Comparative Therapeutic Properties of Garlic Extract and Metformin on Hyperglycaemia, Hypercholesterolaemia, and Hypertriglyceridaemia in Alloxan-induced Type 1-like Diabetic Rats

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Abstract
The current pilot study was conducted to compare the triple effect of garlic extract and metformin on hyperglycaemia, hypercholesterolaemia, and hypertriglyceridaemia in alloxan-induced type 1-like diabetic rats. Wistar rats were randomly divided into four groups. Control group included normal rats. The second group included alloxan-induced type 1-like diabetic rats, receiving no treatment. The other two groups of diabetic rats were orally treated with 0.75 g of garlic extract per kg of body weight and 0.05 g of metformin per kg of body weight respectively for four weeks. The pharmacological impact of garlic compounds on serum glucose and lipids as opposed to the glucose-lowering and lipid-lowering was highlighted in these experiments. These results revealed that garlic extract has a triple action on hyperglycaemia, hypertriglyceridaemia, and hypercholesterolaemia. Its effect on hyperglycaemia is long lasting, and more pronounced compared to the metformin. Interestingly, it had a regulatory effect on glycaemia as highlighted in the control group.

Keywords
Alloxan, diabetes, garlic extract, glycaemia, metformin

1 Introduction
Garlic (Allium sativum) has been exploited for its therapeutic properties for centuries. The advent of new scientific tools has enabled further in-depth scientific investigations and, in turn, the identification of several biological effects of garlic in cancer, atherosclerosis, infection, and diabetes mellitus (DM). The number of studies comparing metformin and garlic extract on glycaemia and blood lipid levels is limited. There are three types of diabetes mellitus (DM) – type 1: resulting from insulin deficiency, type 2: characterised by insulin insensitivity, and gestational diabetes, which develops during pregnancy and disappears after delivery. Chronic diseases such as cancer and diabetes affect millions of individuals worldwide and their pathophysiology is characterised by hyperglycaemia coupled with metabolic disorders. DM is defined according to the criteria proposed in 2006 by the World Health Organization (WHO) as blood sugar greater than 1.26 g l−1 (7.0 mmol l−1) after 8 h of fasting, the presence of clinical symptoms of diabetes (polydipsia, polyuria, polyphagia), and a serum glucose level greater than or equal to 2 g l−1 (11.1 mmol l−1) 2 h after an oral load of 75 g of glucose. Studies on the hypoglycaemic properties of garlic was carried out by Quesada et al., Lawson and Hunsaker, showed that similar effects were observed with the extract of garlic obtained by the organic solvent diethyl ether. Other studies have shown that garlic has a modulating effect by regulating the amount of blood glucose and that garlic extract has a hypoglycaemic effect like that of glibenclamide, another anti-diabetic drug. The hypoglycaemic property of garlic is dependent on the extraction procedure. Furthermore, research carried out by Lima et al., showed that garlic compounds have, in addition to hypoglycaemic properties, a significant ability to improve glycaemic control. The study on type 1-like diabetic rats induced by the drug streptozotocin showed an improvement in insulin secretion through the skeleton muscle. Another study showed that treatment of diabetic rats with S-allylcysteine, a constituent of garlic, reduces the hyperglycaemic state in plasma, liver, and kidneys. The purpose of the present pilot study was to experimentally evaluate the triple effect of garlic extract on hyperglycaemia, hypercholesterolaemia, and hypertriglyceridaemia in alloxan-induced type 1 diabetic and normoglycemic rats, and compare its effect with the first-line anti-hyperglycaemic drug metformin.

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2 Materials and methods

2.1 Preparation of garlic extract
Fresh garlic bulbs (A. sativum L.) were collected in the region of Benchicaou. The cloves were peeled, sliced, and ground into a paste, and suspended in distilled water for 24 h at room temperature. The macerate obtained was filtered through a gauze and then through 0.45 μm diameter microfilter. The obtained aqueous garlic extract was stored at 4 °C until use.

2.2 Chemicals
Alloxan (2,4,5,6-tetraoxypyrimidine; dioxycarb 5-6) was provided by the Pasteur Institute, Algiers, while the antidiabetic drug (metformin HCl 850 mg) was kindly provided by the Laboratory of Animal Toxicology of the antibiotic producing company SAIDAL, Médéa, Algeria.

2.3 Animal material
Eight-week-old male Wistar rats were purchased from the National Centre for Research and Development of El-Harrach (CRD) and housed in the SAIDAL Animal Toxicology Laboratory with free access to water and standard rat food. The rats were left to adapt to the new environment for one week before starting the experiments.

2.4 Selection criteria
Healthy adult (eight weeks old) male Wistar albino rats aged between 2 and 3 months and weighing 200–260 g were used for the study. Their blood glucose, cholesterol and triglyceride levels were standard (Table 1). Abnormal rats were excluded from the study.

2.5 Induction of diabetes
Diabetes was induced by subcutaneously injecting (0.80 g kg⁻¹ body weight) alloxan monohydrate 98 % at a concentration of 40 mg ml⁻¹. This drug is commonly used to induce type 1 diabetes in laboratory animals. It causes necrosis of β-pancreatic cells, thus inhibiting insulin secretion. Hyperglycaemia was confirmed by the elevated glucose levels measured at 48 h.

2.6 Testing blood glucose correction
Animals with a blood glucose level higher than 1.50 g l⁻¹ were considered diabetic. The diabetic and normal rats were divided into four groups; six rats were used in each group and received through gavage feeding garlic aqueous extract.

Group 1: Diabetic rats with no treatment.
Group 2: Diabetic rats receiving 0.75 g kg⁻¹ body weight of aqueous extract of fresh garlic by gavage feeding.

Group 3: Diabetic rats receiving 0.05 g kg⁻¹ body weight of antidiabetic drug (metformin HCl 850 mg) by gavage feeding.

Group 4: Normal control rats, treated with garlic extract.
Group 5: Normal control rats, not treated with garlic extract.

During the three weeks of treatment, a daily investigation of the evolution of diabetic rats during the treatment period was carried out. Water consumption, food and rats’ weight were monitored, and standard analysis for blood sugar, cholesterol, and triglycerides was performed (Table 1). A glucose assay (glycaemia) was performed every week during the treatment period and after treatment cessation.

Table 1 – Standards for blood sugar, cholesterol, and triglycerides in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Weight / g</th>
<th>Blood glucose / g l⁻¹</th>
<th>Cholesterol / g l⁻¹</th>
<th>Triglyceride / g l⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCR</td>
<td>248 ± 25b</td>
<td>1.15 ± 0.10b</td>
<td>1.20 ± 0.40b</td>
<td>1.10 ± 0.20b</td>
</tr>
<tr>
<td>DR</td>
<td>235 ± 15b</td>
<td>1.20 ± 0.15b</td>
<td>1.30 ± 0.50b</td>
<td>1.35 ± 0.45b</td>
</tr>
<tr>
<td>DRG</td>
<td>240 ± 25b</td>
<td>1.00 ± 0.20b</td>
<td>1.15 ± 0.35b</td>
<td>1.15 ± 0.25b</td>
</tr>
<tr>
<td>DRM</td>
<td>245 ± 25b</td>
<td>1.10 ± 0.30b</td>
<td>1.05 ± 0.25b</td>
<td>1.25 ± 0.35b</td>
</tr>
</tbody>
</table>

NCR – normal control rats treated with garlic extract; DR – diabetic rats untreated; DRG – diabetic rats receiving garlic extract; DRM – diabetic rats treated with metformin. Values are the mean ± standard deviation for six rats per group.

Statistical significance p < 0.01; Statistical significance p < 0.05.

2.7 Biochemical analysis of blood
Retro-orbital blood collection in a heparinised capillary tube was followed by glucose analysis. Glucose concentrations in the blood were analysed enzymatically using the Bergmeyer method. The kit consisted of a standard (1 g l⁻¹) glucose solution and reagent of the following composition: phosphate 70 mmol l⁻¹, phenol (5 mmol l⁻¹) glucose oxidase > (10 ul ml⁻¹), peroxides > (1 ul ml⁻¹), 4-aminoantipyrine (0.4 mmol l⁻¹), pH 7.5.

2.8 Equipment
Water bath set at 37 °C (MemmertType WNB 14 F-NR LY 12.0671 230v-freq 50/60 HZ). Spectrophotometer (Model Sp 3000 nano OPTIMA leq 50/60 HZ 1A) was used in the experiments.

2.9 Statistical analysis
All experiments were performed in triplicate. The results of the hypoglycaemia effect of garlic extract and Metformin...
were expressed as mean ± SD (n = 6). One-way ANOVA was used to analyse the differences between experimental
groups (PAST version 1.7). Differences were considered
significant when p-values were below 0.05, and more sig-
ificant when p-values were below 0.01.

2.10 Sample
Serum or plasma was collected by standard procedure,
quickly separated from red blood cells to avoid cell gly-
colysis. The reagent was stored at room temperature and
 aliquoted in tubes. The tubes were incubated in a shaking
water bath at 37 °C for 10 min. The absorbance of the
samples was measured against a blank at 500 nm. The glu-
cose concentration of the sample was calculated by Eq. (1),
as described by GOD-POD colorimetric method.

\[
glucose (g \cdot l^{-1}) = \frac{\text{absorbance of sample}}{\text{absorbance of standard}} \cdot \text{conc. of std (g \cdot l^{-1})}
\]  

(1)

3 Results
Diabetes was induced in rats following alloxan treat-
ment resulting in a significant increase in blood glucose
(4.75 g l⁻¹) (Fig. 1). Garlic extract had a lowering serum glucose and lipids in type 1 diabetic rats, as illustrated in
Fig. 2. A slight decrease in glycaemia after 72 h of treat-
ment with metformin was noticed. Garlic extract had a
more effective and lasting effect than metformin (Fig. 3).

3.1 Effect of alloxan on rats’ serum glucose
To establish a model of diabetic rats, a group of six animals
were injected once subcutaneously with (0.80 g kg⁻¹ body
weight) alloxan monohydrate 98 %. The results of glyca-
emic rats measured for five weeks, as shown in (Fig. 1), in-
dicated that the glucose level measured in 48 h post-in-
jection (4.16 ± 0.21 g l⁻¹) increased during the first four
weeks, reaching 5.00 (± 0.11 g l⁻¹). A slight decrease in
blood sugar level was observed during the fifth and sixth
week. A possible explanation for hypoglycaemia is either
limited effect of alloxan or beta cell regeneration, as re-
ported by Gorray et al.⁵¹

3.2 Effect of garlic extract on serum glucose
in diabetic rats
The effect of garlic extract in lowering serum glucose and
lipids in type 1 diabetic rats is illustrated in (Fig. 2). In al-
loxan-treated rats, the administration of the garlic extracts
at a dose of 0.75 g kg⁻¹ of body weight induced a signifi-
cant hypoglycaemia after 24 h of garlic extract treatment,
i.e., from (4.47 ± 0.44) g l⁻¹ to (2.00 ± 0.19) g l⁻¹. The
blood glucose stabilized after one week of treatment,
reaching (1.04 ± 0.08) g l⁻¹ and (0.86 ± 0.10) g l⁻¹ in the
second and third week, respectively. It is very important to
emphasize that a gradual, but slight re-increase in blood
glucose was noticed one week after the treatment was in-
terrupted. The registered rates were (1.23 ± 0.11) g l⁻¹,
(1.33 ± 0.04) g l⁻¹, and (1.35 ± 0.06) g l⁻¹ in the first week,
the second and third week, respectively.
Fig. 3 – Effect of oral administration of metformin (0.05 g kg\(^{-1}\) body weight) on the serum glucose in diabetic rats. Each column represents the mean of six rats. **I** – 48 h after the injection of alloxan monohydrate 98 %, **II** – after 24 h of treatment, **III** – one week of treatment, **IV** – two weeks of treatment, **V** – a week without treatment, **VI** – two weeks without treatment, **VII** – three weeks of treatment discontinuation, **VIII** – four weeks of treatment discontinuation. Statistical significance vs. normal control (\(p < 0.05\)).

### 3.3 Effect of metformin on blood glucose levels in type 1-like diabetic rats

The aim of the study was to compare the triple effect of garlic extract with that of the reference anti-diabetic drug: metformin (Fig. 3). A slight decrease in glycaemia after 72 h of treatment was noticed, from (3.87 ± 0.61) g l\(^{-1}\) observed in 48 h to (2.58 ± 0.43) g l\(^{-1}\). A decline in blood glucose levels was observed thereafter to reach a minimum level two weeks of treatment. In fact, the glucose levels recorded were (1.98 ± 0.22) g l\(^{-1}\), (2.58 ± 0.43) g l\(^{-1}\) and (0.98 ± 0.26) g l\(^{-1}\) in one week, two weeks, and three weeks of treatment, respectively. It was noticed that the glucose level recorded in the second week of treatment was comparable to that of normal rats. We can therefore say that the garlic extract, at an optimised dose of 0.75 g kg\(^{-1}\) body weight, was more effective than metformin (0.05 g kg\(^{-1}\) body weight). It should be noticed that discontinuation of treatment led to a rapid re-emergence of hyperglycaemia, which reached (3.42 ± 0.400) g l\(^{-1}\) in the fourth week of treatment discontinuation, as opposed to the effect of our aqueous garlic extract.

### 3.4 Effect of the garlic extract on serum glucose in normal control rats

The garlic extract did not induce severe hypoglycaemia in either normal rats or normal rats receiving garlic extract, but played a regulatory role. The control group received a daily treatment of garlic extract, with 0.75 g kg\(^{-1}\) of body weight for eight consecutive weeks. As a result, the glycaemia remained stable and close to normal level throughout the experimental time (Fig. 4).

### 3.5 Physiological and lipid monitoring

The hyperglycaemic increase caused by alloxan was accompanied by a physiological change in rats, namely, weight loss and nutritional disturbance (Table 2).

<table>
<thead>
<tr>
<th>Table 2 – Changes in the body weight (g) of the rats during eight weeks of the experiment</th>
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<tr>
<td></td>
</tr>
<tr>
<td>NCR</td>
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<tr>
<td>DR</td>
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<tr>
<td>DRG</td>
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<td>DRMG</td>
</tr>
</tbody>
</table>

NCR – normal control rats treated with garlic extract; DR – diabetic rats untreated; DRG – diabetic rats receiving garlic extract; **bold** indicates cessation of treatment; DRM – diabetic rats treated with metformin – **bold** indicates cessation of treatment. Values are the mean ± SD for six rats per group.

\(^a\) Statistical significance \(p < 0.01\) vs. normal control rats untreated; \(^b\) Statistical significance \(p < 0.05\) vs. normal control rats untreated.
A significant decrease in body weight was observed from the first week in the group of diabetic rats, untreated with garlic extract. On the other hand, oral administration of garlic extract to the alloxan-induced diabetic rats (0.75 g kg⁻¹ of body weight) significantly reduced the loss of body weight from the second week of treatment (p < 0.05) compared to baseline values. In contrast, daily administration of metformin (0.05 g kg⁻¹) induced a statistically significant change (p < 0.05) in cholesterol and triglyceride levels, observed in rats of groups 1, 2, and 3 over five weeks of observation (Figs. 5a and 5b).

An insignificant difference in body weights of group 1 diabetic rats was found compared to the baseline. Rats in group 1 had the highest cholesterol and triglyceride levels, compared to those of group 5 (non-diabetic rats). This increase was observed in the second week. In alloxan-induced diabetic rats, treatment with garlic extract showed a significant decrease in total cholesterol and triglycerides compound levels (p < 0.05) from the third week of treatment. In the metformin-treated group of rats, no significant improvement was observed. On the contrary, total cholesterol and triglyceride levels continued to ascend. Furthermore, a positive correlation was observed between the concentration values of total cholesterol and triglycerides with glycaemia in the garlic-extract treated rats, whereas in the metformin treatment group there was a weak positive correlation (Figs. 6a and 6b).

4 Discussion

The present study mainly revealed that the triple action of garlic extract, compared to metformin, are more pronounced, long-standing, and have a regulatory and regenerative property. Garlic extract contains a myriad of molecules having a wide range of therapeutic properties. For the purpose of this study, these compounds were used to investigate their triple impact in alloxan-induced, insulin-dependent type-like diabetic rat models by impairing insulin-secreting β-cells in the islets of Langerhans, which is an irreversible effect. Initially, alloxan-induced severe hypoglycaemia, which is attributed to the hyperinsulinaemia following beta-cytolysis caused by altered redox potential, is ephemeral. The results obtained show that the garlic extract significantly reduced blood glucose, cholesterol, and triglycerides in alloxan-induced diabetic rats. The same effect has been confirmed by several studies.

Other studies have shown that the therapeutic effect of garlic is attributed mainly to allicin-type compounds. A research carried out by Behrouj et al. showed that garlic extract had, in addition to the hyperglycaemic effect, an antioxidant property capable of reversing liver injury following exposure to nitric oxide (NO) induced by hyperglycaemia. Allicin, the main active ingredient in garlic, inhibits autophagy, which plays a key role in the pathogenesis of diabetes, through activation PI3K/Akt/mTOR, and MAPK/ERK/mTOR signalling pathway. Activation of mTOR stimulates beta cells growth, which may explain the pancreas recovery. A possible role of garlic extract in glucose oxidation explains the hypoglycaemic effect observed. Glucose reacts with oxygen to produce water and carbon dioxide. Another study revealed the effect of garlic compounds in reducing hyperglycaemia, enhancement of serum insulin, and glycogen synthesis in the liver and skeletal muscle. The mechanism of hypoglycaemic action probably involves either direct or indirect stimulation of insulin secretion. The exact mechanisms of garlic as an antihyperglycemic

![Graph](https://example.com/graph.png)

**Fig. 5** — a) Total cholesterol levels (g l⁻¹) in normal, treated, and untreated diabetic rats, for eight weeks. Cholesterol levels in diabetics rats treated with garlic extract (0.75 g kg⁻¹ of body weight) and diabetic rats treated with metformin (0.05 g kg⁻¹) are shown during treatment and after treatment cessation. Each point indicates the mean ± s.e.m. of six rats from each group. (p < 0.05) compared to untreated diabetic rats and to control rats (Group 5). b) Total triglyceride levels (g l⁻¹) in normal, treated, and untreated diabetic rats for eight weeks. Triglycerides in diabetic rats treated with garlic extract (0.75 g kg⁻¹ of body weight) and diabetic rats treated with metformin (0.05 g kg⁻¹) are shown during treatment and after treatment cessation. Each point indicates the mean ± s.e.m. of six rats from each group. (p < 0.05) compared to untreated diabetic rats and to control rats (Group 5).
agent are still unclear.\textsuperscript{36} It may play a role in increasing insulin activity, enhancing insulin secretion, or increasing the number of beta cells in the pancreas by activating the process of their regeneration.\textsuperscript{31,42} The soluble fibre in the garlic extract may interfere with carbohydrate absorption, thereby affecting glycaemia.\textsuperscript{43} Liu et al.\textsuperscript{41} suggested that the increased activity of insulin may be caused by direct stimulation of pancreatic β cells or indirect production of gastrointestinal hormones associated with the pancreas.

Our results have demonstrated that the control group treated with garlic compounds developed no severe hypoglycaemia (Fig. 5). The stable blood glucose level after treatment cessation in the garlic treated group may probably be attributed to a regulatory effect involving insulin, rather than an orchestrated control of glucose metabolism. Likewise, the comparative study concerning the effects of garlic extract and metformin on type 1 diabetic rats revealed that, during the initial phase of treatment, a significant decrease in glycaemia had occurred. Moreover, metformin did not stimulate insulin secretion, and subsequently had no severe hypoglycaemic effect among normal rats.\textsuperscript{44} This study has shown that metformin induced hypoglycaemia without a lasting effect following treatment cessation, whereas garlic extract had more pronounced effects after treatment discontinuation, as illustrated in (Fig. 2). In contrast, rats previously treated with garlic extract had glycaemia levels close to the average value, and almost remained constant during this phase, which could be an insulin-dependent mechanism. Metformin has been used for decades, but its entire molecular mechanism of action is not completely understood. According to Qiu et al.\textsuperscript{45} and Scarpiello et al.,\textsuperscript{46} the glycaemic reduction action is largely attributed to the improvement in hepatic insulin resistance, leading to a reduction in hepatic gluconeogenesis. To explain the physiological and lipid evolution observed in this study, several mechanisms could be proposed. According to some studies, the destruction of pancreatic β cells can lead to weight loss in diabetic animals.\textsuperscript{47} The increase in triglyceride levels observed in diabetic rats could be due to a lack of insulin, which normally stimulates the enzyme lipoprotein lipase.\textsuperscript{48} Other studies have suggested that garlic extract helps prevent the absorption of cholesterol from the gut by binding to bile acids.\textsuperscript{49} Moreover, it can act by reducing cholesterol biosynthesis, particularly by decreasing the activity of the coenzyme 3-hydroxy-3-methyl-glutaryl reductase (HMG-CoA reductase) (a key enzyme of cholesterol biosynthesis).\textsuperscript{50} Further investigative studies are needed to fully explain the mechanism of action and the pharmacological properties of garlic compounds.

5 Conclusions
The results of this study have indicated that garlic compounds had triple action since they significantly reduced hyperglycaemia, hypercholesterolemia, and hypertriglyceridaemia in alloxan-induced type 1-like diabetic rats compared to the rats treated with diabetogenic drug of choice: metformin. Furthermore, a therapeutic effect persisted for several weeks after treatment cessation, suggesting a possible regeneration mechanism of the pancreatic, insulin-producing beta cells. Moreover, the garlic extract had no severe hypoglycaemia effect on normal rats, suggesting a regulatory function of glucose and lipids homeostasis.
Further investigative research is needed to fully comprehend the mechanism of action of garlic compounds and their pharmacological implications.

ACKNOWLEDGEMENTS

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Author contributions: D.A. designed and carried out experiments in collaboration with D.O. O.B. and K.B. contributed to the writing of scientific inputs of the manuscript. M.H. and S.H. contributed to data acquisition and statistical analysis. All authors read and approved the final version.

Competing interest: The authors declare that they have no competing interests.

Ethical approval: This work received the ethical approval from the ethical authority of the University of Médéa, Algeria.

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Komparativna terapeutska svojstva ekstrakta češnjaka i metformina na hiperglikemiju, hiperkolesterolemiju i hipertrigliceridemiju kod štakora s dijabetesom tipa 1 induciranim aloksanom

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Ovo pilot-istraživanje provedeno je da bi se usporedio trostruki učinak ekstrakta češnjaka i metformina na hiperglikemiju, hiperkolesterolemiju i hipertrigliceridemiju kod štakora s dijabetesom tipa 1 induciranim aloksanom. Štakori Wistar nasumično su bili podijeljeni u četiri skupine. Kontrolna skupina sadržavala je normalne štakore. Druga skupina bili su netretirani štakori s dijabetesom (dijabetes tipa 1, induciran aloksanom). Preostale dvije skupine štakora s dijabetesom liječene su četiri tjedna ekstraktom češnjaka doziranim 75 g po kilogramu tjelesne težine, odnosno metforminom u dozi 0,05 g po kilogramu tjelesne težine. Navedeni spojevi unosili su se oralnim putem. U istraživanjima naglašen je farmakološki utjecaj spojeva iz češnjaka na glukozu i lipide u serumu. Dobiveni rezultati pokazali su da ekstrakt češnjaka ima trostruko djelovanje na: hiperglikemiju, hipertrigliceridemiju i hiperkolesterolemiju. Njegov učinak na hiperglikemiju dugotrajan je i izraženiji u usporedbi s metforminom. Zanimljivo je da je djelovao regulacijski na glikemiju.

Ključne riječi
Aloksan, dijabetes, ekstrakt češnjaka, glikemija, metformin

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