CASSIA ABBREVIATA EXTRACTS PREVENT THE ENSUING OF PRE-DIABETES IN ALBINO RATS SUBJECTED TO SUCROSE LOAD

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ABSTRACT: Two extracts; water extract (CAW) and methanol extract (CAM) from the bark of *Cassia abbreviata* were prepared. Oral glucose tolerance test was carried out with both the extracts in normal albino SD rats to measure their effects in normal rats and to have the potent dose. Potent doses of CAW and CAM were administered to rats of two experimental groups; EXW and EXM, respectively against a sucrose load of 4g/kg body weight. Two control groups were also included in this experiment; normal control group (NC) received distilled water and sucrose control group (SC) received sucrose (4g/kg body weight). The experiment was run for 30 days. In the end, rats were killed under ether anesthesia and blood was collected to measure random glucose, % glycosylated hemoglobin and lipid profile. Oral glucose tolerance was done on these rats one day prior to their sacrifice. The results indicate that both CAW and CAM maintained normal glucose level, glycosylated hemoglobin and normal lipid profile in both experimental groups and results differed significantly

INTRODUCTION

Diabetes is one of the non-communicable diseases, often referred as a "silent killer". Many people have blood sugar levels higher than normal but not high enough to be classed as diabetes. This condition is pre-diabetes, also commonly referred to as borderline diabetes. It is classified as a metabolic condition that is closely tied to obesity. If undiagnosed or untreated, pre-diabetes can develop into type 2 diabetes (SU *et al.*, 2008). Pre-diabetes is often described as the "gray area" between normal blood sugar and diabetic levels, a situation which is thought to be wide spread, such individuals are thus at a high risk for developing type 2 diabetes. Pre diabetic condition not only a fore runner of diabetes but it can also contribute significantly to cardiovascular complications via hyperinsulinemia (HAFFENER *et al.*, 1990)

The projected increase in the number of new cases of pre-diabetes is of concern as it implies a large increase in the cost of providing public health care for this additional numbers of patients that will inevitably progress to being diabetic. The current advice to patients is to eat a balanced diet and engage in regular exercise and such interventions are often ineffective. Alternatively, drug interventions that make use of pharmaceutical interventions that inhibit activity of lipase, inhibit appetite are available for prescription. However the unpleasant side effects and the high cost of such medication provide the incentive to explore alternative treatment of this disease. Use of plant derived treatments for diabetes is being widely studied as they provide a viable alternative in the development of safe and cheaper treatment.

Plant extracts inevitably consist of mixtures of phytochemicals, from which isolated molecules that can be identified and developed into therapeutics for the disease (BIRARI and BHUTANI, 2007; KWAPE and CHATURVEDI, 2014). There are many

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reports regarding the hypoglycemic effects of botanicals in diabetes but reports regarding the role of botanicals in prevention of pre-diabetes are not many. It is proposed that botanicals which are effective in management of diabetes could also be used in the prevention of pre-diabetic condition. *Cassia abbreviata*, is a very common in Botswana locally known as Monepenepe (SEKEP *et al.,* 2013). It is a perennial tree and belongs to family Caesalpiniaceae. Decoction of root and stem are used in stomach pain, dysentery, fever and malaria (MUTHAURA *et al.,* 2007) and powdered stem is used in some gynecological abnormality (SETSHOGO and MBEREKI, 2011). Recently, *Cassia abbreviata* has also been reported for its in vitro inhibitory effects on yeast alpha–glucosidase (SAI *et al.,* 2010) and rat's intestinal alpha-glucosidase (SHAI *et al.,* 2011).

Therefore, it is proposed that *C. abbreviata* might also inhibit these enzymes in vivo condition and play a significant role in prevention of pre-diabetes. Hence, the present study has been planned with an aim to evaluate the effects of *C. abbreviata* bark extracts on plasma glucose and glycosylated hemoglobin which are predictors of pre-diabetes. The effects had also been observed on lipid profile as dyslipidemia is a good predictor of ensuing cardiovascular complications which are expected during pre-diabetic condition.

MATERIALS AND METHODS

Collection of plant material and preparation of extracts: Plant material, the bark had been collected from the University campus, dried in the sun crushed to powder. Two types of extracts, water extract (CAW) and methanol extract (CAM) were prepared. For the preparation of CAW, the crushed powder was mixed with water in the ratio of 1:8 and boiled until the volume of water reduced to 1/8th of its original volume. This extract was prepared every day for oral administration. For the preparation of CAM, the crushed powder was mixed with 70% methanol in the ratio of 1:3 for 3 days and then filtered and rotaevaporized at 40^{0}C under reduced pressure. The solid gel like extract was recovered after evaporization which was further dried in fume hood to get the dry flakes of the extract. The yield was 7%.

Experimental designs: Male albino rats of SD strain, approximately 200g weight were used for experiments. Initial weights of all the rats were recorded. They were kept in plastic cages at an ambient temperature of 25°C ± 2°C with 12 Hours cycle of light darkness and had free access to food and water ad libitum. All the administrations were given orally with the help of syringe and a blunt tube. Experiment was conducted as per internationally accepted principles for laboratory animal care Unit, Department of Biology, University of Botswana and permission to perform this experiment on albino rats was obtained from Ministry of Communication Science and Technology (CST 16/9 III (25).

Experiment 1: Oral glucose tolerance test was performed with 25 rats to establish the potent dose for long term trial. Animals were bled at 0 hour and glucose level was measured. After that rats were administered glucose plus graded doses of CAW and CAM. Animals were grouped into 5 groups each with five rats as follows;

Group 1 : Rats administered glucose (2g/kg body weight) in distilled water
Group 2 : Rats administered glucose (2g/kg body weight) plus CAW (5ml/kg body weight) in distilled water
Group 3 : Rats administered glucose (2g/kg body weight) plus CAW (10ml/kg body weight) in distilled water.
Group 4 : Rats administered glucose (2g/kg body weight) plus CAM (50mg/kg body weight) in distilled water

Group 5   :   Rats administered glucose (2g/kg body weight) plus CAM (100mg/kg
                  body weight) in distilled water

Experiment 2: Potent doses of two extracts, CAW and CAM were inferred from OGTT experiment and were tried in 20 rats for 30 days. Twenty rats were used for this experiment and were grouped as follows;

Group NC :   Normal control group administered distilled water
Group SC :   Sugar control group administered sugar solution (4g/kg body weight)
Group EXW: Experimental group administered CAW extract (10ml/kg body weight)
                  plus sugar (4g/kg body weight)
Group EXM: Experimental group administered CAM extract (100mg/kg body weight)
                  plussugar4g/kg body weight)

The experiment was run for 30 days and in the end, animals were sacrificed using ethyl ether anesthesia and blood was collected in heparinized tube. Plasma was collected after centrifugation and stored at -70^0C for biochemical estimation. OGTT was performed in these rats before they were sacrificed.

Biochemical measurements: Blood glucose was measured using glucometer. Glycated hemoglobin, total cholesterol, high density lipoprotein, light density lipoprotein and triglycerides were measured using kits from Aggape Diagnostics, India.

Statistical analysis: Data was subjected to descriptive statistics and differences among the groups were analyzed using one way ANOVA. Tukeytest was run as post hoc test for multiple comparisons. Results were considered statistically significant at $p<0.05$.

RESULTS AND DISCUSSION

Effects on CAW and CAM oral glucose tolerance test (OGTT): The effects of graded doses of CAW and CAM on OGTT, presented in Figure 1 shows that the two extracts have significant effects on OGTT at both doses but results are more significant at higher doses The pattern of graphs are different for CAW and CAM. In case of CAW, the glucose levels for two doses did not shoot up (in group Gr. 2 and Gr3) like the graph for Gr 1. In Gr. 2, where lower dose was administered, the pick for the glucose curve showed a glucose level of 6.73 mmol/L and similar levels were maintained up to 3^{rd}hour. In Gr 3, where higher dose was administered, a slow shooting of glucose level was noticed up to 2^{nd}hour and there after the level was maintained up to 3rd hour. The only difference between the two doses was in the rising levels of glucose at first hour but it was not significant. It appears that, CAW might be contributing its effects on the delayed gastric emptying and hence the slow peaking of glucose levels. Sustained maintenance of the levels at 1st hour up to 3rd hour also an indicative of the fact that CAW, does not have any effects on glucose clearance from the circulation. In case of CAM, the graph showed different patterns. The glucose levels in Gr 4 and Gr 5 at first hour did not show any significant difference from the glucose level in Gr 1. But at 2nd hours, glucose levels fell down sharply in Gr 4 and Gr 5 and the levels differed significantly when compared with Gr 1 ($p<0.05$)In Gr 1, the glucose level at 2nd hour was maintained up to 3rd hour but in Gr 4 and Gr 5, it continued to fall up to 3rd hour. It appears that CAM has no effects on glucose levels at the gut level but it had definitely some effects at the circulation level in clearing the glucose. There could be a possibility at the insulin level; either at synthesis and/or secretion level or at the level of insulin sensitivity. From this result, two higher doses were selected for long term trial on OGTT and lipid profile against a chronic sugar load for 30 days.
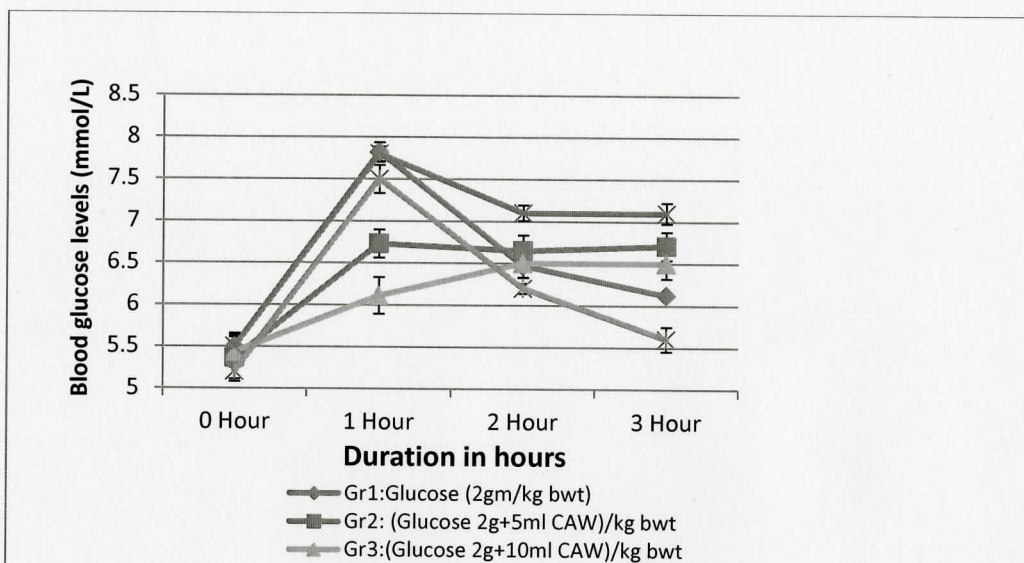
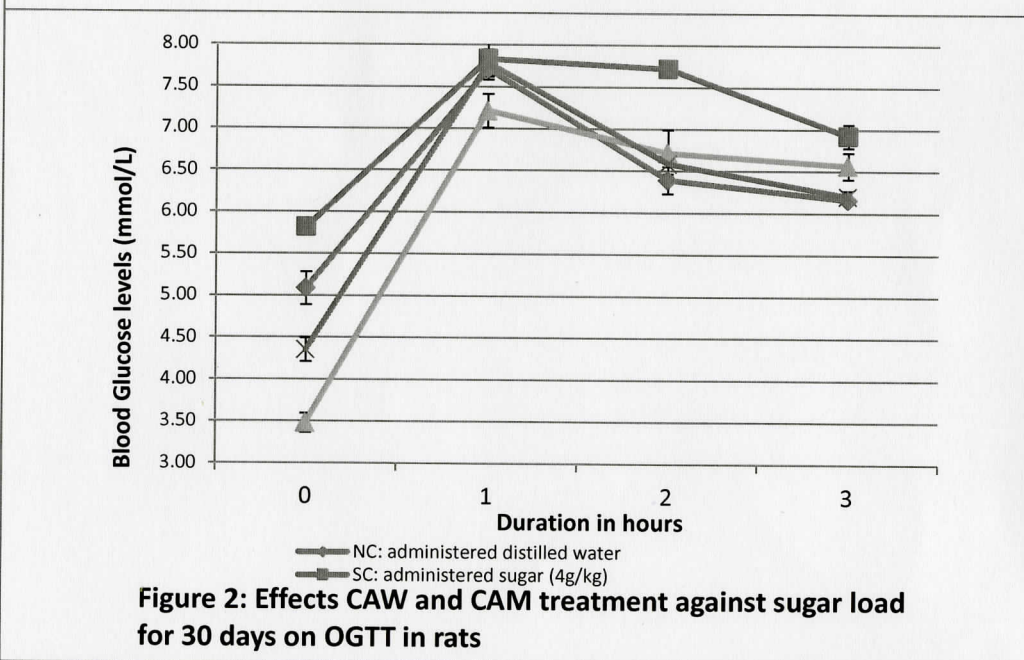Figure 1: Effects of CAW and CAM on OGTT in normal rats

Gr1:Glucose (2gm/kg bwt)
Gr2: (Glucose 2g+5ml CAW)/kg bwt
Gr3:(Glucose 2g+10ml CAW)/kg bwt

NC: administered distilled water
SC: administered sugar (4g/kg)

Figure 2: Effects CAW and CAM treatment against sugar load for 30 days on OGTT in rats

Effects of CAW and CAM on OGTT against a chronic sucrose load (4g/kg body weight) for 30 days: Results of chronic administration of CAW and CAM against sucrose load on OGTT presented in Figure 2, shows that both the extracts have significant contribution in maintaining the OGTT curve within the normal range. The patterns of the curve in EXM showed the similar pattern like the curve for NC at 1st, 2nd

and 3rd hour. In EXW, the curve differed slightly from normal OGTT curve in NC. At 1st hour, the shooting of the glucose level was less than the levels in EXM and NC ($p<0.05$) and again the lowering was also with a slower pace as compared to lowering in NC and CAM after 1st hour. The curve for SC showed a different pattern. The peak for glucose level at 1st hour was matching with the glucose levels in CAM and NC but after that it maintained the higher level (7.72mmol/L) up to 2nd hour. At 3rd hour, it did not show a significant reduction (6.92mmol/L). Thus the results of OGTT test again provide a support to the conclusion of first OGTT in normal rats.The extract CAW, appears to influence the gastric emptying and might not have any effects on clearing and this could be the reason of lower glucose peak at 1st hour as compared to the 1st hour peak in all the groups and slow lowering of glucose levels after 1st hour. The extract CAM, appears to influence the clearing of post prandial glucose and this could be the reason behind maintenance of normal OGTT curve even though, the rats were subjected to chronic sucrose load. In the absence of CAW and CAM, the group SC has almost reached the pre diabetic condition.

Effects of CAW and CAM on random glucose, glycosylated hemoglobin and per cent weight gain against a chronic sucrose load (4g/kg body weight) for 30 days: Effects of CAW and CAM administration for 30 days on random blood glucose and glycosylated hemoglobin presented in Table 1, shows that the random glucose levels in both the experimental groups, EXW and EXM, are significantly lowered as compared to the normal level in NC ($p< 0.001$ for both groups) and elevated level in the group SC ($p<0.01$ for both groups). The results of glycosylated hemoglobin showed the similar trends. Effects on per cent weight gain had different scenario. The weight in NC group showed a non-significant increase while all the three groups; SC, EXW and EXM showed a significant increase in the body weight from the base line value. Group EXW showed a slightly less increase in the weight which is statistically non-significant.

Table-1: Effects of CAW and CAM on blood glucose, HBA1c and per cent increase in weight against a chronic sucrose load (4g/kg body weight) for 30 days

Parameters Groups	Blood glucose (mmol/L)	HBA1c%	% increase in weight
NC	4.8±0.18	4.4±0.17	0.46±0.07
SC	7.98±0.12	6.46±0.21	7.52±0.18
EXW	3.85±0.14$^{a^*, b^*}$	4.46±0.17$^{a^*, b^*}$	6.91±0.21
EXM	3.62±0.12$^{a^*, b^*}$	4.64±0.25$^{a^*, b^*}$	7.11±0.22

a* is $p<0.01$ when compared with NC and b* is $p<0.01$ when compared with SC

Effects of CAW and CAM on lipid profile: Results of chronic administration of CAW and CAM on lipid profile against sucrose load, presented in Table 2, showed that the two extracts had significant contribution in keeping the lipid profile in normal range. Plasma triglycerides (TG) levels were significantly hiked in SC group as compared to NC ($p<0.05$). Levels had also increased in EXW and EXM but they differed significantly from SC group ($p<0.05$). Plasma total cholesterol (CHO) and LDL also increased significantly in SC group as compared to N, EXW and EXM ($p<0.05$). Although the levels of LDL were reduced in two experimental groups but still had elevated levels. Plasma HDL levels were decreased significantly in SC as compared to other groups ($p<0.001$). The levels of HDL were maintained in two experimental groups. In EXW, actually it showed slight increase as compared to NC.

Table- 2: Effects of CAW and CAM on plasma TG, CHO, HDL and LDL against a chronic sucrose load of 4g/kg body weight) for 30 days

Parameters Groups	Triglycerides (mg/dl)	Total CHO (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
NC	96.58± 2.51	115.43±2.84	48.32±3.45	21.62±2.47
SC	221±4.80	152.882±2.21	28.60±2.11	61.42±3.31
EXW	135.43±1.92$^{a**, b*}$	109.06±4.9$^{a, b*}$	50.46±1.40^{b**}	36.10±1.89$^{a, b**}$
EXM	143.26±3.39$^{a**, b*}$	112.20±4.22$^{a, b*}$	43.84±1.72	33.28±1.35$^{a, b**}$

a= $p<0.05$, a* $p<0.01$ and a** $p<0.001$ when compared with control and b is $p<0.05$, b* $p<0.01$ and b** $p<0.001$

Both the extracts were successful in maintaining the normal glucose levels, lipid profiles and prevented pre diabetic condition in both experimental groups. They also significantly maintained the normal oral glucose tolerance curve (Figure 2) as compared to the impaired OGTT curve in group SC. Normal glucose levels apart from a chronic glucose load might be due to inhibition of alpha-glucosidase (SAI et al., 2011) and less or slow absorption of glucose. Careful consideration of OGTT curve before trial and after trial of CAW and CAM, indicated that although both the extracts had similar effects in preventing pre-diabetes, there is a possibility that the two extracts work in different way. CAW appeared to work on gastric level and might be causing the delayed gastric emptying that resulted in the slow peaking up of both OGTT curves. CAM reduced glucose level after 2 hours but a quick shooting was noticed and this could be due to quick clearing of glucose from circulation via insulin. Significant gain in the weight of both groups indicated that there could be a possibility of stimulating the insulin either at secretion/ synthesis level or at the level of it action. Thus to conclude, both CAW and CAM prevented ensuing of pre-diabetes against a chronic glucose load of one month and maintained normal random glucose level, % glycosylated hemoglobin and also prevented dyslipidemia to occur.

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