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Effects of hydroethanolic formulations based on *Garcinia kola* kernel and *Chrysophlum cainito* leaves on the organs of diabetic mice

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Abstract

Plant extracts and their concentrates are natural bioactive compounds that are widely and effectively used as antibiotic alternatives in traditional medicine. This study was conducted to investigate the effects of formulated extracts of *Garcinia kola* kernel and *Crhysophylum cainito* leaves on the organs of mice. To this end, an experimental study was conducted over three weeks in the laboratory on eight batches of mice, 7 of which were made diabetic by intraperitoneal injection of 150 mg/kg of alloxane. The hydroethanol formulation of the extract was administered daily by gavage to 5 batches of diabetic rats at a concentration of 4000 mg/kg body weight. The non-diabetic batch and one diabetic batch received physiological water and were used as untreated normoglycaemic and diabetic controls. The remaining diabetic batch was treated with a reference drug, glibenclamide. The results showed that in the lungs, formulation extracts F2 and F4 significantly reduced lung weight, with respective values of (0.05 g) and (0.10 g) compared with the reference weight of 0.18 g of the normoglycaemic control (TNG). However, extracts of formulations F3 and F5 had no effect on lung weight in the treated groups. As for the heart, the results showed that during the 3 weeks of treatment, formulations F2, F3, F4 and F5 had no negative effect on heart weight. From the results obtained, we can conclude that the hydro-ethanol formulation extracts of *Garcinia kola* kernel and *Chrysophylum cainito* leaves have hepatoprotective properties.

Keywords: Diabetes; *Garcinia kola*; *Chrysophylum caimito*; Hydroethanol extract; Mice

1. Introduction

Diabetes mellitus (DM) is a serious, chronic and complex metabolic disorder with multiple aetiologies and profound consequences, both acute and chronic [1]. Diabetes and its complications affect populations in both developing and developed countries, representing a major socio-economic challenge. It is estimated that 25% of the world's population is affected by this disease [2]. It is characterised by chronic hyperglycaemia with disturbances in the metabolism of macromolecules due to impaired insulin secretion. It causes long-term damage, dysfunction and failure of various organ systems (heart, lung, eyes, kidneys and nerves), leading to disability and premature death [3].

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There are currently many pharmacological treatment options for diabetic patients. Metformin (MET) has been widely used as a first-line approach for patients with diabetes mellitus due to its long-lasting anti-hyperglycaemic effects, low risk of hypoglycaemia, strong cardiovascular safety profile and low cost.

However, 25% of patients are unable to tolerate sufficient amounts of the drug due to gastrointestinal side effects associated with metformin. Metformin can have a direct serotonergic effect or alter serotonin transport, which is associated with nausea, vomiting and diarrhoea [4]. To overcome the limitations of current diabetes treatment, certain bioactive components isolated from different plants could be used as a solid base for the synthesis of new anti-diabetic drugs, with the ultimate aim of improving the condition of diabetics and reducing drug-induced side effects. The aim of the present study was to investigate the influence of extracts from formulations based on *Garcinia kola* kernel and *Crysophylum cainito* leaves on vital organs in the prevention and treatment of diabetes and its complications.

2. Materials and methods

2.1. Plant material

The plant material consisted of *Chrysophylum cainito* leaves (Figure 1) and *Garcinia kola* kernel (Figure 2). Fresh *Garcinia kola* seeds were collected in the Boka-Oh area in the department of Agboville. Young *Chrysophyllum cainito* leaves were collected at the Swiss Research Centre in Côte d'Ivoire.

Figure 1 *Crysophylum cainito* leaves

Figure 2 *Garcinia kola* almond

2.2. Preparation of the formulation

The 02-component simplex lattice mixing plan according to Goupy [5] was adopted to determine the optimum formulation. The formulations are as follows: the mixture of A and B=100 (Table 1). The (controlled) variables are *Garcinia kola* kernels (A) and *Chrysophyllum cainito* leaves (B).

Table 1 Formulations of *Chrysophyllum cainito* leaf extracts and *Garcinia kola* kernel

TESTS	*Garcinia kola* kernel	*Chrysophyllum cainito* leaf extracts
1	100	0
2	100	0
3	75	25
4	50	50
5	50	50
6	25	75
7	0	100
8	0	100

2.3. Extraction process for hydro-ethanolic extracts

To prepare the hydro-ethanolic extracts for the formulations, the various organs (leaves and kernels) were cleaned, then dried in an air conditioner at 18°C for a fortnight for the leaves and 5 days for the kernels. Finally, they were finely ground in a mechanical grinder at the Botany and Plant Diversity Development Laboratory at Nanguy Abrogoua University (Abidjan, Côte d'Ivoire). The 80% hydroethanol extractions were then determined by cold maceration using the method of Zirihi et al [6] (Figure 3).

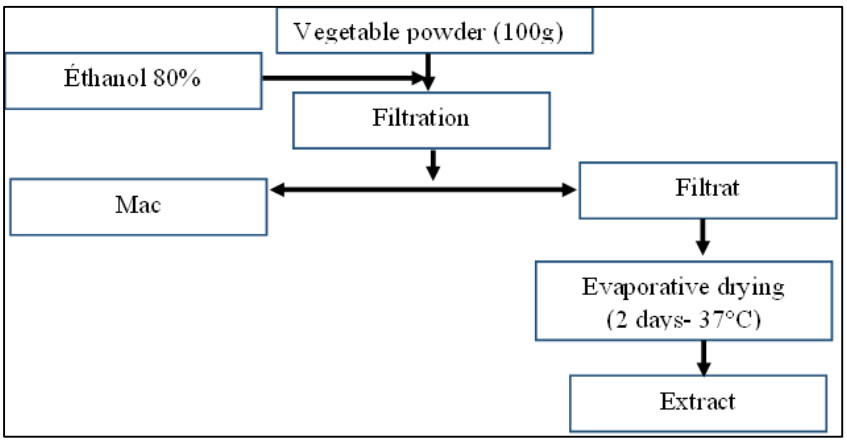


Figure 3 Flow chart for the preparation of hydroethanol extracts

2.4. Conditioning and distribution of the mice

The animals were placed in plastic cages with stainless steel lids and fitted with feeding bottles. They were subjected to a room temperature of 28 ± 2°C with alternating 12 hours of light and 12 hours of darkness. The difference in weight of the animals in the batches did not exceed ± 20% of the mean weight. The animals were fed commercial pellets (Ivograin®) and tap water ad libitum. They were acclimatised in cages for 5 days.

Forty (40) mice were used. These animals were divided into 8 batches of 5 mice. Batch 1 consisted of normal non-diabetic mice, which were the normoglycemic controls (NGC) receiving 1 ml of distilled water throughout the experiment. Batch 2 consisted of diabetic mice, the diabetic controls (Dc), treated with 1 ml of distilled water throughout the experiment. Batch 3 consisted of diabetic control mice treated with a daily dose of 5mg/kg body weight of glibenclamide® (TG), the reference hypoglycaemic substance. Batch 4 consisted of diabetic mice treated with

formulation 4 (25g Garcinia kola + 75g *Chrysophylum cainito* leaves) at a dose of 400 mg/kg. bw throughout the experiment. Batch 5 consisted of diabetic mice treated with formulation 2 (75g *Garcinia kola* +25g *Chrysophylum cainito* leaf) at a dose of 400 mg/kg bw throughout the experiment. Lot 6 consisted of diabetic mice treated with formulation 5 (100 g of *Chrysophylum cainito*) at a dose of 400 mg/kg bw throughout the experiment. Batch 7 consisted of diabetic mice treated with formulation 3 (50 g *Garcinia kola* + 50g *Chrysophylum cainito*) at a dose of 400 mg/kg body weight throughout the experiment. Batch 8 consisted of diabetic mice treated with formulation1 (100g Garcinia kola) at a dose of 400 mg/kg bw throughout the experiment.

2.5. Diabetes induction in mice

Diabetes was induced using the method of Natarajan et *al* [7] at a dose of 400 mg/kg body weight.To do this, 35 mice were deprived of food for 12 hours but given water ad libitum.After 12 hours of fasting, diabetes was induced in these mice by intraperitoneal injection of alloxane.

2.6. Organ harvesting

The organs were harvested immediately after the sacrifice of the treated and control animals (on day 21).The lung and heart were removed by opening the thorax.The kidneys were removed by opening the abdominal cavity. The organs were weighed and the relative mass was determined according to the formula described by kharchoufa et *al* [8] :

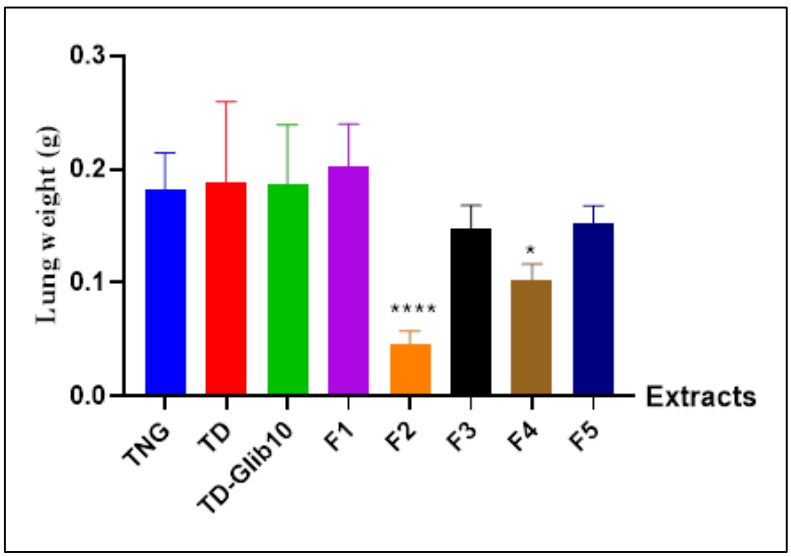
$$\text{relative weight (\%)} = \frac{\text{weight of organ (g)}}{\text{weight of animal (g)}} \times 100$$

2.7. Statistical analysis

Graph Pad prism 5 software (San Diego, California, USA) was used for statistical analysis, calculating means and standard deviations. Analysis of variance (ANOVA) followed by the Newman-Keuls multiple comparison test of means was used to classify and compare the means. Means are always followed by their standard deviations. Two means are significantly different if the probability derived from the statistical tests is less than or equal to 0.05 ($P \leq 0.05$). Otherwise, the differences are not significant ($P>0.05$).

3. Results and discussion

The aim of this study was to show the effects of the formulations on the vital organs of the mice on day 21. The evaluation of the effects of the hydro-ethanolic formulation extracts of the combination of Garcinia kola kernel and *Chrysophylum cainito* leaves on the lung, heart and kidney of mice at day 21 is presented in Figures 4, 5 and 6 respectively.

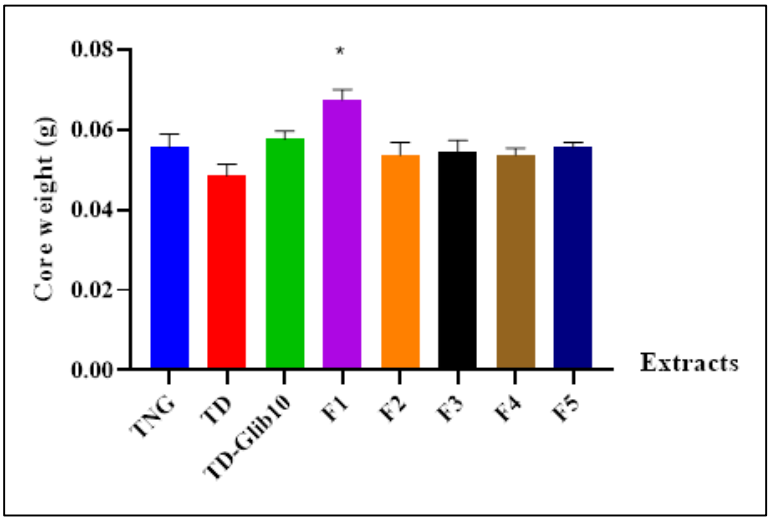


P<0.0001 compared with normoglycaemic control (TNG) mice.(Mean ± SEM, n = 5) TNG =Normoglycaemic control, TD =Diabetic control, TD-Glib10=Diabetic control treated with glibenclamide F1 =100g Garcinia kola, F2=combination of 50g Garcinia kola+50g *Chrysophylum cainito* leaves , F3=, combination of75g Garcinia kola+25g *Chrysophylum cainito* leaves F4=25g Garcinia kola+75g *Chrysophylum cainito* leaves, F5=100g *Chrysophylum cainito* leaves,

Figure 4 Effect of hydroethanol formulations on relative lung weights (g) in mice

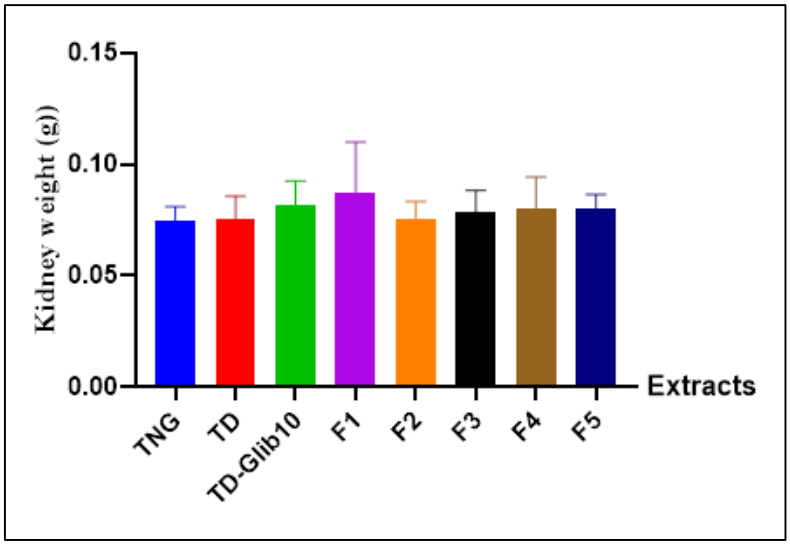
The results showed that in the lung, extracts of formulations F2 and F4 significantly reduced lung weight, with values of (0.05 g) and (0.10 g) respectively compared with a reference weight of 0.18 g for the normoglycaemic control group (TNG) and the group treated with glibenclamide (TD-Glib10). On the other hand, extracts from formulations F3 and F5 had no effect on lung weight in the treated groups, due to the absence of damaging effects on this organ. The absence of harmful effects could be due to the presence of phenolic compounds, which could have protective effects [9]. Because these formulations contain high concentrations of total polyhenols, each F5 (483.796 ± 22.49 mgEAG / g) and F3 (447.421 ± 25.15 mgEAG / g) (Figure 4).

As for the heart, the central organ of blood circulation, it was examined to assess the effects of the formulations on the organs.



P<0.0001 compared with normoglycaemic control mice (TNG); (Mean ± MSE, n = 5)bNGC =Normoglycaemic control, DM =Diabetic control, DM-Glib10=Diabetic control treated with glibenclamide F1 =100g Garcinia kola, F2=combination of 50g Garcinia kola+50g *Chrysophylum cainito* leaves , F3=, combination of75g Garcinia kola+25g *Chrysophylum cainito* leaves F4=25g Garcinia kola+75g *Chrysophylum cainito* leaves, F5=100g *Chrysophylum cainito* leaves,

Figure 5 Effect of hydroethanol formulations on relative heart weight (g) in mice



P<0.0001 compared with normoglycaemic control (TNG) mice. (Mean ± SEM, n = 5) TNG =Normoglycaemic control, TD =Diabetic control, TD-Glib10=Diabetic control treated with glibenclamide F1 =100g Garcinia kola, F2=combination of 50g Garcinia kola+50g *Chrysophylum cainito* leaves , F3=, combination of75g Garcinia kola+25g *Chrysophylum cainito* leaves F4=25g Garcinia kola+75g *Chrysophylum cainito* leaves, F5=100g *Chrysophylum cainito* leaves,

Figure 6 Effect of hydroethanol formulations on relative kidney weight (g) in mice

The results showed that over the 3 weeks of treatment, formulations F2, F3, F4 and F5 had no negative effect on heart weight.This observation suggests that these formulations contain nutrients, such as polyphenols, which have a

protective effect on the heart. These results are similar to those of Salvamani et al [9] who showed that polyphenols promote a reduction in the genesis, differentiation and proliferation of adipocytes (Figure5).

With regard to relative kidney weight, no significant difference was observed between control mice treated with glibenclamide (TD-Glib10) and those treated with the formulations.

This suggests that plant extract-based formulations of G. kola kernel and C. cainito leaves could help mitigate the toxic effects of glibenclamide by inhibiting free radical formation and restoring antioxidant defence systems. However, Enogieru et al [10] found that Ephedra alata affects nephron excretory function, highlighting the importance of further research into the effects of formulations on renal function (Figure 6).

4. Conclusion

Oral administration of formulation extracts (F1, F3, F4 and F5) of *Garcinia kola* kernel and *Crysophylum cainito* leaves for 21 days in Mus musculus mice protected the lung and heart from the radical damage caused by injection of experimental diabetes (alloxane). Investigation of renal function suggests that formulation extracts have nephroprotective properties.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors hereby declare that there is no conflict of interest.

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