therefore, more effective and less ADRs hypoglycemic drugs are still the goal of global pharmaceutical researchers.

Fructus Arctii is the dried ripe fruit of *Arctium lappa L.* (family Asteraceae) and has been used to treat diabetes since ancient times in China. We have reported the effects of hypoglycemia and weight loss of TLFA (content is about 10% in *Fructus Arctii*) in KKAy mice [4]. However, at the same dose, the hypoglycemic effect of TLFA is not as good as that of metformin.

CA is another main component of *Fructus Arctii* (content is about 2%), and there are some reports about its anti-diabetic activity [5]. CA and TLFA are present in *Fructus Arctii* in

a ratio of 1:5, in this study, TLFA and CA were combined according to several content ratios that different from the ratio they exist in *Fructus Arctii,* hypoglycemic and weight loss effects of these formulas, TLFA and CA were observed, metformin was adppted as a positive control drug.

RESULTS AND DISCUSSION

Measurement of blood Glucose and body weight

KKAy mouse is a type II diabetic mouse with a mutation in the coat color gene (ay), the ay gene not only affects the coat color of mice, but can also cause metabolic disorders, such as obesity, hyperglycemia, lipid metabolism disorders, and hyperinsulinemia. Its onset is induced by the addition of environmental factors on the basis of genetic susceptibility [6], which is very similar to human type II diabetes.

Figure 1 A shows FBG levels of KKAy mice in each week throughout the experiment. Compared to the age-matched C57 BL/6J mice, KKAy mice had higher FBG levels, and the FBG levels of the KKAy mice in the administration group were significantly lower than those in the model group.

At the end of the experiment, the decreased values of FBG and weight gain in each administration group are shown in Figure 1 B and Figure 1 C respectly.

We found that when CA and TLFA are used alone, the hypoglycemic effects of both are significantly weaker than metformin. However, after the two are administered in combination, the hypoglycemic effect of the composition is significantly improved, so that there is no statistical difference with metformin. We also noticed that although the hypoglycemic index of CA is better than that of TLFA, the hypoglycemic index of formula 2 (TLFA:CA=2:1) is better than that of formula 1 (TLFA:CA=1:1), indicating the medicinal effect caused by the combination of the two drugs is not a simple additive relationship. The correlation between the formula ratio and hypoglycemic efficacy of the two drugs is worthy of further study. In terms of weight control, the combined use of TLFA and CA is also better than the two used alone.

Oral glucose tolerance test (OGTT)

Figure 1 D and Figure 1 E shows the blood glucose-time curve and the area under the blood glucose-time curve (AUC) of each group of mice after giving a glucose load. The blood glucose level of the mice in the administration groups at each time point was lower than that of the model group. The AUC of the formula 1 and formula 2 groups were lower than the positive drug group, but there was no statistical difference.

Serum biochemical indexes of the experimental mice

As shown in Table 1, the levels of TC, TG, HDL-C and LDL-C of mice in the model group were remarkably higher than those in the normal group ($P<0.05$), indicating that KKAy mice have obvious symptoms of hyperlipidemia and lipid metabolism disorders. Compared with the serum biochemical indexes of the CA group and the TLFA group, the data of the formula 1 and formula 2 group were closer to the normal group. Moreover, the levels of TC, TG and LDL-C in the formula 2 group are significantly different from those in the model group ($P<0.05$).

As shown in Figure 1 F, the serum insulin level of mice in the model group was significantly higher than that of the normal group ($P<0.05$), and the serum insulin level of mice in the administration group was significantly lower than that of the model group ($P<0.05$). Among them, the serum insulin levels of mice in the TLFA group and the CA group were higher than the formula 1, the formula 2 group and the positive group. The serum insulin levels of mice in the formula 2 group and the positive drug group were the closest to those in the normal group.

Histopathological examination

As shown in Figure 2, the islet cells of C57 BL/6J mice (A-1) have clear boundaries, a large number, and abundant capillaries between the cells and their nuclei present circular cell clusters of different sizes. The pancreatic islet cells of the model mouse (B-1) were atrophied, with irregular contours, small numbers and sparse distribution. While the number

Table 1: TC、TG、HDL-C、LDL-C in the experimental mice.

Group	TC mmol/L	TG mmol/L	HDL-C mmol/L	LDL-C mmol/L
Nomal	2.452±0.12	0.5±0.07	1.984±0.06	0.352±0.05
Positive	5.484±1.08*	6.232±1.65*	3.986±0.60	0.850±0.12*
Model	7.612±0.82	8.358±1.09	4.828±0.29	1.020±0.13
TLFA	6.012±0.76*	6.282±0.86	4.356±0.48	0.870±0.16*
CA	5.850±0.56*	6.554±1.54	4.234±0.41	0.776±0.13*
Formula 1	5.600±0.65*	7.002±1.94	4.044±0.39	0.656±0.08*
Formula 2	5.594±1.32*	4.916±2.36*	3.996±0.59	0.738±0.11*

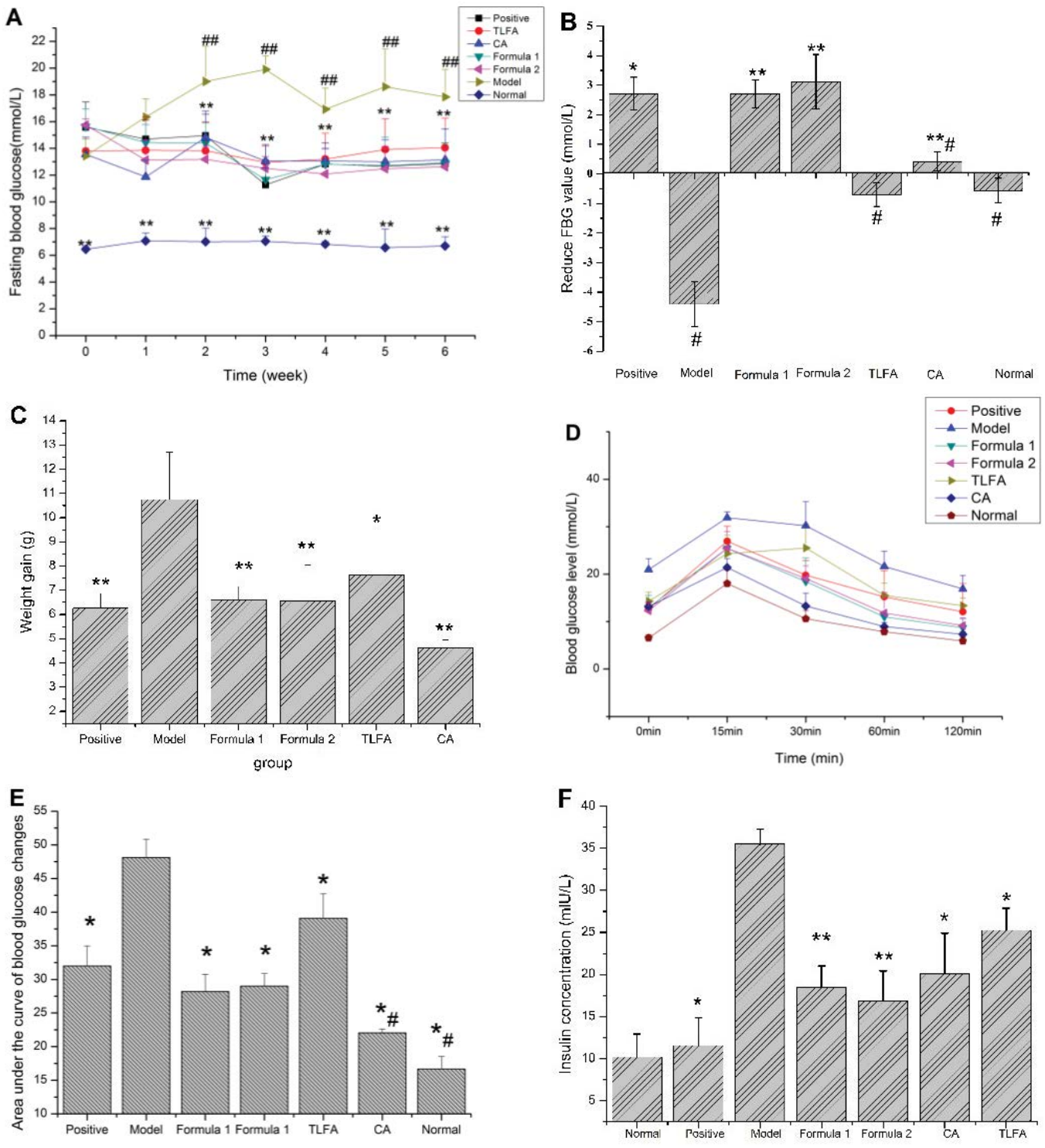

Figure 1: Effects on blood sugar, body weight and OGTT in KKAy mice.

(A) FBG levels. (B) Decreasement of blood glucose level. (C) Increment of body weight.(D) Response of blood glucose to OGTT in KKAy mice. (E) AUC in OGTT of experimental mice. (F) Serum insulin level of experimental mice.

Results were presented as mean ± standard error of the mean; n = 8. Groups: Normal = normal control; Model = Type 2 diabetic control; Positive group = metformin, TLFA = Total lignans from *Fructus Arctii*, CA = chlorogenic acid, Formula 1 = TLFA:CA (1: 1), Formula 2= TLFA:CA (2 :1),

*p < 0.05, **p <0.01 versus the model control. #p <0.05 versus the normal control.

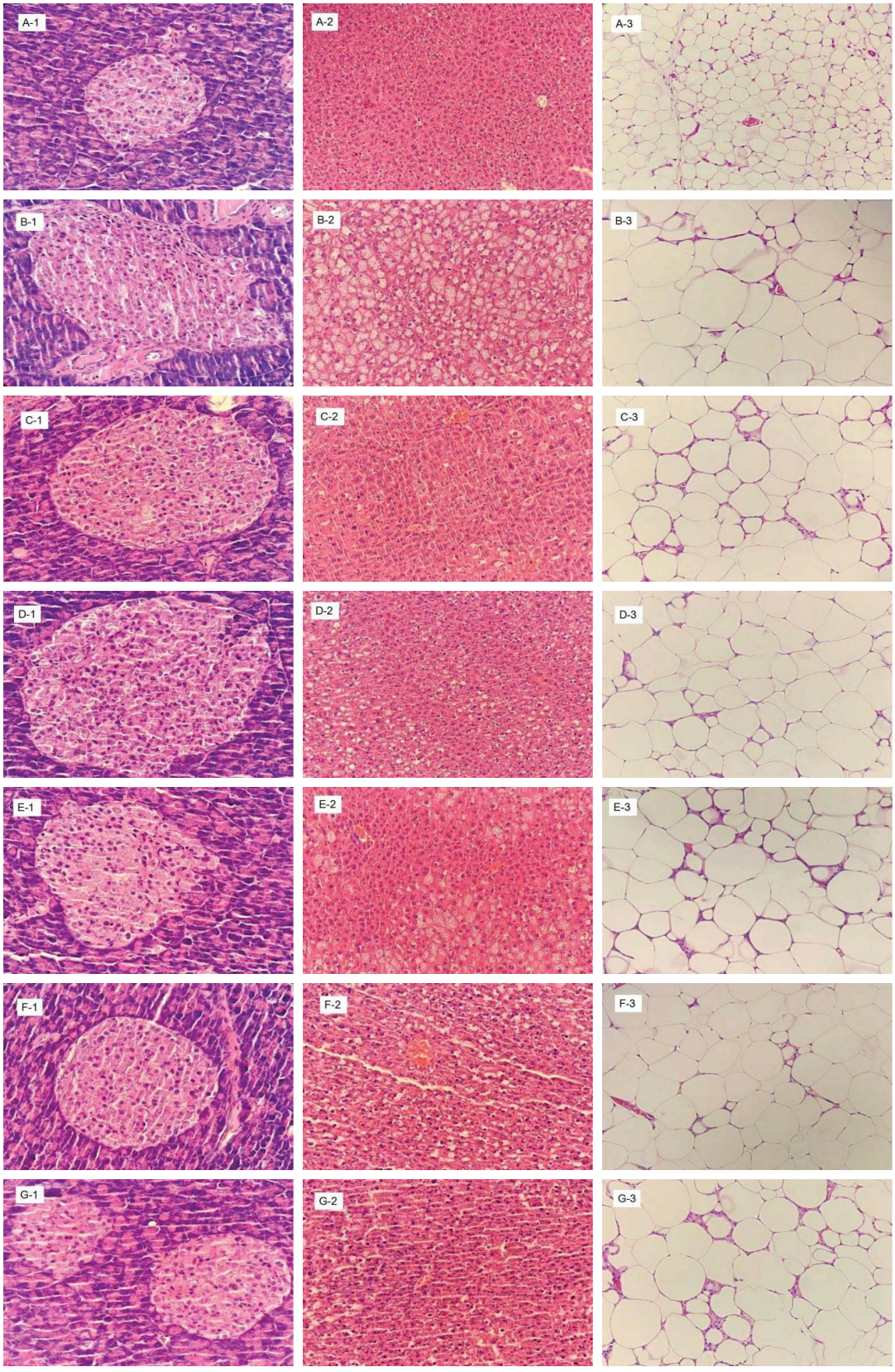

Figure 2: Paraffin sections of pancreas (magnification 200×), liver (magnification 400×), and adipose tissue (magnification 400×) of KKAy mice. A-1 to A-3: normal control; B-1 to B-3: model control; C-1 to C-3: positive group; D-1 to D-3: CA group; E-1 to E-3: TLFA group; F-1 to F-3: formula 1 group (1:1; TLFA: CA); G-1 to G-3: formula 2 group (2:1; TLFA: CA)

of islets in the positive control group (C-1) was significantly increased, and the boundaries were obvious, which had better roundness. Compared with the model group, the pancreatic cells of the mice in the formula 1 group (F-1) and the formula 2 (G-1) group increased significantly. Compared with the TLFA group (E-1) and the CA group (D-1), their boundaries are clearer, and the roundness is better, which is closer to the normal group.

As shown in Figure 2, the liver structure of the normal control group (A-2) mice is complete and clear, there are no obvious vacuoles, and the liver cells are very small. The mice in the model group (B-2) had obvious hepatocyte swelling, cytoplasmic porosity, obvious vacuoles, more lipid droplets and fatty liver. Compared with the model group, each administration group improved the phenomenon of liver disease. The lipid droplets in the positive drug group (C-2) were significantly reduced, which was close to the normal group. Compared with the model group, the appearance of fatty liver in the formula 1 group (F-2) and formula 2 group (G-2) is not obvious, and the lipid droplets are reduced, which is close to the normal group. The formula group is better than the TLFA group (E-2) and the CA group (D-2) used alone.

As shown in Figure 2, the (A-3) fat cells of mice in the normal group are small in size, tightly connected, and have the same cell size. The volume of (C-2) fat cells in the model group mice was significantly increased, and the arrangement was relatively loose. Compared with the model group, the (C-3) adipocytes of the mice in each administration group decreased in volume.

In addition, CA and TLFA have high safety, and there is no report on the adverse reactions caused by oral CA. Powder of *Fructus Arctii* was not found to be toxic after clinical oral administration for 30 days [7]. However, there are reports in the literature that the incidence of adverse reactions in patients taking metformin for the first time is as high as 40.17% [8,9].

CONCLUSIONS

In summary, the combination of CA and TLFA is a hypoglycemic and lose weight candidate drug worthy of further study.

CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

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