Inhibitory Effects of *Datura innoxia* Mill Extracts on two Enzymes Involved in The Control of Diabetes and Inflammation

ABSTRACT

Aims: Thisworkaimed to evaluate the inhibitory effects of *Datura innoxia* Mill leaf and root extracts on two enzymesinvolved in the control of diabetes and inflammation.
Study design: The α-amylase and trypsin are two key enzymes involved in the regulation of diabetes and inflammation. Inhibiting the activity of these two enzymes is one of the keys to managing the diabetes and inflammation.
Place and Duration of Study: The leaves and roots of *D. innoxia* were harvested at Safo, village located in the commune of Kati, Region of Koulikoro, Mali in January 2022. Experimental studies were conducted at the Laboratory of Food Biochemistry and Natural Substances (LBASNa), Faculty of Sciences and Techniques (FST),University of Sciences, Techniques and Technologies of Bamako (Mali) from February to September 2022.
Methodology: The phytochemical composition of the extracts was performed based on classical qualitative methods. The colorimetric method was used to determine the anti-diabetic and anti-inflammatory activity of the extracts using the amylase and trypsin inhibition test, respectively. The statistical tests employed with Minitab software.
Results: The phytochemical screening revealed the presence of numerous bioactive compounds. The inhibitory potential against α-amylase and trypsin varied according to the extract concentrations and plant parts (p-value < .05). The hydroethanolic extracts exhibited more significantly α-amylase inhibitory activities than the ethyl acetate and aqueous ones. The leaves were more active than roots. With the extract concentrations tested (20 to 640 µg/mL), the inhibition rates ranged from 12.98±0.56 to 89.65±4.18% against amylase and from 2.70±0.43 to 75.19±3.54% against trypsin. The concentrations inhibiting 50% (IC_{50}) of enzyme activity were 182.22±20.12 to 323.27±7.01 µg/mL for α-amylase and 333.86±10.38 to 461.25±27.41 µg/mL for trypsin. These registered potential inhibitors of amylase and trypsin conferred on these extracts the ability to modulate the diabetes and inflammation, respectively.
Conclusion: These data show that *D. innoxia* extracts would possess hypoglycemic and anti-proteolytic potential. Therefore, the species are hopeful for the conception of phytomedicines using its hydroethanolic leaf extracts to contribute to the traditional management of diabetes and inflammation in Mali.

Keywords: Datura innoxia, diabetes, inflammation, alpha-amylase, trypsin.

1. INTRODUCTION

According to the International Diabetes Federation (IDF), diabetes represents the number one non-infectious epidemic affecting the humanity. Nowadays, it has become an enormous health problem and is an increasing economic burden hampering the social and economic

development of many countries, especially in low incomes ones [1]. It is a multifaceted metabolic disorder that affects the glycemic status of the human body. The impaired glucose tolerance and hyperglycemia are known as the main clinical and diagnostic features and result from the total or relative insulin deficiency or the resistance to its action [2].

Currently, the role of chronic inflammation in the pathogenesis of type 2 diabetes and its complications is well documented. It has been reported that certain molecules like as cytokines, chemokines and interleukins, responsible for inflammatory and immune responses, are highly involved in process leading to diabetes [3]. Therapeutic interventions reducing metabolic inflammation improve insulin secretion and action, glucose control, and may prevent long-term complications [4]. For instance, the proteases, enzymes that digest the proteins, are specific in their action, and need to be tightly regulated by inhibitors such as trypsin [5]. These protease inhibitors (PIs) inhibit the proteases produced during the wounds or infections, which are the main initiators of inflammation [6].

To face up to this scourge, many synthetic inhibitors (acarbose, metformin, etc.) have been developed. The limitations and side-effects observed with these synthetic molecules have given new impetus to the scientific community to investigate other sources of natural anti-diabetic and anti-protease agents. The medicinal plants are renowned to be a priceless source of bioactive compounds with these potentialities. Among these plants, those belonging to the *Datura* genus are highly prized in the Malian traditional medicine.

Among the species inventoried in Mali, *Datura innoxia* Mill are highly coveted by the local populations. An ethnobotanical survey recently conducted byTogola et al.[7] in southern of Mali reported that many traditional healers used this species (alone or in combination with other plant species) for the treatment of snakebites and venomous insects, wounds, diabetes, etc. Numerous studies have also demonstrated its antioxidant, anti-diabetic and anti-inflammatory properties. Numerous studies have also demonstrated its antioxidant, antidiabetic[7][8], anti-inflammatory and anticancer properties [9]. While many studies praise its biological properties of *D. innoxia* extracts, their mechanism of action in inflammatory and anti-diabetic processes remains under-investigated. This study was initiated to fill this gap. It is part of a strategy to enhance the value of medicinal plants in the Malian pharmacopoeia.

2. MATERIAL AND METHODS

2.1. PLANT MATERIAL

The plant material used in this work was constituted of leaves and roots of *D. innoxia*. They were harvested from Safo, located in the commune of Kati, Region of Koulikoro, Mali. After cleaning, the samples were air-dried at 25-30 °C during two weeks about. An electric grinder (Hausberg, HB 7567, 150 W) was used to reduce the dried samples into powder, kept at cold (0 °C) before further use.

2.2 METHODS

2.2.1Preparation of extracts

The extracts were prepared by macerating in the three types of solvents (aqueous, Hydroethanolic at 70%, and ethyl acetate). After solubilization of sample powders (10%), the mixture was stirred magnetically at room temperature for 6 h, then filtered under vacuum. The same operation was repeated three times for each sample. The filtrates obtained were added and concentrated using a rotary evaporator (Bucchi B-100), before stored cold for further analysis.

2.2.2Phytochemical screening

The phytochemical major groups were detected in the extracts by using the standardized qualitative tests [10][11]. The alkaloids were detected by the Dragendorff and Mayer reaction while the flavonoids were detected by the alkaline reagent test; the tannins by Braymer test; the coumarins by the NaOH test; the sterols and terpenoids by Salkowski test; the anthraquinones by Borntrager test; and the saponins by the foam test.

2.2.3 Evaluation of inhibitory effects on enzymes

2.2.3.1 Inhibitory effects on α-amylase

The inhibitory activity of α-amylase was performed based on the protocol reported byKonaré et al.[12]with slightly modifications. An extract volume of 125 µL at different concentrations (from 20 to 640 µg/mL) was mixed with 125 µL of alpha amylase solution (10 µg/mL) and 125 µL of sodium phosphate buffer (0.1 M pH6.9). After incubated the reaction mixture at 37° for 10 mn, 125 µL of substrate (strach 1%) were added. The mixture was re-incubated at 37° for 20 mn, and the reaction was stopped by adding 500 µL of DNS reagent (3.5 dinitrosalicylicacide). After being cooled at room temperature (25-30 °C), the absorbances were read at 540 nm using a spectrophotometer (Thermo Scientific, Biomate 3S).

Acarbose, a reference anti-inflammatory drug, was used as positive control and distilled water, the solubilizing solvent for extracts, as negative control. The results were expressed as inhibition rate (%) using the formula below.

Inhibition rate (%) = [1 - $\frac{Absorbanceofextract}{Absorbanceofnegativecontrole}$] × 100

2.2.3.2. Inhibitory effects on trypsin

The protocol described byKennedy[13] allowed to assess the inhibitory potential on trypsin.
A volume of 125 µL of extract at different concentrations (from 20 to 640 µg/mL was added to 25 µL of trypsin (10 µg/mL) and 25 µL distilled water. Then, 225 µL of casein (3%) were added into the mixture and incubated at 37 °C for 30 min. A solution of 150 µL of trichloroacetic acid was introduced into the mixture to stop the reaction. The read of absorbances was carried out at 280 nm. Quercetin was used as positive control. The inhibition rates (%) were calculated using the same formula above.

2.3. Data analysis

The statistical analysis was performed with Minitab statistical software version 18.1 (Minitab Inc., PA., USA).ANOVA based on Tukey test was used to compare the means at .05 threshold.

3.RESULTS

3.1. Phytochemical composition

The results of phytochemical screening are summarized in (Table1)

Table 1. Phytochemical constituents

Chemical groups	Leaves			Roots		
	Aqueous	Ethanol	Ethyl acetate	Aqueous	Ethanol	Ethyl acetate
Alkaloids	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+
Coumarins	+	+	+	+	+	+
Saponins	+	+	+	-	-	-
Tannins	+	+	+	+	+	+
Triterpenes	+	+	+	+	+	+

(+) = Present, (-) = Absent

All the sought major chemical groups have been found in all the extracts excepted the saponin in the root extracts.

3.2 Anti-diabetic and Anti-inflammatory potential

The enzyme inhibition rates of the various extracts at different concentrations, in addition to their 50% inhibitory concentrations (IC_{50}), are presented in (Tables 2 and 3). All leaf and root extracts showed the inhibitory effects on the activity of the two tested enzymes (amylase and trypsin). For each enzyme, this inhibitory potential varied according to organs and extract types, and also according to the extract concentrations (p-value<.05). For each organ and enzyme, the hydroethanolic extracts showed the best inhibition rates. For amylase, the inhibition rates ranged from 31.77±0.75 to 89.65±4.18% for leaf hydroethanolic extracts and from 9.72±1.77 to 87.69±0.84% for root hydroethanolic extracts, with extract concentrations ranging from 20 to 640 µg/mL. With trypsin, the variations of 21.94±2.14 75.19±3.54% for hydroethanolic leaf extracts and 9.72±1.77 to 74.54±1.05% for root extracts were recorded.

Table 2. Inhibitory effects of *Datura* extracts against α-amylase's activity (%)

Plant parts	Types of extracts	Concentrations (µg/mL)						IC_{50} (µg/mL)
		20	40	80	160	320	640	
Leaves	Aqueous	19.02±3.23^{bc}	25.30±1.82^{bc}	39.70±2.86^{a}	48.09±5.67^{ab}	62.14±2.09^{ab}	86.63±1.51^{a}	241.31±2.32^{b}
	Hydroalcoholic	31.77±0.75^{a}	36.88±2.42^{a}	42.62±1.49^{a}	49.72±2.34^{a}	64.54±4.26^{a}	89.65±4.18^{a}	182.22±20.12^{c}
	Ethyl acetate	10.45±1.06^{d}	16.06±1.29^{d}	28.20±2.12^{c}	43.36±4.89^{b}	57.13±1.74^{bc}	81.64±0.93^{c}	306.03±6.62^{a}
Roots	Aqueous	27.80±5.32^{a}	31.21±1.86^{b}	36.24±0.33^{b}	42.98±1.69^{b}	61.35±1.61^{ab}	84.26±4.89^{b}	240.44±14.61^{b}
	Hydroalcoholic	24.62±0.96^{ab}	30.02±4.06^{ab}	39.34±4.72^{a}	47.44±2.28^{b}	66.99±1.75^{a}	87.69±0.84^{b}	216.37±6.30^{b}
	Ethyl acetate	12.98±0.56^{cd}	19.43±3.02^{cd}	31.42±2.94^{b}	38.65±2.78^{b}	53.97±2.01^{c}	77.30±0.44^{c}	323.27±7.01^{a}
p-value		0.002E-3<.05	0.004 E-3<.05	0.003E-1<.05	0.024	0.003E-1<.05	0.002	0.002E-5<.05
F value		30.70	27.32	11.77	3.95	11.68	8.01	68.48

**For each column, the means values of extract that do not share the same letters are considered to be significantly different at 0.05 threshold.*

Table 3. Inhibitory effects of *Datura* extracts against trypsin's activity (%)

Plant parts	Types of extracts	Concentrations (µg/mL)						IC_{50} (µg/mL)
		20	40	80	160	320	640	
Leaves	Aqueous	13.69±1.17^{b}	20.64±1.13^{b}	25.11±0.93^{b}	37.16±2.56^{a}	49.57±3.54^{a}	71.63±3.44^{ab}	364.52±11.89^{c}
	Hydroalcoholic	21.94±2.14^{a}	26.29±1.21^{a}	32.79±5.26^{a}	37.64±3.10^{a}	46.43±3.23^{ab}	75.19±3.54^{a}	333.86±10.38^{c}

	Ethyl acetate	2.81±1.35^{d}	12.53±1.25^{d}	21.85±2.11^{b}	27.68±2.22^{b}	42.08±4.58^{ab}	62.35±5.19^{cd}	461.25±27.41^{b}
Roots	Aqueous	6.42±0.77^{cd}	16.24±1.38^{c}	23.55±2.38^{b}	29.52±2.36^{b}	36.88±2.20^{b}	65.37±1.96^{bcd}	452.17±11.13^{b}
	Hydroalcoholic	9.72±1.77^{c}	13.48±1.25^{cd}	18.87±1.78^{bc}	33.40±2.13^{ab}	48.37±2.68^{a}	74.54±1.05^{ab}	373.80±3.71^{c}
	Ethyl acetate	2.70±0.43^{d}	5.51±0.78^{e}	13.97±1.00^{c}	19.04±2.82^{c}	27.11±4.15^{c}	59.58±3.70^{d}	543.10±44.29^{a}
p-value		0.001E-5<.05	0.001E-6<0.05	0.005E-1<.05	0.001E-2<.05	0.003E-2<.05	0.003E-1<.05	0.009E-5<.05
F calculated		84.09	110.10	16.82	22.41	17.97	11.19	35.43

**For each column, the means values of extracts hat do not share the same letters are considered to be significantly different at 0.05 threshold*

4. DISCUSSION

This work aimed to contribute to the search for natural inhibitors of α-amylase and trypsin through an estimation of the inhibitory effects of extracts from the leaves and roots of *D. innoxia* on their activity.
The preliminary phytochemical screening tests revealed the presence of the same chemical groups (alkaloids, flavonoids, tannins, coumarins, and triterpenes) in the leaf and root extracts. On the other hand, we note the absence of saponins in the root extracts. The presence of these metabolites had been highlighted by several authors[8][9][14].

All the extracts of*D. innoxia*showed an inhibitory poweragainst α-amylase and trypsin (protease) activities. With extract concentrations ranged from 20 to 640 µg/mL, the inhibition rates of amylase activity varied from 12.98±0.56 to 89.65±4.18% respectively for the root ethyl acetate extracts and leaf hydroethanolic extracts. With the same range of concentrations, the antitrypsin rates were from 2.70±0.43 to 75.19±3.54% for the root ethyl acetate extracts and the leaf hydroethanolic extracts. The highest anti-amylase inhibitory activity (p-value = 0.002E-5<.05; F-value = 68.48) was observed with the hydroethanolic extracts of the leaves (IC_{50} = 182.22±20.12 µg/mL). On the other hand, with trypsin, it was the hydroethanolic extracts with IC_{50} = 333.86±10.38 µg/mL and aqueous extracts with IC_{50} = 364.52±11.89 µg/mL from the leaves and the hydroethanolic extracts from the roots with IC_{50} = 373.80±3.71 µg/mL which simultaneously showed the best inhibitory activities (p-value = 0.009E-5<.05; F-value = 35.43). Globally, the leaves showed the best inhibitory power towards these enzymes.
An effective manner to better manage the diabetes is to inhibit or reduce the α-amylase's activity, which collapsed the starch to more simple carbohydrates such as glucose, maltose, dextrin [15]. As a result, the increase in level of blood sugar by inhibiting or limiting the action of this enzyme, *D. innoxia* extracts could be helpful to regularizing blood sugar.

Furthermore, it is well known that the inflammation and the oxidative stress encountered inthe diabetic patients are directly associated with the body's insulin resistance [16]. Likewise, many authors strongly incriminated the oxidative stress and inflammatory factors in the pain experienced during the diabetes mellitus [13][16]. The natural antioxidant agents are endowed with the ability to protect the human body from free radicals and consequently retard the progress of many chronic diseases [12].
These inhibitory activities exhibited by our extracts would be due to their phytochemical composition. The literature reports that most of the chemical groups highlighted in these extracts have anti-inflammatory and antidiabetic potential [17][6][12]. Previous studies have reported that the inhibitory effects of amylase and trypsin of Datura species would be linked to their alkaloid contents, and above all to a synergy of action between the different molecules such as phenolic compounds, coumarins, terpenes [18].Togola et al. [14]had shown the richness of *D. innoxia* extracts in polyphenols and flavonoids which are endowed with anti-amylase activity, synonymous with hypoglycemic potential[12]. This explanatory hypothesis corroborates the scientific data from the literature which demonstrated a strong involvement of polyphenols and flavonoids in the inhibition of the activities of α-amylase and trypsin[12][19].The hydroethanolic extracts of *D. innoxia*leaves showed the best antitrypsin and anti-amylase activities. According to Taylor et al. [20], the high antitrypsin or antiprotease potential in the leaves would be linked to their protein richness. These data confirm the numerous therapeutic claims attributed to the organs of *D. innoxia* in Mali. For example, Togola et al.[9] reported that this species was used in Mali as an anti-inflammatory through its ability to modulate the production of nitric oxide by murine macrophages, main agents inducingthe inflammatory reactions. These authors recorded 30.05±3.11% of inhibition rate of nitrite secretion with only 0.5 µg/mL of extracts without any obvious toxicity. They work also reported very promising inhibitory effects on cancer cells with *D. innoxia* extracts [9]. This anti-inflammatory and anti-cancer potential would be linked to the presence of triterpenes (in our extracts) which are produced by plants in response to infection or mechanical damage[17]. Indeed, the triterpenes are powerful inhibitors of protease, an inflammatory molecule produced in excess in tumor cells[21].
Other previous studies have revealed that the protease inhibitors also have the ability to trap the reactive oxygen species due to their antioxidant power[5]. These results support the hypothesis that the anti-inflammatory and antioxidant treatment could positively impact the treatment and prevention of diabetes [3]. Despite the existence of synthetic inhibitors such as acarbose and metformin which are widely used by diabetic patients, our data show that the leaf and root extracts of *D. innoxia*are hopeful to compensate for the side effects, generated by these synthetic molecules [22]. With this ability to inhibit amylase and trypsin, *D. innoxia* extracts could be proposed as a possible alternative for the therapy of diabetes and tumors.

Besides, based on their antifungal and antibacterial properties[5], the protease inhibitors can curb the microbial alteration of foodstuffs[6], which is a global concern due to the enormous post-harvest losses registered each year. These same authors reported that these natural inhibitors can delay several deterioration processes such as protein degradation caused by the proteases action, during food preservation. Therefore, their proper use appears to be an effective tool for extending the storage time of foodstuffs.

4. CONCLUSION

This work assessed the anti-diabetic and anti-inflammatory potential of *D. innoxia* extracts via their ability to inhibit alpha-amylase and trypsin *in vitro* activity. The phytochemical study revealed the richness of the leaves and roots in various bioactive compounds. The activity-inhibiting effects observed with alpha-amylase and trypsin show that the extracts from*D. innoxia*are endowed with hypoglycemic and anti-inflammatory potential. This potential was more pronounced in the hydroethanolic leaf extracts, which is an asset for safeguarding the species. Consequently, the hydroethanolic leaf extracts of *D. innoxia*could be useful in the management of diabetes and inflammation. In addition, based on theirobserved trypsin-inhibiting effect, these extracts could be also helpful to preserve foodstuffs.

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