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Research Article

Oral Glucose Tolerance of Traditional Medicines in a Diabetes Induced Rat Model.

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ABSTRACT

Background: The purpose of this study was to investigate the oral glucose tolerance activity of aqueous extracts of *Icacina tracantha (tuber) (fam Icacinaceae,) Ananas cosmos* (*fam. Bromeliaceae),* and *Uraria picta (*leaves*) (fam leguminosae)* on a high calorie diet fed animal model to induce type II diabetes. These plants have a long history of use as anti-diabetic agents in western Nigeria. 120 male Sprague-Dawley rats were assigned into two major groups. One group was fed on normal rat chow with the other group fed on a high calorie diet for four months. The plant crude extracts were prepared according to the traditional healer method of boiling, filtering, drying and reconstituting. A non-treated group as well as a metformin dosed group of rats were used as the control and comparator respectively. Over a 3 week period, all the animals were orally dosed with the different doses of plant extracts daily. Blood was collected from of each rat prior to dosing and thereafter weekly, and analysed for glucose concentration. During this period, the animals were weighed weekly and food intake was measured every three days. An oral glucose tolerance test (OGTT) was performed after the dosing period and fasting, 0, 30, 60 and 120 minute blood samples were taken and assayed for glucose concentration. Only *UP* showed a significant difference in its effect on the plasma glucose lowering in the normal chow fed rats. The effect of the plant extracts on the weight, and food consumed, was minimal and in most of the groups was not significant.The administered doses of IC and UP in the normal diet fed animals significantly improved the glucose clearance rates. None of the different plant extracts had any significant effect on the glucose clearance rate in the high calorie diet fed animals. Higher doses need to be checked in future studies as well as the insulin sensitivity of the plant extracts.

Key words: OGTT; plant extracts; in vivo; high calorie diet; toxicity.

INTRODUCTION

The prevalence of diabetes throughout the world has increased dramatically over the recent past, and the trend will continue for the foreseeable future. One of the major concerns associated with diabetes relates to the development of micro- and macro-vascular complications, which contribute greatly to the morbidity and mortality associated with the disease[1]. While diabetes is most common among the elderly in many populations, prevalence rates are significantly rising among comparatively young and productive populations in the developing world[2].Though conventional synthetic drugs have made considerable progress in the management of DM, traditional plant treatments for DM are also being used throughout the world and the search for natural anti-diabetic plant products for controlling DM is on-going[3]. Various ethno-pharmacological surveys have shown that a number of medicinal plants have been used for the treatment of diabetes with various authors attesting to the efficacy of the plants in the control of both type 1 and II diabetes[4]. Although medicinal plants have been historically used for diabetes treatment throughout the world, few of them have been validated by scientific criteria[5]. Many studies have been done on the isolation of compounds from plants that have been used traditionally, but very few have actually been challenged with *in vivo* animal and human models. We believe that it makes more scientific sense to first test plants to confirm their activity, and then to try and isolate the active compounds to see which ones in the plant material are pharmacologically active. The medicinal plants investigated in this study, *Icacina trichantha (*IC*), Ananas cosmos* (AC) *and Uraria picta* (UP) have been used in Western Nigeria for managing diabetes (personal observation at Complete Cure Herbal Clinic, 19 Igbobi College Road, Jibowu, Lagos) but without any scientific validation to confirm their anti-diabetic effects.The aim of this study was to investigate the ability of IC, AC, and UP individually, and in combination to test the oral glucose sensitivity in a rat

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Table 1. Estimated nutritional value of the high calorie diet

Food description	*Calorie (cal)*
Carbohydrate from sugar	6591
Carbohydrate other from plant sources	3595
Protein	2032
Fats	2835

model of induced DM. Increased glucose concentrations have been associated with DM.

MATERIALS AND METHODS

An oral glucose tolerance test (OGTT) was used to determine the ability of the body to metabolize and clear glucose out of the blood stream. Administering the test after the period of dosing will help determine the effect of the plant extract on glucose clearance. Where the plant extracts have the ability to improve glucose clearance rate, it is expected that the area under the curve for the plant extract will be lower compared to the control. The ability of the plant extracts were compared to metformin, a commonly used drug in the treatment of DM in current therapy.The model was characterised by first feeding the animals on a high calorie diet for a period of four months to induce glucose intolerance.

Materials: The plants used in this study were procured from a market in Mushin, a suburb in Lagos, Western Nigeria. The procedure for procuring the plants from the Mushin market was undertaken to simulate the procedure of the traditional herbalist, who regularly buys plants from the same seller who in turn obtains the plants from villages in a particular geographical area. The plants were identified at the Institute for Lagos University Herbarium, Nigeria and voucher specimens were deposited for reference and preservation at the Universities Herbarium. The voucher numbers for the plants are;

Ananas cosmos – LUH 4570
Uraria picta – LUH 4571
*Icacina tric*antha LUH - 4572

An Accu-check® active glucometer and test strips were purchased from Roche Diagnostics, Basel, Switzerland. Glucose obtained from Saarchem chemicals, Johannesburg, South Africa. Methods: Procedure for preparation of crude plant extracts- A 1.25 kg batch of each dried macerated individual plant was weighed and washed in 5 litres of cold distilled water. The water was then removed by filtering the cleaned plant material through a 710 µm sieve (Endocotts, England). In traditional use the aqueous extract is used by filtering through muslin. We needed to add more control to eradicate a possible source of variation. The clean plant material was then placed in a 3 litre beaker. Distilled water was added to adequately cover the plant in the beaker. The beaker containing the plant and distilled water was then heated and allowed to boil at a temperature of 100^{o}C for 2 hours. Distilled water was added intermittently to ensure the plant material was always covered. The preparation was allowed to stand at ambient temperature and cool for 12 hours. It was then filtered through a 710µm sieve. The filtrate containing the plant extract was poured into a number of preweighed 250ml beakers and allowed to dry in a drying oven (Memmert Schwabach, Laboratory Marketing Services (Pty) Ltd, Johannesburg, South Africa) at a temperature of 50^{o}C until the filtrate was evaporated to dryness leaving the crude plant extract.

The quantity of the plant extract obtained from the plant was determined by calculating the difference between the weight of the preweighed beakers and the weights of the beakers containing the dried plant extract. The dried extract was stored at a temperature of 4^{o}C in a Labcon® low temperature incubator (Laboratory Marketing Services, Johannesburg, South Africa) for appropriate use when the doses were formulated and administered to the animals as required. The extraction ratio was determined using the weight of the extract as a percentage of the original dried plants. To obtain a mixture of the plant extracts, the plants were combined based on the ratio used for treating diabetic patients as used in the traditional herbal clinic in Nigeria (Complete Cure Herbal Clinic, 19 Igbobi College Road, Jibowu, Lagos). Thus, 3 parts of UP to 2 parts AC to 1 part of IT was weighed, combined and extracted.

Toxicity studies:Toxicity studies were conducted to ascertain the safe doses of the plant extracts to be administered to the rats. This was necessary because previous work on the plants has only been able to identify the acute toxicity doses of some of the plants[6-8], whereas the present study involved the chronic administration of the plant extracts for a period of three weeks. The toxicity study was performed using doses of IC of 100mg/kg, AC of 200mg/kg, and UP of 300mg/kg body weight. These doses were chosen based on published acute toxicity studies[6-8].For the toxicity study, nine male Sprague-Dawley rats weighing approximately 600g were used. The animals were divided into groups of three animals per group. The maximum dose of the extracts (100mg/kg for IT, 200mg/kg for AC, and 300mg/kg body weight for UP) to be administered per kilogram body weight in the main study was administered to the animals daily for three weeks. During this period, the animals were observed for adverse symptoms, rate of movement, general activity, behavioural changes and death.

Preparation of rats for extract dosing:Male Sprague-Dawley (SD) rats weighing between 500 – 650g were used for the investigation of the possible anti-diabetic activity of IC, UP and AC. Two rats were housed in each cage. All cages were numbered for proper identification, while all animals were marked with water resistant makers on their tails. After one week of acclimatization to the facility, feeding of the animals commenced. The first batch of animals (n=54) was made up of the control animals fed on normal rat chow. All animals were weighed before the commencement of feeding. Animals were then allowed free access to food and water for the next four months. All animals were weighed monthly.

Table 2. Groups of rats and the daily dosing regimen administered to both the normal rat chow fed and high calorie diet fed (HCD) rats. (n=6 rats per group x 9 dosing groups x 2 fed groups = 108)

Plant extract	Dose given(mg/kg body weight)	Code
Icacina tracantha	50	IC50
	100	ICI00
Ananas cosmos	100	AC100
	200	AC200
Uraria picta	150	UP150
	300	UP300
AC/IC/UP (1:1:1)	300	MIX300
AC/IC/UP (1:1:1)	300	MET300
Distilled water (Control)		control

Table 3. Differences between HCD and normal diet fed animals at (time = 0 weeks) baseline and after 3 weeks (t=3)

Variable	**Normal diet fed rats (N=54)**		**High calorie diet fed rats (N=54)**	
	Time = 0 weeks	Time = 3 weeks	Time = 0 weeks	Time = 3 weeks
Mean body weight ± s.d (g)	643 ± 55	627 ± 55[1]	622 ± 55[*]	592 ± 54[1*]
Mean fasting glucose ± s.d(mmol/l)	6.30 ± 1.11		7.17 ± 2.29	

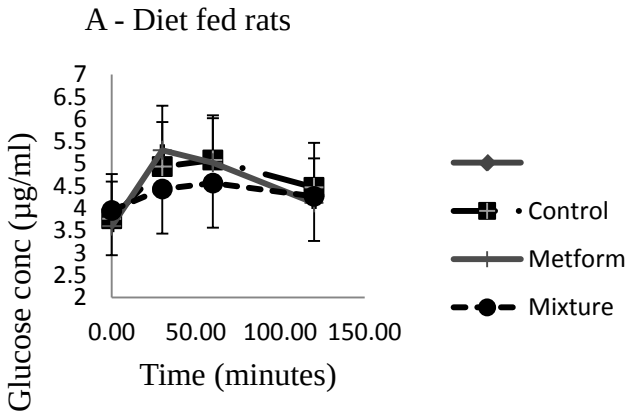
[1] *$p<0.05$, versus time = 0 weight of rats.* [*] *$p<0.05$ versus normal diet fed rats*

This same procedure was applied to the animals (n=54) given the high calorie diet.

Recipe for the special diet: The second experimental batch of animals (n=54) was fed on a special high calorie diet. The diet consisted of 1.32kg of normal rat chow, 812ml of distilled water, 280 grams of brown sugar (Selati® golden brown sugar) and 4 cans of normal condensed milk (Clover® condensed milk, 370 grams) that was mixed together to produce a homogenous paste. The paste was fed to the animals daily in a bowl. All rats in this experimental group were placed on the diet for four months. This formulation was made every three days and the remnant discarded to avoid the growth of fungus.

To calculate the amount of food consumed per day per rat, the food in the cages was weighed before it was given to the animals and then the remnant weighed every three days. Enough food was left to last beyond the three day period. The difference in weight between the initial quantity of food and the remnant was calculated and was taken as the food consumed by the animals in the cage. After taking measurement of the food consumed, fresh food was weighed to replenish the consumed food. This figure was divided by the number of animals per cage and by the number of days to estimate the quantity of food consumed by each animal per day; it is assumed that each rat consumes roughly the same amount of food. At the end of the feeding period of four months, all the animals were randomly divided into groups containing six animals (n=6) per group as shown in table 2. For the next three weeks of the dosing of the rats with the various doses of the plant extracts, food consumption was measured and calculated. It was also done to determine the effect of the plant extract on the quantity of food consumed by the animals. Weighing of rats- After feeding for a period of four months, dosing commenced. During the period of dosing, all animals were weighed on a weekly basis using a Mettler PM4600 electronic scale. Dosing rats: After the animals were weighed, doses of the plant extract to be administered were calculated and formulated per body weight for each of the animals. This was done by dissolving the plant extract in distilled water, making the appropriate concentration and determining the dose to be administered. The animals were then each given the calculated dose (2 -5ml depending on concentration and weight of rat) of the plant extract daily between 7 – 10 am in the mornings by oral gavage with the aid of a dosing needle and syringe for 3 weeks. For the metformin dosing, 300mg/100g/day was dosed in a 50ml volume of drinking water as done by Kim et al[9]. When the 50ml was finished, the rats were given normal water. This was done to not put the rats under unnecessary stress. It normally took the rats about 4 hours to drink. The doses given of each plant extract are shown in Table 2.

Measurement of glucose concentration: After the daily dosing period of three weeks, an oral glucose tolerance test (OGTT) was carried out on all the animals. All rats were fasted overnight and the following morning 2g/kg body weight of glucose solution was administered to each animal by oral gavage. Blood glucose concentrations were measured by pricking the tail tip and using an Accu-check Active® (Roche Diagnostics, Basel, Switzerland) blood glucose meter to take the readings. The glucose level was read by allowing a drop of blood from each



animal's tail to drip on the glucometer strip Accu-Check Active strips® inserted into the glucometer. The glucose concentration was then read and documented from the glucometer readings. This method was chosen because it used a very small quantity of whole blood (<5 µl) and gave immediate results and thus an indication of whether the glucose load had been administered successfully via gavage. After the initial measurement of blood glucose was taken using the glucometer (at time 0), glucose was administered orally to each rat. Blood glucose concentrations were then measured at 30, 60, and 120 minutes after the loading dose of 2g/kg glucose was given.

STATISTICAL ANALYSES

Area-under-the-curve (AUC) values for glucose during the OGTT were calculated using the trapezoid rule. Fasting and AUC glucose concentrations were compared to see if there was a significant difference between the glucose concentration concentrations versus the control, the metformin standard, and normal fed versus HCD fed rats. The Microsoft Excel® (2007) student - t double tailed, homoscedastic variance test was used to determine the probability of a significant difference between the various variables measured, and a paired variance for comparing the normal chow fed values to the HD fed values under measurement. The Pearson correlation coefficient was used to determine if there was any relationship between the amount of food consumed and the weight difference of the rats (Week 3 value – Week 0 value)

RESULTS

Toxicity test : After the 3 week period of dosing with the plant extracts all the rats in the toxicity test showed no signs of distress, discomfort and no deaths were recorded. All the animals were adjudged healthy by the animal house veterinarian. Ethical approval from the university of the Witwatersrand Animal Ethics Committee was granted for the commencement of the work.

Description of body weight results: Table 3 shows differences at baseline, for body weight and fasting glucose concentration between the normal diet fed and the HCD fed animals. Body mass was slightly higher in the normal diet fed animals whilst fasting glucose concentrations were significantly higher in the high calorie diet fed animals

During the dosing period of three weeks prior to the OGTT, the average weight of the rats did not change by an appreciable amount. Although the weight differences were mathematically significant ($p<0.05$) the difference between the average weights were only -4.67% for the normal chow fed group and -4.35% for the HCD fed group.

Table 4 gives the mean weight percentage difference of normal diet fed rats in comparison to control group, metformin group, and HCD fed rats during the 3 week period of dosing of plant extracts. In the normal chow fed rat group, the metformin, AC100, UP150 and UP 300 dosed rats decreased significantly ($p < 0.05$) when compared to the control group in the normal chow fed rats. The percentage mass decrease however was very small, with the highest percentage mass decrease being only 8.22 ± 1.08 percentage for the metformin dosed rats. When comparing the percentage weight loss of the rats given the different doses of plant extracts were compared to the metformin dosed rats, all showed a significant difference with *p* values below 0.05. Only the mixture dosing regime in the normal chow fed rats showed a minimal increase in weight (0.69 ± 2.11%) which was not significantly different from the control group of rats.

In the HCD fed rat group, metformin once again produced the highest percentage weight loss at -6.98 ± 6.57%. This however, was not significantly different when compared to that of the control group. All other rats in the HCD fed group showed a small percentage loss in weight over the 3 weeks of dosing of the different plants. Only the mixture of plant extracts and the UP150 dosed rats showed a significant difference to the water (control) dosed rats ($p=0.0043$ and $p=0.0085$ respectively). When comparing the percentage weight loss of the HCD fed rats dosed with the various plant extracts as compared to the metformin dosed group, only the AC200 dosed rats showed a significant difference ($p=0.0391$) to that of the metformin dosed rats. If one compares the percentage weight change of the normal chow fed rats and the HCD fed rats, only the metformin ($p=0.3120$) ,the AC200 ($p=0.2517$), and UP300 ($p=0.1307$) groups did not show any significant difference in their percentage weight losses. All the others showed a significant percentage weight loss. Interestingly, the difference between the two controls in the groups showed the biggest variation with the control mean difference from time = 0 to time 3 weeks of 0.14 ± 2.05% for the normal fed rat chow group, and -6.09 ± 2.27% difference. However, once again, the differences are small percentages.

Effect of food intake on weight of rats during the three week dosing period: In order to check whether the weight gain or loss in the rats was due to the amount of food consumed, a linear regression analysis was done and the Pearson Correlation coefficient was calculated. In the normal rat chowder fed rats the average amount of food consumed by the rats was 26.09 ± 2.91g per week and

Table 4. Mean weight percentage difference of normal diet fed rats in comparison to control group, metformin group, and HCD fed rats during the period of dosing

Group(mg/kg)	Mean % mass difference ± s.d.	p value[1] vs control	p value[1] vs metformin	p value[2] vs HCD
Normal chow fed rats				
Control	0.14 ± 2.05		0.0000	0.0056
Metformin	-8.22 ±1.08	0.0000		0.3120
Mixture	0.69 ±2.11	0.3284	0.0000	0.0217
I T 50	-0.39 ±1.38	0.3064	0.0000	0.0007
I T 100	-1.17 ±1.36	0.1277	0.0000	0.0072
A C 100	-2.39 ±1.02	0.0129	0.0000	0.0313
A C 200	-0.91 ±1.41	0.1630	0.0000	0.2517
U P 150	-5.91 ±1.47	0.0001	0.0056	0.0023
U P 300	-4.23 ±2.05	0.0021	0.0009	0.1307
High Calorie Diet fed rats				
Control	-6.09 ±2.27		0.7734	
Metformin	-6.98 ±6.57	0.3806		
Mixture	-2.74 ±1.09	0.0043	0.2065	
I T 50	-5.04 ±1.74	0.1946	0.5263	
I T 100	-4.53 ±1.13	0.0816	0.4576	
A C 100	-4.22 ±1.36	0.0567	0.3811	
A C 200	-2.16 ±3.20	0.0169	0.0391	
U P 150	-2.58 ±1.98	0.0085	0.2237	
U P 300	-5.89 ±1.31	0.4259	0.7076	

[1]p value calculated from student t-test with double tail and unmatched pairs
P[2] value calculated from student t-test with double tail and matched pairs

Table 5. Mean AUC's for the OGTT as compared to the control, metformin, and the HCD fed rat groups.

Dosage	Mean AUC ± sd(mmol/l/min)	AUC ± vs Control p value	vs Metformin	Diet vs normal p value
HCD group				
Control	567.25 ± 31.68		0.4160	0.1050
Metformin	562.50 ± 42.98	0.4160		0.3697
Mixture	525.75 ± 59.17	0.0804	0.1233	0.1068
I T 50	600.00 ± 48.50	0.0981	0.0934	0.1675
I T 100	559.25 ± 47.79	0.3698	0.4519	0.1661
A C 100	524.75 ± 38.21	0.0312	0.0695	0.0011
A C 200	578.25 ± 77.54	0.3772	0.3363	0.0710
U P 150	577.50 ± 64.24	0.3666	0.3224	0.0606
U P 300	575.25 ± 54.74	0.3815	0.3316	0.0006
Normal diet fed group.				
Control	609.25 ± 82.08		0.0864	
Metformin	557.00 ± 29.42	0.0864		
Mixture	563.25 ± 70.48	0.1611	0.4226	
I T 50	555.00 ± 79.95	0.1366	0.4776	
I T 100	596.80 ± 71.08	1.3923	0.1169	
A C 100	656.25 ± 37.13	0.1151	0.0002	
A C 200	525.00 ± 60.44	0.0352	0.1353	
U P 150	530.25 ± 30.12	0.0256	0.0753	
U P 300	483.75 ± 54.44	0.0054		

29.62 ± 2.51 g per week for the HCD group. Although the difference in the average amount of food consumed per week is only 3.53g, it was mathematically significant (p=0.0141). However, there was no correlation between the mean amounts of food consumed for the normal fed rats (r^2 = 0.3893) and the HCD rats (r^2 = 0.0146) per week over the three week period of dosing. This result is similar to those obtained by Kanazawa et al[10]. They reported that feeding rats on a high sucrose (60% sucrose) diet for 2 weeks did not induce obesity in lean rats or enhance weight gain in obese rats. Santuré et al[11] demonstrated that, after 4 weeks of feeding, rats displayed comparable final body weight regardless of whether they had been fed high sucrose or the normal chow diet. However, the average daily *ad libitum* intake was significantly lower for the sucrose-fed rats compared

to their control chow-fed rats. Long term feeding of rats for up to 42 weeks on a high sucrose diet (63% sucrose) as reported by Fortino et al[12], induced dyslipidemia, glucose intolerance, and adiposity. Rats fed on a high sucrose diet were also observed to consume lesser amounts of food when compared to animals fed on the normal rat chow[12].

In this study, the animals were fed for 16 weeks before dosing with the plant extracts commenced. The average weight of the animals fed on the high calorie diet was observed to be slightly lower than those of rats fed the normal rat chow before dosing with the plant extracts. Despite this, the high calorie diet fed animals had higher glucose concentrations than the normal diet fed animals before the plant extracts were administered to them, a result similar to that reported by other researchers[13,14]. Also, weight gain is affected and determined by the diet composition and the duration of feeding. Lombardo et al[15] showed that weight gain in rats fed on a rich sugar diet for the first 15 weeks did not differ between groups. A significant increase in weight gain was however observed in animals fed from 15 – 30 weeks. In the present study, the animals were fed for 16 weeks before dosing.

Meformin was observed to bring about a sustained decrease in the body weight of the animals in the normal diet fed group without affecting food intake[16]. A decreased weight loss in all animal groups except the control group in the normal fed rats was observed over the 3 weeks of dosing. This effect is interesting, and suggests that the extracts have effects on the metabolism that are altered directly or indirectly by diet intake. Energy expenditure was not measured in this study so we cannot make any conclusions on whether the weight effects of the plant extracts are mediated by effects on energy expenditure. The IC plant extract also caused weight loss relative to the control animals, but had no effect on food intake. Other studies have shown that medical plants decrease body weight in rats[17] and the ability of some of the plant extracts to decrease body weight portends a possible functionality in the improvement of type II diabetes associated with obesity and weight gain[16].

Glucose concentrations in normal fed and HCD diet fed rats:Table 5 compares the mean AUC's of the various glucose blood levels for the period of 0, 30, 60 and 120 minutes after the glucose dose to the various groups of rats. In the normal rat chow fed groups, all the plant extract doses lowered the glucose levels more than the control group with the exception of AC100. Although most of the mean AUC's were less than the control mean AUC, only AC 200 ($p=0.0352$), UP150 ($p=0.0256$) and UP300 ($p=0.0054$) were significantly lower. When compared to metformin, only AC200, UP150, and UP300 had lower mean AUC's. Only UP 300 had a significantly lower AUC than metformin ($p=0.0079$).

If one compares the mean AUC between the normal and HCD groups, only AC 100 ($p=0.0011$) for the HCD group had a significantly lower mean AUC when compared to the mean AUC for the normal fed group. UP 300 ($p = 0.006$) had a significantly larger mean AUC in the normal fed group as compared to the HCD fed group.The results obtained from the OGTT therefore show that the various plant extracts had a greater effect on glucose clearance (smaller AUC) in the animals fed on normal rat chow compared to the animals fed the high calorie diet (HCD). Figure 1 shows a typical AUC plot of glucose concentration versus time from which AUC's were calculated.

Figure 1. Typical plot of glucose concentration versus time curves used to calculate the AUC's for comparison of glucose lowering effect. The curve is for diet fed rats and is for control, metformin and mixture glucose levels.

The metformin treatment in the normal diet fed group led to a significant reduction in glucose concentration in the last week when compared to the control group, whilst metformin in the high calorie diet group caused no significant change in glucose concentration although they did tend to be lower than the concentrations observed in the plasma of the control animals at week 1 and week 3. It is possible that the dose of metformin used in the high calorie diet group was too low to bring about a significant change in glucose concentration.

The fluctuation of the glucose concentration during the period of dosing may be an indication that the plant extracts contain glucose lowering substances, but not in sufficient quantities to maintain a sustained reduction in glucose concentration. A major reason why the plant extracts could not bring the glucose concentration under control may be due to inadequate dosages and the route of administration. Rouru et al[18] demonstrated that chronic administration of metformin subcutaneously improves efficacy of the drug. Oral administration exposes the extracts to a first pass effect leading to lesser availability of the drug.

CONCLUSION

The results in this study demonstrate the glucose lowering effect of some of the plant extracts. The administered doses of IC and UP in the normal diet fed animals significantly improved the glucose clearance rates. None of the different plant extracts had any significant effect on the glucose clearance rate in the high calorie diet fed animals.

While a number of medicinal plants have been shown to have hypoglycaemic effects and improve the glucose clearance rate in animals[19], studies such as those conducted by Al-Awadi and Gumaa[20], investigating the antidiabetic effect of five Kuwaiti medicinal plants, concluded that only two of the plant extracts showed glucose lowering effect using the OGTT. A similar pattern is seen in this study.

The marginal effect of the plant extracts on the OGTT glucose concentration in the HCD fed animals may be due to the low dose being dosed to the animals. We believe that doses more frequently than once per day could have a much more significant effect on the OGTT. Also, the toxicity level of these pants seems to be much higher than the doses we tested, and further studies on the

toxicity of the plants need to be conducted, so that higher doses can be administered.

The high calorie diet did not lead to obesity or type II diabetes. Among out-bred Sprague Dawley rats fed on a high fat diet, approximately one-half developed diet-induced obesity and one-half were diet resistant on a diet relatively high in fat and energy content, gaining weight and fat at the same rate as chow-fed controls however having increased fat pads, higher insulin and leptin concentration when compared to animals fed on normal rat chow[13,14]. This may have been the case why not all rats increased in weight and glucose levels were variable in this study. Also, the animals were fed on a high sucrose diet. The estimated percentage of sucrose in the diet is 18 % may not have profound effects on glucose concentrations, but it may have more effect on insulin concentrations. Obviously, the efficacy of these plants in human diabetic subjects would need to be tested in a clinical trial, however the fact that these plants are used for treating diabetes in African populations suggests there may be some clinical benefit. Although the OGTT is an important measurement in determining the effect of the plant extracts on type II diabetes, one also needs to check the effect of the plant extracts on insulin sensitivity. The use of diabetic Zucker rats may also give better results as this model of feeding rats on a HCD might not be optimal in inducing type II diabetes.

The plants used in the present studies were observed to be safe for use in all the animals, even in large doses. This present study also suggests that the crude plant extracts may have value in modifying insulin resistance, a syndrome that predisposes to type II diabetes.

Contribution of authors

M. P. Danckwerts – supervisor of student's research work done, analysis of results, write-up of publication.

Femi Fatokun – carried out the research work.

Nigel Crowther – co-supervisor of research work done, advised on research methodology and interpretation of results.

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