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Multisystemic effects of the herbal formulation cholesterol defence in high-fat diet-induced dyslipidaemic rats

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Abstract

Aim: This study evaluated the multisystemic effects of the herbal formulation cholesterol defence, in high-fat diet-induced dyslipidaemic rats.

Methodology: A total of 35 male albino rats weighing between 160 to 180g were used for the study. The rats were weighed and grouped into 5 groups of 7 rats each. They were fed high fat diet for 6 weeks. 2 weeks before commencement of treatments and then 4 weeks alongside the treatments. Group 1 was negative control and group 2 positive control. Group 3 was administered metformin, group 4 administered CholesDefence, and group 5 administered a combination of metformin and cholesDefence. Fasting plasma glucose (FPG) was determined using Glucose oxidase method. Fasting plasma insulin (FPI) and C-reactive protein (CRP) levels were quantitatively determined by a rat-specific sandwich-enzyme linked immunosorbent assay (ELISA) method. Insulin resistance (IR) was determined using the homeostatic model assessment for insulin resistance (HOMA-IR) method. Total Cholesterol (TC), Triglyceride (TG) and High Density Lipoprotein Cholesterol (HDL-C) were determined by enzymatic methods. Low Density Lipoprotein Cholesterol (LDL-C) was calculated from the Friedewald's equation. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined using the Reitman-Frankel method. Alkaline phosphatase (ALP) was determined using the Colorimetric endpoint method. Phytochemical analysis was done on the herbal tablet using classical methods.

Results: The results revealed the presence of flavonoids, cardiac glycosides, saponins, tannins and terpenoids in the herbal tablet. FPG, insulin and HOMA-IR values were significantly higher in the positive control group, compared to the negative control and treatment groups. Metformin and CholesDefence significantly reduced FPG levels. Metformin significantly reduced insulin and HOMA-IR values, which were significantly higher in the group 3 administered CholesDefence, compared to the negative control. ALT, AST and ALP were significantly higher in the positive control, but was significantly reduced to normal levels by the treatments. CRP was significantly reduced by metformin, but not by CholesDefence. Metformin and the combination therapy significantly improved TC, TG, HDL-C, but had no impact on LDL-C levels. CholesDefence had no effect on TC, HDL-C, and LDL-C levels, but significantly reduced TG levels.

Conclusion: Dyslipidaemia induced by high-fat diet elevated glucose and insulin levels, causing significant insulin resistance. It impacted the liver, leading to elevated liver enzymes and systemic inflammation. CholesDefence and its combination therapy was not as effective as metformin in improving the dyslipidaemia, hyperinsulinaemia, insulin resistance and the resulting inflammation. It however had equipotent anti-hyperglycaemic and hepatoprotective effects compared to metformin.

Keywords: Dyslipidaemia; High fat diet; Metformin; CholesDefence; Herbal formulation

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1. Introduction

There is a rise in the consumption of high fat and high sugar diets, due to the westernisation of society, and the demand for fast and pre-packaged processed foods. This has led to a gradual increase in metabolic diseases such as diabetes, obesity, hypertension and dyslipidemia [1, 2]. Dyslipidaemia is characterised by defective cholesterol metabolism and an abnormal lipid profile. It is a global public health problem that affects millions of people and increases the risk of cardiovascular disease, the leading cause of death worldwide. The epidemiology of dyslipidemia varies by region, age, sex, and ethnicity and is influenced by genetic and environmental factors [3].

Dyslipidaemia is associated with insulin resistance, inflammation, obesity, hepatic lipid accumulation and non-alcoholic fatty liver disease (NAFLD), which are also directly linked to the consumption of high sugar and/or high fat containing foods. In general, a high-fat diet induces the development of metabolic syndromes, which consists of insulin resistance, oxidative stress, atherogenic dyslipidemia, a pro-inflammatory and pro-thrombotic state, high blood pressure, central obesity and cardiovascular disease [4, 5]. Experimental high-fat-diet rat models have become important to biomedical researchers, as rats are easy to manage in terms of dietary feeding and controlling environmental factors. Also, the high fat diet-induced hyperlipidemia model is suitable because the biochemical and pathological changes as well as the clinical manifestations resemble human pathology and associated complications [6, 7, 8].

Increase in the disease burden of non-communicable diseases (NCDs), and metabolic syndromes have led to a corresponding increase in the use of herbal therapeutics. Worldwide, and more especially in local settings, there is a growing acceptability and perception on the use of herbal/alternative therapies. This is due in part to the availability, perceived efficacy and safety of herbal medicines, compared to the high cost and side effects of orthodox medicine [9, 10]. Metformin is a widely used biguanide, which is derived from the plant *Galega officinalis.* It is a first-line treatment for type 2 diabetes and has been used for insulin resistance, pre-diabetes and other metabolic syndromes. Human and animal studies have reported beneficial effects of metformin on metabolic syndromes, liver dysfunction and other diseases, though its mechanism of action is poorly understood [11, 12, 13]. This study evaluates the effects of a commonly used herbal formulation *CholesDefence* (Cholesterol Defence), in high-fat diet fed rats, in comparison to the effects of metformin.

2. Materials and methods

2.1. Experimental Animals

A total of thirty-five (35) male Albino rats weighing between 160 to 180 g were used for the study. The rats were housed in standard cages at regulated room temperature, with controlled 12-hour light-dark cycles, and allowed access to feed and water *ad libitum*. The rats were allowed to acclimatize for two (2) weeks prior to the commencement of study.

2.2. Drugs

The drugs used for the study were CholesDefence (Cholesterol Defence) and metformin. CholesDefence is a commonly used polyherbal drug manufactured by Nature's Field Company, Nigeria, and commercially sold as a dietary supplement. Metformin, a biguanide is manufactured by LEK SA, Poland.

2.3. Acute Toxicity study

Acute Toxicity Study was done by the fixed dose procedure [14], using a group of 3 rats. 2000 mg/kg body weight of CholesDefence was orally administered to each of the rats. The rats were then observed for signs of toxicity for 48 hours. After observation, there were no signs of toxicity, hence the polyherbal tablet was deemed safe up to a dose of 2000 mg/kg body weight. Metformin is a standard anti-diabetic drug, and the doses were translated from the human dose.

2.4. Dose Calculation

2.4.1. CholesDefence

The administered rat dose was extrapolated from the human daily dose [15] as shown below:

Human daily dose is 2 tablets (1722.2 mg each) daily, which is 3444.4mg/day.

Rat dose (mg/kg) = Human daily dose × 0.018 × 5

=3444.4 × 0.018 × 5

= 310mg/kg body weight/day

2.4.2. Metformin

Human daily dose is 1 tablet (500mg) twice daily, that is, 1000mg/day.

Rat dose (mg/kg) = Human daily dose x 0.018 x 5

=1000 x 0.018 x 5

= 90mg/kg body weight/day

2.4.3. High-fat Diet

The high-fat diet was prepared by mixing 3g of normal rat feed with 2g of dietary fat (margarine). The high-fat diet had 42.1% fat content compared to the 3.9% fat content in the normal chow diet.

2.5. Experimental Design

The rats were weighed and grouped into 5 groups of 7 rats each. They were fed high-fat diet for six (6) weeks, two (2), before commencement of treatments and then four (4) weeks alongside the treatments. Treatments (drugs) were administered daily according to the groupings by means of oral gavage for 28 days.

- Group 1: Negative control group (Fed normal chow diet and received no form of treatment)
- Group 2: Positive control group (Fed high-fat diet)
- Group 3: Fed high-fat diet and administered Metformin
- Group 4: Fed high-fat diet and administered CholesDefence
- Group 5: Fed high-fat diet and administered Metformin and CholesDefence

On the 29th day, the rats were fasted for 6 hours, anaesthetized and later sacrificed. Blood samples were collected by means of cardiac puncture. All the animal experiments were conducted according to the ethical norms approved by the Institutional Ethical Committee.

2.6. Reagents and Biochemical Analyses

All reagents were commercially purchased and the manufacturer's standard operating procedures strictly followed. Quality control (QC) samples were run together with the biochemical analysis. Fasting plasma glucose (FPG) was determined using Glucose oxidase method [16], as modified by Randox Laboratories Limited (UK). Fasting plasma insulin (FPI) and C-reactive protein (CRP) levels were quantitatively determined by a rat-specific sandwich-enzyme linked immunosorbent assay (ELISA) method [17] as described by Elabscience Biotechnology Company limited, China. Insulin resistance (IR) was determined using the homeostatic model assessment for insulin resistance (HOMA-IR) method [18]. Total Cholesterol (TC) was determined by enzymatic method [19], as modified by Randox laboratories limited (UK). Triglyceride was determined by enzymatic method [20], as described by Randox laboratories limited (UK). High Density Lipoprotein Cholesterol (HDL-C) was determined by enzymatic method [21], as modified by Randox laboratories limited (UK). Low Density Lipoprotein Cholesterol (LDL-C) was calculated from the Friedewald's equation [22]. The liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined using the Reitman-Frankel method [23], as modified by Randox laboratories limited (UK). Alkaline phosphatase (ALP) was determined using the Colorimetric endpoint method [24] as modified by Randox laboratories limited (UK). Semi-quantitative phytochemical analysis was done on the herbal mixture using classical methods [25].

2.7. Statistical Analysis

Data was analysed using Graph Pad Prism version 8.0.2. Differences between groups were compared using one way analysis of variance (ANOVA), followed by Tukey's multiple comparison test. Results were considered statistically significant at 95% confidence interval ($p \leq 0.05$). Values are expressed as Mean ± SD.

3. Results and discussion

Table 1 Semi-quantitative Phytochemical Analysis of the Herbal Tablet CholesDefence

Phytochemicals	Presence
Flavonoids	+
Cardiac glycosides	++
Saponins	++
Tannins	+
Phenols	-
Terpenoids	+
Alkaloid	-
Glycoside	-
Phlobatannins	-
Anthraquinones	-

++ Absolutely detected, + Moderately detected, - Not detected

Table 1 shows results of phytochemical analyses of the polyherbal tablet CholesDefence. The results revealed the presence of flavonoids, cardiac glycosides, saponins, tannins and terpenoids. Phytochemicals/plant secondary metabolites possess the ability to modulate therapeutic targets and metabolic pathways. This is the basis for their use and application in human healthcare systems, as they bring about drug-like responses [26, 27]. This is consistent with earlier works [28, 29], in which commercially sold herbal formulations were shown to possess active phytochemicals.

Table 2 Fasting Plasma Glucose (FPG), Insulin (INS) and Insulin Resistance (HOMA-IR) Values of the Rats after Treatment

Groups (n=7)	FPG (mmol/L)	INS (mU/L)	HOMA-IR
Group 1 (Negative Control)	4.43 ± 0.36^{b}	0.43 ± 0.10^{b}	0.08 ± 0.02^{b}
Group 2 (Positive Control)	5.83 ± 0.39^{a}	0.69 ± 0.08^{a}	0.19 ± 0.02^{a}
Group 3 (Metformin)	3.98 ± 0.68^{b}	0.47 ± 0.12^{b}	0.10 ± 0.03^{b}
Group 4 (CholesDefence)	4.30 ± 0.62^{b}	0.51 ± 0.17^{ab}	0.14 ± 0.01^{ab}
Group 5 (Metformin + CholesDefence)	4.39 ± 0.52^{b}	0.38 ± 0.13^{b}	0.09 ± 0.02^{b}
p-value	0.0117	0.0103	0.0041
F-value	3.933	4.051	4.676
Remark	S	S	S

n- Number of rats, S- Significant, NS- Not significant, [a]- Significant difference versus negative control, [b]- Significant difference versus positive control

Table 2 shows the results of fasting plasma glucose (FPG), insulin (INS) and insulin resistance (HOMA-IR) levels of the rats after treatment. The results revealed significantly higher (P <.05) FPG levels in the positive control, compared to the negative control and treatment groups. This indicates high-fat diet caused significant hyperglycaemia in the rats. This could be due to increased hepatic lipid metabolism and gluconeogenesis. This is in-line with the works of Moreno-Fernández *et al*. [30], in which a high-fat diet increased plasma glucose levels in rats and made the rats obese. Administration of metformin and Cholesdefence significantly improved the glycaemia caused by the high-fat diet. This implies the herbal tablet and metformin were effective in reducing high-fat diet-induced hyperglycaemia. This is in agreement with the works of Huang *et al*. [31] and Isdadiyanto *et al*. [32], in which metformin and herbal neem leaf extract reduced glucose level in high fat fed rats.

Plasma insulin levels and HOMA-IR values were significantly higher (P <.05) in the positive control group, compared to the negative control and treatment groups. This indicates the high-fat diet caused hyperinsulinaemia and significant insulin resistance. This could be due to increased free fatty acids in circulation, causing increased beta-cell secretion of insulin and/or accumulation of lipids in muscle and adipose tissue leading to insulin resistance. Also, oxidative stress induced by hyperglycaemia and lipid metabolism pathways could affect beta-cell function as seen in diabetes [33, 34]. From the results, administration of metformin significantly reduced the insulin and insulin resistance levels. CholesDefence reduced insulin and insulin resistance, but not to negative control levels, as the values were significantly higher (P <.05), compared to the negative control. Metformin potentiated the actions of the herbal tablet CholesDefence, as the combination therapy significantly improved plasma insulin and insulin resistance. This is consistent with the works of Zabielski *et al*. [35] and Na *et al*. [36], in which metformin and the herbal formulation Seyoeum ameliorated and improved systemic insulin resistance in high-fat diet fed animal models.

Table 3 C-Reactive Protein (CRP) and Liver Enzymes of the Rats after Treatment

Groups (n=7)	CRP (mg/L)	ALT (IU/L)	AST (IU/L)	ALP (IU/L)
Group 1 (Negative Control)	0.59 ± 0.28^{b}	29.71 ± 5.22^{b}	108.86 ± 6.64^{b}	38.43 ± 7.66^{b}
Group 2 (Positive Control)	0.76 ± 0.12^{a}	37.83 ± 5.94^{a}	116.33 ± 7.08^{a}	41.67 ± 8.52^{a}
Group 3 (Metformin)	0.63 ± 0.08^{b}	25.00 ± 7.85^{b}	102.33 ± 4.32^{ab}	39.50 ± 3.83^{b}
Group 4 (CholesDefence)	0.71 ± 0.14^{a}	28.20 ± 5.32^{b}	100.57 ± 6.53^{ab}	36.43 ± 7.18^{b}
Group 5 (Metformin + CholesDefence)	0.66 ± 0.11^{b}	29.43 ± 7.63^{b}	93.04 ± 4.45^{ab}	37.14 ± 6.31^{b}
p-value	0.0241	0.036	< 0.001	< 0.001
F-value	1.459	2.984	12.652	22.83
Remark	S	S	S	S

n- Number of rats, S- Significant, NS- Not significant, [a]- Significant difference versus negative control, [b]- Significant difference versus positive control

Table 3 shows results of C-reactive protein (CRP) and the liver enzymes after treatment. It showed significantly elevated (P <.05) CRP and liver enzymes (ALT, AST, ALP) in the positive control, compared to the negative control and treatment groups. This implies the consumption of high-fat diet caused systemic inflammation, thus the elevation of plasma CRP level. It also, impacted the liver, leading to elevated liver enzymes levels. This could be due to the overproduction and deposition of free fatty acids in tissues including the liver, linking dyslipidaemia to non-alcoholic fatty liver disease. The liver is also responsible for the production of CRP in response to inflammatory stimuli [37, 38]. Administration of metformin and the combination therapy was effective, as it significantly reduced (P <.05) the inflammatory CRP and liver enzyme levels. The polyherbal tablet cholesDefence had no impact on CRP, as the levels were not significantly different (P >.05) from the positive control levels. However, cholesDefence was hepatoprotective, as it significantly reduced (P <.05) the elevated liver enzyme levels. Metformin reduces inflammation through the AMPK activation and suppression of pro-inflammatory cytokines [39]. The results are in consonance with the works of Yasmin *et al*. [40], in which metformin treatment reversed diet induced non-alcoholic fatty liver disease and inflammation in rats.

Table 4 Lipid Profile Parameters of the Rats After Treatment

Groups (n=7)	TC (mmol/L)	TG (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)
Group 1 (Negative Control)	4.56 ± 0.51^{b}	1.05 ± 0.29^{b}	1.29 ± 0.21^{b}	2.80 ± 0.48^{b}
Group 2 (Positive Control)	5.83 ± 0.63^{a}	2.14 ± 0.11^{a}	0.87 ± 0.22^{a}	3.93 ± 0.53^{a}
Group 3 (Metformin)	4.64 ± 0.43^{b}	1.01 ± 0.18^{b}	1.04 ± 0.21^{ab}	3.41 ± 0.45^{a}
Group 4 (CholesDefence)	5.11 ± 0.38^{a}	1.57 ± 0.15^{ab}	0.89 ± 0.19^{a}	3.59 ± 0.52^{a}
Group 5 (Metformin + CholesDefence)	4.58 ± 0.34^{b}	0.99 ± 0.20^{b}	0.92 ± 0.12^{b}	$3.27 \pm .40^{a}$
p-value	0.017	0.035	0.045	0.0028
F-value	1.706	2.106	0.959	1.904
Remark	S	S	S	S

n- Number of rats, S- Significant, NS- Not significant, [a]- Significant difference versus negative control, [b]- Significant difference versus positive control

Table 4 shows the lipid profile of the rats after treatment. The positive control was dyslipidaemic, with significantly (P <.05) elevated total cholesterol (TC), triglycerides (TG), and low-density cholesterol (LDL-C), compared to the negative control. High density cholesterol (HDL-C) was significantly higher (P <.05) in the negative control, compared to the positive control. High-fat diet models have been seen to induce dyslipidaemias in animals, leading alterations in many organ systems, due to the changes in energy metabolism and the deposition of lipids in tissues [40, 41]. Metformin was the most effective treatment, followed by the combination therapy, as they significantly (P <.05) improved TC, TG, HDL-C, but had no impact on LDL-C levels. CholesDefence did not restore the dyslipidaemic conditions to normal, as it only impacted triglyceride levels. Metformin through its antioxidant and anti-inflammatory mechanisms ameliorated the pathologic condition [40]. However, in a similar study, metformin and a polyherbal tablet did not have impact on dyslipidaemia induced by the combination of high-fat diet and streptozotocin, in a type 2 diabetic animal model [29]. The different animal models could be responsible for the different outcomes.

4. Conclusion

Dyslipidaemia induced by high-fat diet elevated glucose and insulin levels, causing significant insulin resistance. It impacted the liver, leading to elevated liver enzymes and systemic inflammation. CholesDefence and its combination therapy was not as effective as metformin in improving the dyslipidaemia, hyperinsulinaemia, insulin resistance and the resulting inflammation. It however had equipotent anti-hyperglycaemic and hepatoprotective effects as metformin and reduced the elevated liver enzyme levels. Herbal medicines should be properly evaluated, with utmost care taken in their combination and use.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

All animal experiments were carried out following ethical norms approved by the Institutional Ethical Committee.

Authors' Contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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