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IN VITRO α-AMYLASE AND α -GLUCOSIDASE INHIBITORY EFFECTS AND *IN VIVO* ANTI-DIABETIC EFFECTS OF THE HYDROALCOHOLIC SEED AND LEAF EXTRACTS OF POT CASSIA (*CASSIA TORA*)

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Keywords: *Cassia tora*, Diabetes, α-amylase, α-glucosidase

Abstract

Owing to the lifestyle of many individual, diabetes mellitus (especially type 2 diabetes mellitus, T2DM) had been a global health challenge of economic importance over the years one of whose etiologies has been linked to the activities of carbohydrate degrading enzymes such as α-amylase and α-glucosidase. This work studied the *in vitro* inhibitory effects of seed and leaf extracts of *Casia tora*, (pot cassia) on α-amylase and α-glucosidase enzymes as well as its ability to lower blood glucose level *in vivo*. The latter was carried out using 24 healthy wistar rats grouped into 4 groups. Group I injected with the two separate extracts (seed and leaf) at 100mg/kg, Group II at 200mg/kg, Group II received metformin (standard drug) while Group IV received normal saline, serving as control. The effects were compared across the groups by measuring the blood glucose level using glucometer before

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and after each treatment. Both extracts from the plant showed higher lowering effects at 200mg/kg than 100mg/kg. Meanwhile, at 100mg/kg, higher lowering effects than metformin was observed. For the *in vitro* α-amylase and α-glucosidase inhibitory effects at IC_{50} (Mg/ml), the test extracts also showed higher effect than metformin with the range 0.295mg/ml and 0.287mg/ml for the seed extract, 0.290mg/ml and 0.281mg/ml for leaf extract while for metformin, 0.27mg/ml and 0.286mg/ml against α-amylase and α-glucosidase enzymes respectively. Invariably, both the seed and leaf extracts of *C. tora* have a high inhibitory effect on these enzymes and administration of at least 200mg/kg of the extracts also has a high anti-diabetic effects. With further pharmacological studies on the actual chemotherapeutic index, the seed and leaf extracts of *C. tora* is highly recommended for the treatment (management) of hyperglycemia.

INTRODUCTION

According to Wild *et al.,* (2004), the prevalence of type 2 diabetes mellitus (T2DM), the hallmark of which is hyperglycemia (High glucose level in the blood) is increasing at an alarming rate to the extent that it is currently being estimated to be responsible for about 90 to 95% of all diabetes cases globally. This development is not unconnected to lifestyle and socioeconomic changes mainly characterized by lower physical activity and higher intake of high fat as well as saturated carbohydrate-containing diets among many other factors (International Diabetes Federation, IDF, 2011).

Hyperglycemia is a condition that prevails when there is decreased insulin sensitivity or decreased insulin secretion from pancreatic β-cells, which reduce insulin-mediated glucose uptake in peripheral tissues (Ali *et al.,* 2002). All diabetic complications (nephropathy, neuropathy, micro and macro angiopathy, retinopathy, as well as cataract) are strongly linked to hyperglycemia. For this reason, improved treatment of hyperglycemia,

T2DM-related risk factors and the long-term degenerative disorders is of utmost importance so as to lower the risk of both micro-and macro-vascular complications. An important strategy which has proven to be greatly effective against hyperglycemia is the inhibition of key carbohydrates digestive enzymes such as α-amylase and α-glucosidase, because they also play a vital role in preventing diabetes complications. The inhibitors of these enzymes delay the digestion of carbohydrates and reduce the rate of glucose absorption from the small intestinal tract, thereby reducing postprandial blood glucose level. Therefore, inhibition of α-amylase and α-glucosidase enzymes is very vital to the management and treatment of hyperglycemia and T2DM (Ali *et al.,* 2002). Currently, several conventionally prescribed α-glucosidase inhibitors are available in the market, which include acarbose, voglibose, and miglitol, but these kind of inhibitors have been shown to have some undesirable side effects such as flatulence, diarrhea, and abdominal pain (Ali *et al.,* 2002). As a result of this, there is an urgent need for the development of newer alternatives. A better clinical outcome could be derived from specific agents with inhibitory activity against the α-amylase and α-glucosidase enzymes and are not cytotoxic to target cells, but still effective to reduce the postprandial hyperglycemia.

As stated by Sanni *et al.,* (2009) the use of medicinal plants has been adopted since ancient times to treat a number of ailments, and some of these plants and plant-derived products have shown impressive potentials including the discovery of some conventional medicines. Dependence on medicinal plant in the treatment of disease is common especially among a large proportion of the rural populace because of its availability and affordability. Some medicinal plants such as *Momordica charantia* (bitter gourd or balsam pear), *Vernonia amygdalina* (Bitter leaf) etc. have traditionally and conventionally shown to possess α-amylase and α-glucosidase inhibitory activities (Ortiz *et al*., 2007). However, the presence

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of some potential toxic and carcinogenic agents in some of these plants made them unsuitable for therapeutic applications.

Of all the important antidiabetic plants that possess the α-amylase and α-glucosidase inhibitory activities, plants like *Cassia tora* (now called S*enna tora*), common name; Pot cassia, a legume in the Family **Fabaceae** and the Subfamily **Caesalpinioideae** had demonstrated a very high potential (Bruneau *et al*., 2001).

C. tora is an annual herb, 30-90 cm high which occurs as wasteland rainy season wild plant. It is a wild crop. The main useful parts of *Cassia tora* are leaves, roots and seeds. This plant according to Nikhil (2013) is one of the well-known herb as well as a common weed in most of the Asian countries. For instance, in India, different parts of these plants are known for its meditative value as an antioxidant, antimutagenic, antidiuretic etc.

In Ayurveda, it helps in the treatment of skin diseases like ring worm, leucoderma, eczema etc. It is an anthroquinone containing plant which also has a certain bioactive compounds such as emodin, rhein, palmatic, isostearic, etc (Albasarah *et al*., 2010).

C. tora has been reported to contain many active substances, including Anthraquinone, Quarcetine, Chrysophenol, Emodin, Rhein, etc. *Cassia tora* has been reported to exhibit significant antimutagenic activity (Obdoni and Ochuko, 2001). Anthraquinone act as a fluorescence sensor (Bhatt *et al*., 2012) or fluorophores therefore this plant also shown sensing properties.

STATEMENT OF PROBLEM

The prevalence of diabetes is on a steady increase worldwide, and it is now identified as one of the main threats to human health in the 21st century (Wild *et al*., 2014). Medicinal plants have been used over the years to mitigate and fight against the disease, but not much has been documented on the mechanism of action of these medicinal plants. This research work tends to find solution to this by analyzing the mechanism of action of one

of these medicinal plants with anti-diabetic property pot cassia (*Cassia tora*) both in vitro and in vivo.

AIM AND OBJECTIVES

The aim of this research work is evaluate the *in vitro* α-amylase and α-glucosidase inhibitory effects and *in vivo* anti-diabetic effects of the hydroalcoholic seed and leaf extracts of *Cassia tora*

The objectives of the study are to:

i. extract and determine the phytochemical constituents (qualitative and quantitative) of the hydroalcoholic seed and leaf extract of *Cassia tora*,

ii. evaluate the *in vitro* α-amylase and α-glucosidase inhibitory effects of the hydroalcoholic seed and leaf extract of *Cassia tora* using wistar rats, and

iii. evaluate the *in vivo* anti-diabetic effects of the hydroalcoholic seed and leaf extracts of *Cassia tora* in same wistar rats.

METHODOLOGY

Collection and Authentication of Plant Material

Pot cassia *(Cassia tora)* seed and leaf samples were collected from the local area of Bali, Taraba State in the month of April, 2023 and was identified taxonomically using pertinent taxonomic literature (Plant list, 2015). For professional confirmation, identification and authentication was further done in the Department of Biological Sciences, University of Maiduguri, Borno state. The collected plant parts (seeds and leaves) were cleaned with deionized water and dried at room temperature for weeks. These were then grinded into find powder using mortar and pestle separately, the powdered sample was stored in a desiccator until further analysis.

Experimental Animals

Forty (40) apparently healthy albino Wistar rats (*Rattus norvegicus*) of different sexes weighing within the range of 13.09g (0.13kg) to 33.00g

(0.33kg) were obtained from National Veterinary Research Institute (NVRI) Vom, Jos, Nigeria. The rats were housed in cages in the Department of Science Laboratory Technology, Federal Polytechnic Bali, Taraba State, Nigeria and maintained under standard environmental condition; controlled temperature, relative humidity with free access to water and standard food.

Experimental Design

Preparation and Extraction of Senna tora *(Cassia tora)* seeds and leaves

The powdered sample was extracted by soxhlet extraction method. About 5g of the powdered plant sample *(Cassia tora seeds)* was packed into a thimble and extracted with 250ml of ethanol. The time allowed for the extraction was 6 hours till the solvent in the siphon tube of an extractor become colorless. After that, the extract was transferred into the beaker and kept on the water bath at 68°C till all the solvent got evaporated. The yielded crude was stored for treatment (In vivo study). This was repeated for the leaf samples as well. The samples were also analyzed chemically based on the Association of Official of Analytical Chemist (AOAC) (1990) which was done in duplicate for accuracy.

Phytochemical Analysis

The phytochemical analysis (Screening) of senna tora *(C. tora)* seeds and leaves was carried out according to standard method of Evan (2006).

Test for Alkaloid

About 0.5g of the powdered material was warmed with 10ml of 2% H_2SO_4 (Sulfuric acid) for two minutes and filtered. Three-one portions were treated with a few drop of Drangendroff's reagent, Wagner's reagent and Mayer's reagent.

Test for Tannins

A small quantity of the powdered material was mixed with water and

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heated on a water bath, the mixture was filtered and ferric chloride reagent was added to the filtrate. A dark blue or dark green solution indicates the presence of tannins. For hydrosable tannins, shake 4ml of the extract with 4ml of ammonia solution. Formation of an emulsion on shaking shows the presence of tannins.

Test for Saponin

About 0.5g of the powdered material *(Cassia tora)* seeds extract was shaken with 5ml of distilled water and heated to boiling point. Frothing shows the presence of saponin. The filtrate from the extract was added to 3ml of paraffin oil and thoroughly shaken to form a stablseedse emulsion. This was left to stand for about 5minutes, the presence of stable emulsion indicates the presence of saponins.

Test for Phlobatanins

0.5ml of the filtrate was boiled with 5ml of 1% HCl (Hydrochloric acid), a red precipitate shows the presence of phlobatanins.

Test for Glycosides

a. The aqueous extract of the powdered material was boiled with a drop of Fehling's solution A and B for 2 minutes. The presence of reducing sugar is indicated by an orange red precipitate on boiling with Fehling's solution.
b. The extract is hydrolyzed with hydrochloric acid (HCl) solution. A drop of Fehling's solution A and B were added to the extract, and the presence of further red precipitate indicates the presence of glycosides.

Test for Flavonoid

2ml of dilute sodium hydroxide (NaOH) was added to 2ml of the extract. The appearance of a yellow color indicates the presence of flavonoids.

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Test for Carbohydrates

a. Fehling's Test:

5ml of an equal mixture of Fehling's solution of A and B was added to a small portion of the *C. tora* seedss extract in a testtube and was boiled on a water bath, brick red precipitate indicates the presence of reducing sugar *(Evans, 2006)*.

b. Molisch Test:

To a small portion of the extract *(Cassia tora)* seedss, a few drops of Molisch reagent was added to the test tube containing the extract and the concentrated sulfuric acid (H_2SO_4) was added down the sides of the test tube to form a lower, reddish coloring at the interphase indicates the presence of carbohydrates *(Evans, 2006)*.

Invitro α-amylase and α-glucosidase inhibitory effects

α-amylase inhibition assay

Stock solutions of test plant extracts were prepared in water. As described by Kwon, Apostolidis and Shetty (2006), inhibition α-amylase activity was determined using dinitrosalicylic acid. Test plant extracts (100 µl of 2 to 20 mg/ml) was added to 100 µl of α-amylase (1 U/ml) and 200 µl of sodium phosphate buffer (20 mM, pH 6.9) to get 0.5 to 5.0 mg/ml final concentration. The samples were pre-incubated at 25 °C for 10 min.in addition to this was 200 µl of 1 % starch prepared in 20 mM sodium phosphate buffer (pH 6.9). These mixtures were then carefully incubated at 25 °C for 10 min. The reactions were stopped by incubating the mixture in a boiling water bath for 5 min after adding 1 ml of dinitrosalicylic acid. The reaction mixtures were then cooled to room temperature and was diluted to 1:5 ratio with water. The absorbance of these was measured in a spectrophotometer (Amersham Biosciences, USA) at 540 nm. Percentage of inhibition of enzyme activity was calculated as

% Inhibition = $[(A_{540}^{Control} – A_{540}^{Treatment}) / A_{540}^{Control}] \times 100$

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$A_{540}^{Control}$ = absorbance at 540 nm in control sample without test plant extracts

$A_{540}^{Treatment}$ = absorbance at 540 nm in treatment with test plant extracts.

α-glucosidase inhibition assay

Using the method described by Kim, Wang and Rhee (2004), inhibition of α-glucosidase activity was determined using yeast α-glucosidase and p-nitrophenyl-α-D-glucopyranoside (pNPG). Test plant extracts (100 µl of 2 to 20 mg/ml) was added to 50 µl of α-glucosidase (1 U/ml) which was previously prepared in 0.1 M phosphate buffer (pH 6.9) and 250 µl of 0.1 M phosphate buffer to get a final concentration of 0.5 to 5.0 mg/ml. The mixture was pre-incubated at 37 °C for 20 min. After this period of pre-incubation was an addition of 10 µl of 10 mM pNPG prepared in 0.1 M phosphate buffer (pH 6.9) and incubated at 37 °C for 30 min. The reactions were stopped by adding 650 µl of 1 M sodium carbonate. Measurement of absorbance followed in a spectrophotometer (Amersham Biosciences, USA) at 405 nm. Percentage of inhibition of enzyme activity was calculated as

% Inhibition = $[(A_{405}^{Control} – A_{405}^{Treatment}) /A_{405}^{Control}] \times 100$

$A_{405}^{Control}$ = absorbance at 405 nm in control sample without test plant extracts

$A_{405}^{Treatment}$ = absorbance at 405 nm in treatment with test plant extracts.

Invivo anti-diabetic effects of *C. tora* seed and leaf extract

Preparation of Doses of Test Plant

A quantity of 200g and 400g of *C. tora* seeds extract were suspended/ml tripled distilled water (TDW) respectively containing 2% (W/V) gum of *Cassia tora*. The suspension was given in a volume of 1ml/100g animal BW. (200mg and 400mg drug/kg bw) by oral intubation.

Induction of Diabetes in Rats

After 10 days of acclimatization, the rats were assigned randomly into four

groups; A, B, C and D (6 rats/each) and were subjected to overnight fasting (Addala, *et al.*, 2021). Diabetes was induced by intra peritoneal injection of Alloxan, freshly dissolved in citrate buffer pH 4.5. The animals were allowed to drink water 5% glucose solution overnight to overcome the drug induced hypoglycemic due to massive release of insulin-cells. After the induction, on 3rd day the blood glucose levels were measured and the animals with a blood concentration of more than 250mg/dl were considered as diabetic and taken for the experiment. Administration of the plant extract was started on the 4th day after alloxan injection and this was considered as the 1st day of treatment, which was continued for 21 days.

Wistar Rat Treatment

The fasting glucose and body weight of all animals were recorded at the beginning of the study. The blood glucose was checked by one touch glucometer throughout the study, in the experiments, 40 rats were divided into 4 groups of three rats each.

Group 1: 5 Alloxan induced diabetic rats received 200mg/kg/bw of leaf extract and the other 5 received same concentration of seed extract for 21 days.

Group 2: 5 Alloxan induced diabetic rats received 100mg/kg/bw of leaf and the other 5 received same concentration of seed extract for 21 days.

Group 3: 10 Alloxan induced diabetic rats received the Standard drug (100 mg/kg/bw, of Metformin Oral route) for 21 days.

Group 4: 10 rats served as Normal Control (Normal saline Oral route) for 21 days.

For all rats, body weight was measured before and after the induction of diabetes. Blood glucose level was measured on 1st, 7th, 14th and 21st day throughout the study period by tail tip cutting method and the average was taken. At the end of the experiment, sufficient blood was collected by retro-orbital bleeding from all the animals under mild anaesthesia for estimation

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of glucose level.

Blood samples collection and Blood Sugar Determination

The tail tip cutting method was used to collect the blood samples. The area of blood sampling was shaved scrubbed by disinfectant (70% ethanol) before cutting the tail tip with surgical blade. The collection of blood samples was achieved at days 0, 7, 14, and 21 of the experimental periods, for fasting blood sugar test.

After proper hand cleaning and drying, glucometer was brought out of the meter bag and was placed on a clean table. A test strip was inserted into the meter. The tail tip cutting method was used to collect the blood samples. The area of blood sampling was disinfected with 70% ethanol before cutting the tail tip with surgical blade. A drop of blood was applied to the test strip. The meter displayed the blood sugar level on a screen after a few seconds; it was recorded and repeated with other blood samples. The average inhibitory indicator of the seed and leaf extract was recorded as the drop in the blood sugar level of the rats after treatment.

The following values were taken as indicated:

Normal: 100 mg/dL or lower

Prediabetes: 100 mg/dL to 125 mg/dL

Diabetes: 126 mg/dL or higher

Any raised value was taken to be potential diabetic condition

RESULTS

Table 1: Qualitative and Quantitative Phytochemical Analysis of Senna tora *(Cassia tora)* Seed and Leaf Extract

S/N	PARAMETERS	INFERENCE	EXTRACT QUATITY (%w/w)	
			Seed	Leaf
1	Alkaloid	++	0.86±0.25	0.62±0.23
2	Phenol	+++	0.64±0.02	0.22±0.15
3	Phlobatanins	+	0.15±0.03	0.54±0.05

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4	Flavonoids	++		0.64±0.15	0.86±0.06
5	Glycosides	+++		0.45±0.04	0.04±0.35
6	Saponins	+		0.89±0.21	0.94±0.41
7	Volatile oil	++		0.35±0.10	0.67±0.05
8	Hydrolysable tannins	-		-	-
9	Tannins	+++	0.89±0.07	0.74±0.05	
10	Proteins	+++	0.62±0.13	0.44±0.25	
11	Carbohydrates	-		-	-

Note:

Present: +

Moderately High: ++

Very High: +++

Absent -

Table 2: Invitro α-Amylase and α-Glucosidase Inhibitory effects of the Hydroalcoholic Seed and Leaf Extracts of *C. tora*

Analyte	IC_{50} (Mg/ml)	
	α-Amylase	α-Glucosidase
Metformin	0.286 ± 0.019	0.27 ± 0.022
Seed Extract	0.295 ± 0.020	0.287 ± 0.031
Leaf Extract	0.290 ± 0.022	0.281 ± 0.019

Table 3: Hypoglycemic effect of hydroalcoholic seed extracts of *C. tora*

Groups	Treatment	Average Glucose Level (mg/ml)		
		BI	AI	AT
1	200mg/kg Extracts	71.5	92.6	40
2	100mg/kg Extracts	72.6	90.6	44.5
3	100mg/kg Metformin	71.8	98	60.3
4	Normal Saline	86.6	-	-

BI – Before Induction, **AI** – After Induction, **AT** – After Treatment

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Table 4: Hypoglycemic effect of hydroalcoholic leaf extracts of *C. tora*

Groups	Treatment	Average Glucose Level (mg/ml)		
		BI	AI	AT
1	200mg/kg Extracts	72	91.7	42
2	100mg/kg Extracts	72.6	92.6	42.5
3	100mg/kg Metformin	70.6	97.5	67
4	Normal Saline	86.6	-	-

BI – Before Induction, **AI** – After Induction, **AT** – After Treatment

DISCUSSION

Phytochemical analysis of the seed and leaf extracts of *C. tora* showed the presence of nine (9) phytochemical agents; Alkaloid, phenol, phlobatannins, flavonoids, glycosides, saponins, volatile oil, tannins and protein in varying proportions. Higher in concentrations are alkaloid, flavonoid, saponins and tannins while the least was phlobatannins. This agrees with the work of Edoga *et al.* (2005). The presence of these in large amount according to the work of Mohamed, Bushra and Abdelrahman (2010), justifies its antimicrobial, anti-inflammatory and anti-diabetic activities. Similarly, both the seed and leaf extracts of *C. tora* proved to have higher inhibitory effects on α-amylase and α-glucosidase than the conventional drug, metformin. The higher inhibitory effects of seed and leaf extracts against α-amylase enzyme was established by the concentrations 0.295 ± 0.020 mg/ml and 0.290 ± 0.022mg/ml IC_{50} as against 0.286 ± 0.019mg/ml of metformin. This agrees with the work of Olubomehin, Abo and Ajaiyeoba (2013). Likewise, higher inhibitory effects was also observed in the activities of the test seed and leaf extracts against α-glucosidase enzyme and this was established by the concentrations 0.287 ± 0.031mg/ml and 0.281 ± 0.019mg/ml respectively as against metformin with 0.27 ± 0.022mg/ml. This corroborates the work of Kwon *et al.,* (2006).

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The anti-diabetic potential of seed and leaf extracts of *C. tora*, was established in the sharp drop observed in the blood glucose level of the wistar rats in the two (2) treatment groups (I and II), a trend which followed the pattern of the work of Uebanso *etal.,* (2007). Results of the seed extract treatment in which the average normal blood glucose level of Group I (treated with 200mg/kg seed extract), 71.5mg/ml was raised to 92.6mg/ml after induction and after treatment dropped drastically to 40mg/ml and that of Group II (treated with 100mg/kg seed extract) with 72.6mg/ml normal glucose level, raised to 90.6mg/ml after alloxan induction and drastically reduced to 44.5mg/ml after treatment is in line with Ali, Houghton and Soumyanath (2006). Perfect similar trend was observed for the leaf extract treatment too. This indicated high anti-diabetic activities. Meanwhile, negating the work of Mohamed *et al.,* (2012), the effect of the standard drug, metformin used for Group III treatment was smaller for both seed and leaf extracts. This shows that, the test plant extracts have a high anti-diabetic effect both in reducing the blood glucose level and inhibiting the enzymes (α-amylase and α-glucosidase) which are incriminated in the development of Type 2 diabetes.

CONCLUSION

Since α-amylase and α-glucosidase enzymes digest the carbohydrates and increase the postprandial glucose level in diabetic patients, the activity of these two enzymes needs to be inhibited so as to control postprandial hyperglycemia, and reduce the risk of developing type 2 diabetes mellitus (T2DM). Based on this painstakingly performed experiment, it can be duly inferred that both the seed and leaf extracts of *C. tora* have a high inhibitory effect on these enzymes. Similarly, oral administration of at least 200mg/kg of the extracts has a high anti-diabetic effects on diabetic patient. Therefore, with further pharmacological and pharmacognostic studies as well as the study of the actual therapeutic index of the seed and leaf

extracts of *C. tora*, the extracts is highly recommended for the treatment (management) of hyperglycemia.

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