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A new botanical complex improves blood glycemic control and reduces hepatic steatosis in mice

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Background

Type 2 diabetes (T2D) is a major public health issue. Worldwide, the number of people with T2D is estimated at nearly 592 million[1]. The estimated total cost of healthcare in the United States and in Europe as a result of T2D was 456 billion in 2014. Prediabetes is a risk factor that defines a high chance of developing T2D. According to experts from the American Diabetes Association, potentially 70% of prediabetics will develop type 2 diabetes. Several trials have demonstrated a reduction in the risk of developing T2D among prediabetic individuals following diet and lifestyle changes (DLC). Nevertheless, patients rarely adhere to DLC and there is currently no suitable solution to the management of prediabetes and T2D patients between the DLC and the initiation of a therapeutic treatment. We have developed an innovative botanical food complex (TOTUM-63), a synergistic combination of plant extracts, regulatory status: food supplement) that aims to reverse prediabetes and to prevent each dysfunction and/or its consequences independently.

Objectives

The aim of these preclinical studies was to investigate the effects of TOTUM-63 on the glycemic control in the db/db mouse model and in healthy C57BL6 mice. Moreover, the effects of TOTUM-63 on whole body composition, and circulating and hepatic lipids were investigated. In the db/db mouse model, TOTUM-63 was also compared with metformin. The safety of TOTUM-63 supplementaion in healthy Syrian golden hamsters through circulating and hepatic fats, liver weight and serum transaminases was also investigated.

Research Design and Methods

The protocols were approved by the local ethics committee (C2EA-02, Auvergne, France). All animals aged 5 weeks were obtained from JANVIER (JANVIER LABS, France), and housed in individual cages. Both chow and water were provided ad libitum. Whole body composition was determined by Echo MRI analyses (Houston, USA) at the beginning and at the end of the experiments.The weight of the mice, food intake and fasting glycemia were recorded weekly. Due to the oral starch tolerance test (OSTT) and i.p. insulin tolerance test (ITT) conducted in mice at weeks 0 and 6 or 9, food intake was not determined at these periods. At the end of treatments, animals were fasted and sacrificed. Blood and liver samples were taken immediately and frozen in liquid nitrogen for further analysis.

Treatment of diabetic db/db mice

After 1 week of acclimatization, 31 male db/db mice (BKS(D)-Leprdb/JOrlRj) were randomly assigned to Control (n = 10), Metformin (n = 11) and TOTUM-63 (n = 10) groups based on their fasting glycemia and body weight. Animals were fed a standard diet (A03 enriched with 3% corn oil, Safe, France) or a standard diet incorporating TOTUM-63 (2.7%) or metformin (0.2%) for 6 weeks.

Treatment of healthy C57BL6 mice

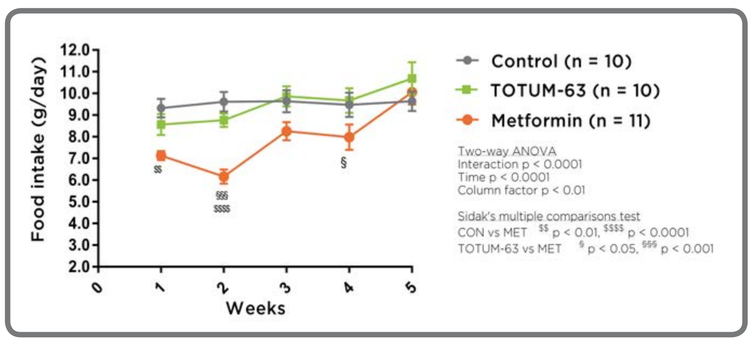
After 1 week of acclimatization, animals (C57BL/6NRJ) were randomly assigned to Control (n = 13) and TOTUM-63 (n = 10) groups. Animals were fed a standard diet (A03 enriched with 3% corn oil, Safe, France) or a standard diet incorporating TOTUM-63 (2.7%) for 9 weeks.

Treatment of hamsters

After 1 week of acclimatization, 20 male Syrian golden hamsters were assigned to Control (n = 10) and TOTUM-63 groups (n = 10). Animals were fed a standard diet (A03, Safe, France) or a standard diet incorporating BC (2.7%) for 6 weeks. The weight of the hamsters and food intake were recorded at weeks 1, 2, 3, 4 and 5.

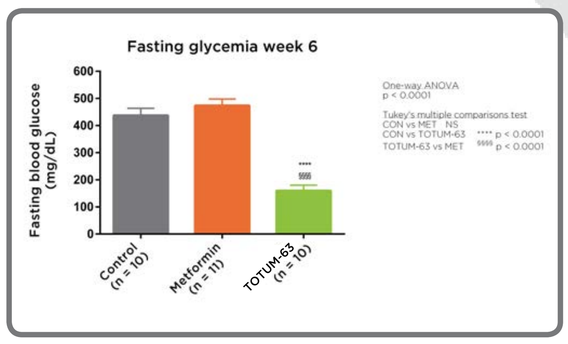
Results and discussion

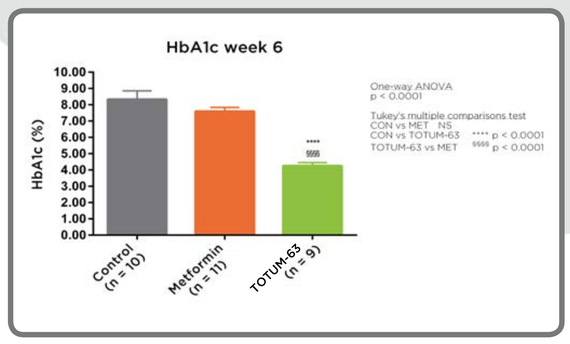
Food intake

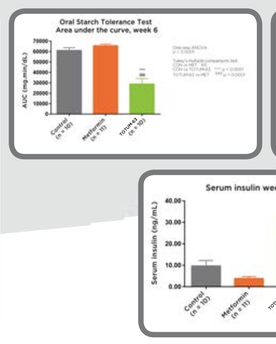


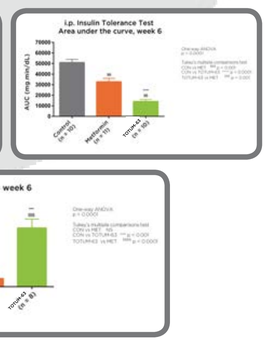
- There was no significant difference between the Control and the TOTUM-63-treated db/db mice.
- The food intake of Metformin-treated mice was lower than that of the Control and TOTUM-63 groups.
- Characterization and quantification of biomolecules in the TOTUM-63 was realized. The amount of Metformin ingested daily by the mice was greater than the total amount of molecules present in TOTUM-63.

Glycemic control



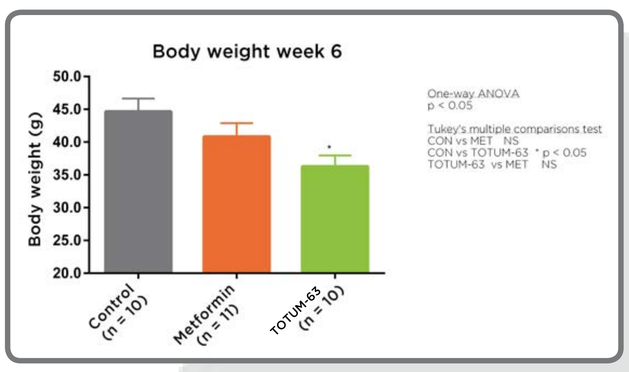


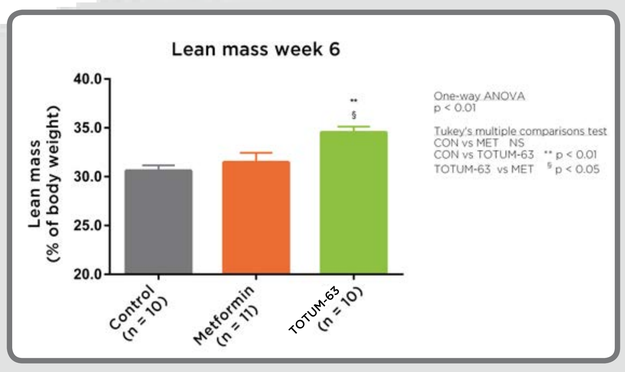


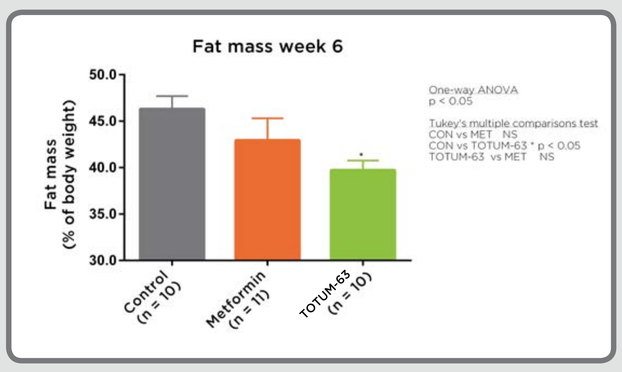


- The TOTUM-63-treated db/db mice exhibited lower fasting blood glucose. Serum insulin concentrations were higher in the TOTUM-63-treated mice. In db/db mice, after an initial rise to compensate for the development of insulin-resistance, circulating insulin rapidly declined as the disease progressed[3,4]. It can be hypothesized that the decline in serum insulin was slowed down in the TOTUM-63-treated mice, which is consistent with slower disease progression. Accordingly, HbA1c levels in TOTUM-63-treated mice were over 48% lower than in the Controls. Moreover, TOTUM-63 significantly improved starch tolerance, as measured by OSTT. No effect of metformin was found, which is in line with other studies conducted in db/db mice[5].
- Insulin sensitivity, determined with an i.p. insulin tolerance, seemed to increase in TOTUM-63- and metformin-treated mice. The effect of TOTUM-63 was higher than that of metformin. A hyperinsulinemic-euglycemic clamp study will be necessary to confirm this effect.

Whole body composition

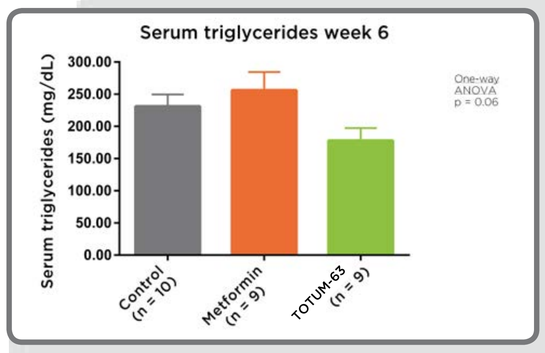


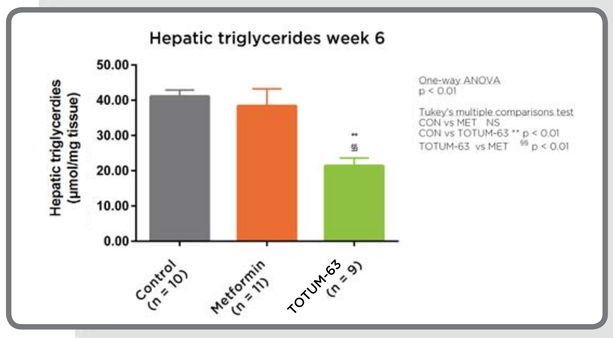




- Body weight in the Control group was over 18% higher than the TOTUM-63-treated db/db mice. Improved body composition by reducing body fat content.
- There was no significant difference in body weight and whole body composition between the control and metformin-treated mice.

Serum and hepatic triglycerides





- Serum triglycerides tended to be lower in TOTUM-63-treated db/db mice (p = 0.06).
- Mice fed a diet containing TOTUM-63 showed lower liver triglyceride content.

TOTUM-63 improves glycemic control in C57BL6 mice

Table 1. Data at week 9

Parameters	Control	TOTUM-63
Food intake (g/day)	4.5 ± 0.0	5.7 ± 0.2 ***
Body mass (g)	26.7 ± 0.5	24.5 ± 0.4 **
Fat mass (% of body weight)	9.21 ± 0.59	7.32 ± 0.28 *
Lean mass (% of body weight)	50.51 ± 0.47	52.41 ± 0.42 **
Free water (% of body weight)	0.26 ± 0.07	0.29 ± 0.04
Total water (% of body weight)	40.02 ± 0.71	39.98 ± 0.43
Fasting glycemia (mg/dL)	141 ± 7	116 ± 10 *
HbA1c (%)	6.00 ± 0.16	5.99 ± 0.20
Serum insulin (ng/mL)	1.66 ± 0.62	0.94 ± 0.21
Area under curve measured during i.p. insulin sensitivity test (AUC, mg.min/dL)	6233 ± 594	6113 ± 594
Lower peak measured during i.p. insulin sensitivity test (mg/dL)	24 ± 6	34 ± 15
Area under curve measured during oral carbohydrate tolerance test (AUC, mg.min/dL)	20377 ± 690	14873 ± 1325 ***
Peak measured during oral carbohydrate tolerance test (mg/dL)	176 ± 15	125 ± 17 ***
Serum triglycerides (mg/dL)	70.74 ± 12.70	56.94 ± 8.95
Hepatic triglycerides (µmol/mg tissue)	11.80 ± 2.23	8.05 ± 0.81
Liver weight (g)	1.13 ± 0.03	1.18 ± 0.05

*Mean values ± SEM. Student's t test for unpaired data. TOTUM-63 versus Control, * p < 0.05, ** p < 0.01, *** p < 0.001.*

Preliminary safety results in hamsters

Table 2. Data at week 6

Parameters	Control	TOTUM-63
Food intake (g/day)	8.3 ± 0.2	8.4 ± 0.2
Body mass (g)	106.6 ± 1.3	106.5 ± 2.6
HbA1c (%)	3.84 ± 0.21	3.79 ± 0.38
Serum insulin (ng/mL)	2.95 ± 0.95	0.88 ± 0.32 p = 0.054
Total cholesterol (mg/dL)	94.81 ± 4.39	98.44 ± 2.99
HDL cholesterol (mg/dL)	45.66 ± 2.77	42.58 ± 2.55
Serum triglycerides (mg/dL)	145.59 ± 9.63	128.05 ± 8.82
Hepatic triglycerides (µmol/mg tissue)	12.12 ± 1.18	11.84 ±1.87
Liver weight (g)	4.17 ± 0.13	4.24 ± 0.11
Serum AST (U/L)	124.30 ± 16.73	113.58 ± 8.30
Serum ALT (U/L)	115.88 ± 9.51	82.47 ± 6.04 **

*Mean values ± SEM. Student's t test for unpaired data. TOTUM-63 versus Control ** p < 0.01.*

The results show no adverse effects of TOTUM-63 supplementation for any of the parameters in healthy hamsters nourished with a standard chow diet. The hamster model was in particular used to ensure that the TOTUM-63 has not deleterious effect on the cholesterol metabolism. Indeed, this model is relevant for studying impact of products on lipoprotein metabolism unlike the mouse model[6].

Conclusion

The results show an extremely broad effect of TOTUM-63 on a set of risk factors of metabolic syndrome, obesity, diabetes, non-alcoholic steatohepatitis (NASH) and cardiovascular diseases, namely:

- A decrease in fasting glycemia and glycated hemoglobin;
- An improvement in carbohydrate tolerance;
- A decrease in body weight via a reduction in fat mass;
- An increase in lean mass;
- And a decrease in hepatic and serum triglycerides (trend for serum triglycerides).

TOTUM-63 is ready for Phase I clinical trial.

References

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