ORIGINAL ARTICLE

INTESTINAL LIPIDS AND MINERALS IN STREPTOZOTOCIN-INDUCED DIABETIC RATS FED BITTER YAM (*DIOSCOREA POLYGONOIDES*) SAPOGENIN EXTRACT

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ABSTRACT

Yam is the leading form of staple for millions of people in the tropical and subtropical countries. They are good sources of carbohydrate. However, the protein content of yam is low. The effect of bitter yam sapogenin extract or commercial diosgenin on faecal minerals and intestinal lipids in streptozotocin-induced diabetic rats was studied. Sapogenin extract or commercial diosgenin (1%) supplemented diets were fed to diabetic male Wistar rats for three weeks. Bitter yam sapogenin extract or commercial diosgenin did not significantly alter faecal magnesium, calcium, and zinc excretion but significantly decreased faecal sodium and potassium excretion. The absorption of iron was impaired by bitter yam sapogenin extract or commercial diosgenin during the first week of feeding. Bitter yam sapogenin extract or commercial diosgenin supplements significantly decreased intestinal lipids towards normal. Faecal lipids excreted was significantly higher in diabetic rats fed bitter yam sapogenin extract or commercial diosgenin for the three weeks period compared to the diabetic control group. These results show that bitter yam sapogenin extract or commercial diosgenin does not have the same effects on mineral excretion in diabetes. There was no direct correlation between the decrease in excretion of mono-valent cations and the activity of intestinal Na^+/K^+ ATPase.

Keywords: Sapogenin; lipid; yam; diabetes; minerals; diosgenin.

INTRODUCTION

The yam tuber is the main economically utilized part of the yam plant (Onwueme, 1978). It is primarily used as a source of energy because of its high carbohydrate content. Carbohydrates account for approximately one-third of the tubers' fresh weight and 80-90% of the yam on a dry weight basis. The protein content is very low and ranges from 2-5% on fresh weight and 4-12% on dry weight basis. Vitamins and minerals (ash) are also minor components of the yam tuber. The tuber is a good source of vitamin C with values ranging from 6-10mg/100g dry wt (Onwueme, 1978). The water content of yam tubers is high and lies between 60-80% [wet weight, (Coursey, 1967)].

Research has shown that some yams contain significant levels of antinutritional factors that may produce acute toxic effects in rats (Panigrahi and Francis, 1982; Prima and Kurup, 1979). The presence of antinutritional factors in yams has raised questions about their suitability as a staple diet (Womack *et al.,* 1976 and Osagie, 1992). Yams contain phenolic compounds which are responsible for the pigmentation and bitterness of yam tubers and the rapid browning reactions that occur when the tissues are injured or otherwise exposed to air (Osagie, 1992). The bitterness and toxicity of yam tubers may be influenced by the presence of alkaloids from the dioscorine group or high levels of saponins. Diosgenin is the main sapogenin in yam and it has been reported to be present in all members of the Dioscoreaceae family (DeGras, 1993). Diosgenin is the most important sapogenin from an economic point of view. Some *Dioscorea* species contain as much as 13% diosgenin (Akahori, 1965). Saponins have been reported to form complexes with minerals with the resultant decrease in the availability of minerals for absorption or metabolism (Southon *et al*., 1988 and West *et al*., 1978). Diosgenin has also been reported to inhibit the absorption of dietary and enterohepatic cholesterol and/or bile acid absorption (Cayen and Dvornik, 1979). Yam is a staple food in the Caribbean and cases of iron deficiency has been reported in Jamaica (Simmons, 1990). This study was aimed at investigating the effects of consumption of bitter yam sapogenin extract on intestinal lipids and minerals with a view to explaining the hypolipidemic action and the intestinal absorption of some minerals in streptozotocin-induced diabetic rats. Commercial diosgenin was used for comparison purposes.

METHODS

Diosgenin was purchased from Sigma-Aldrich, (St. Louis, MO) and the normal diet (PMI Feeds Inc. Lab Diet #5001) was a marketed laboratory rodent diet recommended for rats, mice and hamsters with the approximate chemical composition: protein 23%, fat 4.5%, fibre 6.0%, ash 8.0%

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and carbohydrate 58.5%. All other reagents were of analytical grade.

Sample preparation

Freshly harvested tubers of bitter yam (*Dioscorea polygonoides*) were obtained from Muirhouse, St. Ann, Jamaica. Sapogenin was extracted from Jamaican bitter yam using the method of Morris *et al*. (1958). Freshly harvested tubers (50 g) were peeled, chopped and refluxed with 3.5 M HCl (115 ml) for 3 h. The solution was filtered and the residue was washed with water to neutrality. The filter paper and residue were dried at 65-70°C overnight and then extracted with petroleum ether in a Soxhlet apparatus for 6 h. The petroleum ether extract was concentrated in vacuo. The solid which precipitated was filtered and dried to give the crude sapogenin extract.

The sapogenin extract or commercial diosgenin (purchased from Sigma-Aldrich, St. Louis, Mo.) were supplemented at a level of 1.0g of sapogenin extract (80% diosgenin and the other 20% made up of β-sitosterol, pennogenin, stigmasterol and $\Delta\Delta^3$ diosgenin) or commercial diosgenin/100g of rat basal diet (Cayen and Dvornik, 1979).

Animals

Adult male Wistar rats (32) obtained from the University of the West Indies Animal House, were divided into four groups by weight for a three-week study (8 rats per group, average body weight 249.0±0.3g). The experimental groups were as follows: non-diabetic rats receiving normal diet (Normal); diabetic rats fed normal diet (Diabetic); diabetic rats fed sapogenin extract (Sapogenin extract) and diabetic rats fed commercial diosgenin (Commercial diosgenin).

Diabetes was induced by a single intraperitoneal injection of streptozotocin (60mg/Kg-body weight in 0.05M citrate buffer, pH 4.5). The normal control group was injected intraperitoneally with an equivalent amount of buffer (0.05M citrate buffer, pH 4.5). After 8 days, blood was withdrawn from the tail vein and the level of blood glucose determined using the method of Teller (1956). Diabetes was confirmed when blood glucose was about four times in excess of normal. Initial blood glucose for normal rats was 4.77 ± 0.75 mMol/L, while the initial blood glucose for all three diabetic groups ranged from 17.04 ± 0.98 to 32.52 ± 4.49 mMol/L.

Rats were housed in stainless steel cages in a room kept on a 12-hour light-dark cycle, and were allowed to have access to their respective diets and water *ad libitum.* The cages were cleaned daily. Body weight change, faecal output and total food intake were recorded weekly. The rats were fed on their respective diets for 21 days and sacrificed by decapitation after an overnight fast. Approval for the study was obtained from the Board of Ethical Studies, Faculty of Medical Sciences, University of the West Indies, Mona.

Total lipid in the intestine was extracted by the method of Bligh and Dyer (1959). The determination of cholesterol was by the method of Zlatkis *et al*. (1953). Faecal lipid was extracted using the method of Folch *et al*. (1957). Faecal HDL-cholesterol was measured by precipitating the low density lipoprotein cholesterol with phosphotungstate and magnesium and finding the amount of cholesterol in the supernatant (Lopez-Virella, *et al*., 1977). Total phospholipid was determined with the method of Bartlett (1959). Faecal minerals were determined according to standard AOAC methods (AOAC 2000).

STATISTICAL ANALYSES

Results were expressed as mean ± SEM. Analysis of variance was used to test differences among all the groups tested at $P<0.05$. To determine where the significant difference lies among the groups, the Duncan's Multiple Range test was used to compare the means in descending order (Dixon and Massey, 1957; Sokal and Rholf, 1969).

RESULTS

Table 1 shows the effect of bitter yam sapogenin extract on feed intake, body and intestinal weights. The diabetic control or bitter yam sapogenin extract or commercial diosgenin groups significantly lost weight compared to the normal control group despite consuming similar amount of feed. The groups fed bitter yam sapogenin extract or commercial diosgenin further lost weight compared to the diabetic control group. Similarly, there was a significant decrease in intestinal weight in the diabetic control or bitter yam sapogenin extract or commercial diosgenin groups compared to the normal control group. Bitter yam sapogenin extract further significantly lowered intestinal weight compared to the diabetic control or commercial diosgenin groups.

Tables 2 and 3 show the effect of bitter yam sapogenin extract on faecal minerals. Faecal zinc excretion was not significantly altered in diabetic rats for the three weeks of feeding compared to normal rats. Supplementation of the diet with bitter yam sapogenin extract or commercial diosgenin did not significantly decrease faecal zinc excretion compared to the diabetic control.

Faecal iron was not significantly altered in diabetic rats for the feeding period compared to normal rats. Supplementation of the diet with bitter yam sapogenin extract significantly increased faecal iron excretion by week 1, but did not significantly alter faecal iron by weeks 2 and 3 compared to the diabetic control. Treatment with commercial diosgenin did not significantly alter faecal excretion of iron for the trial period compared to the diabetic controls.

Table 1: Effect of bitter yam sapogenin extract on feed intake, body and intestinal weights (g)

	Normal control	Diabetic control	Sapogenin extract	Commercial diosgenin
Final Body Wt	325.4 ± 9.2	247.3 ± 27.0‡	190.8 ± 17.4‡	193.3 ± 8.8‡
Initial Body Wt.	248.4 ± 16.2	249.3± 9.2	249.8 ± 14.1	248.4 ± 16.8
Daily Feed Intake	14.6 ± 0.0	14.5 ± 0.1	14.6 ± 0.1	14.4 ± 0.1
Intestine Wt	8.6 ± 0.5	6.5 ± 0.4†	5.0 ± 0.2†‡	6.3 ± 0.3†

Values expressed as mean ± SEM.
Final body weight: ‡denotes significant differences (P<0.05) from normal control group.
Intestine weight: † denotes significant differences (P<0.05) from normal control group whereas ‡ denotes significant differences (P<0.05) from diabetic control and commercial diosgenin groups.

Table 2: Effect of bitter yam sapogenin extract on faecal zinc, iron and magnesium levels (mg/kg dry wt.)

Mineral	Normal control	Diabetic control	Sapogenin extract	Commercial diosgenin
Zn				
Week 1	357.9 ± 14.0	311.1 ± 4.0	284.2 ± 1.7	308.4 ± 11.7
Week 2	338.3 ± 12.0	403.3 ± 2.6	372.9 ± 29.1	345.1 ± 4.7
Week 3	364.1 ± 18.7	405.8 ± 4.9	331.8 ± 32.9	338.8 ± 1.6
Fe				
Week 1	818.6 ± 46.3	750.4 ± 53.8	1480.8 ± 104.6†	713.7 ± 38.6
Week 2	704.4 ± 22.4	837.9 ± 30.0	873.7 ± 45.9	789.9 ± 2.8
Week 3	694. 0 ± 34.4	858.9 ± 69.4	1043.7 ± 60.2	1055.9 ± 29.2
Mg				
Week 1	6434.1 ± 880.2	5539.9 ± 324.9	4580.1 ± 378.0	5479.6 ± 282.3
Week 2	6234.8 ± 86.5	6781.6 ± 131.2	7990.2 ± 176.0	6104.1± 305.2
Week 3	6514.4 ± 146.2	6784.9 ± 179.3	5684.8 ± 487.5	5933.7 ± 161.9

Values expressed as mean ± SEM.
Iron (Fe): †denotes significant differences (P<0.05) from normal control, diabetic control and commercial diosgenin groups.

The excretion of magnesium was not significantly altered in diabetic rats for the feeding period compared to normal rats. Supplementation of the diet with bitter yam sapogenin extract or commercial diosgenin did not significantly alter faecal excretion of magnesium for the trial period compared to the diabetic controls.

Faecal sodium was not significantly altered in diabetic rats for the three weeks of feeding compared to normal rats. Supplementation of the diet with bitter yam sapogenin extract or commercial diosgenin significantly decreased sodium excretion for all three weeks compared to the diabetic control.

Faecal potassium was not significantly altered in diabetic rats for the three weeks of feeding compared to normal rats. Supplementation of the diet with bitter yam sapogenin extract or commercial diosgenin significantly decreased faecal potassium excretion compared to the diabetic control. Treatment with bitter yam sapogenin extract did not significantly alter faecal excretion of potassium by week 2 compared to the normal and diabetic controls. Commercial diosgenin significantly decreased potassium excretion in week 2 compared to normal control and diabetic control groups. Bitter yam sapogenin extract or commercial diosgenin significant decreased potassium excretion compared to the normal and diabetic controls.

The excretion of calcium was not significantly altered in diabetic rats for the feeding period compared to normal control rats. Supplementation of the diet with bitter yam sapogenin extract or commercial diosgenin did not significantly alter faecal excretion of calcium for the trial period compared to the diabetic controls.

Table 4 shows the effect of bitter yam sapogenin extract on faecal lipid profile. The mass of faecal cholesterol excreted was significantly increased in weeks 1 and 3 in diabetic control rats when compared to the normal control group. Treatment with bitter yam sapogenin extract or commercial diosgenin resulted in a significant increase in faecal excretion of cholesterol by weeks 2 and 3 but did not significantly alter faecal cholesterol output by week 1 compared to the diabetic control group.

Table 3: Effect of bitter yam sapogenin extract on faecal sodium, potassium and calcium levels in diabetic rats (g/kg dry wt.)

Mineral	Normal control	Diabetic control	Sapogenin extract	Commercial diosgenin
Na				
Week 1	3.9 ± 0.1	3.3 ± 0.1	2.9 ± 0.2†	2.9 ± 0.0†
Week 2	3.5 ± 0.0	3.5 ± 0.2	2.7 ± 0.0†	2.8 ± 0.1†
Week 3	3.3 ± 0.2	3.1 ± 0.0	2.6 ± 0.1†	2.7 ± 0.0†
K				
Week 1	12.6 ± 1.1	10.0 ± 0.0	8.6 ± 0.1†	8.3 ± 0.4†
Week 2	11.3 ± 0.0†	11.5 ± 0.0†	10.3 ± 0.6†‡	9.1 ± 0.2 ‡
Week 3	11.5 ± 0.0	11.1 ± 0.1	7.9 ± 0.1 †	8.5 ± 0.0†
Ca				
Week 1	38.7 ± 6.4	35.7 ± 0.0	45.2 ± 9.2	37.6 ± 3.0
Week 2	45.6 ± 2.2	45.0 ± 5.6	48.9 ± 9.7	37.4 ± 5.1
Week 3	38.3 ± 1.5	43.9 ± 1.8	41.8 ± 2.1	41.6 ± 1.8

Values expressed as mean ± SEM.
Horizontal †denotes significant differences (P<0.05) from normal and diabetic control groups.
Potassium (Week 2): † denotes significant differences (P<0.05) from commercial diosgenin group whereas ‡ denotes significant differences (P<0.05) from normal and diabetic control groups.

Table 4: Effect of bitter yam extract on rat faecal lipid profile

Lipids	Normal control	Diabetic control	Sapogenin extract	Commercial diosgenin
Total Cholesterol				
Week 1	2.3 ± 0.2	3.8 ± 0.2 †	3.5 ± 0.1†	3.9 ± 0.1†
Week 2	3.4 ± 0.3	3.5 ± 0.2	5.1 ± 0.4†	5.0 ± 0.2†
Week 3	2.4 ± 0.1	3.6 ± 0.1†	5.8 ± 0.1‡	6.1 ± 0.1‡
HDL-Cholesterol				
Week 1	2.1 ± 0.2	3.3 ± 0.1†	2.6 ± 0.3	3.0 ± 0.2†
Week 2	2.7 ± 0.1	2.9 ± 0.3	4.4 ± 0.1†	4.0 ± 0.1†
Week 3	1.9 ± 0.1	2.7 ± 0.1	4.4 ± 0.1 †	4.5 ± 0.2†
Total Phospholipids				
Week 1	0.6 ± 0.02	0.6 ± 0.04	1.1 ± 0.05†	0.3 ± 0.01†‡
Week 2	0.8 ± 0.02†	3.5 ± 0.30	0.9 ± 0.02 †	0.6 ± 0.02†
Week 3	0.5 ± 0.02	0.9 ± 0.02†	0.5 ± 0.02	0.8 ± 0.20†

Values expressed as mean ± SEM.
Total cholesterol & HDL-cholesterol (Week 1): †denotes significant differences (P<0.05) from normal control group.
Total cholesterol (Week 2) and HDL-cholesterol (Weeks 2 & 3): †denotes significant differences (P<0.05) from normal and diabetic control groups.
Total cholesterol (Week 3): † denotes significant differences (P<0.05) from normal control group whereas ‡ denotes significant differences (P<0.05) from normal and diabetic control groups.
Total phospholipids (Week 1): †denotes significant differences (P<0.05) from normal and diabetic control groups whereas ‡ denotes significant differences (P<0.05) from sapogenin extract group.
Total phospholipids (Week 2): †denotes significant differences (P<0.05) from diabetic control group. Total phospholipids (Week 3): †denotes significant differences (P<0.05) from normal control and sapogenin extract groups.

Faecal HDL-cholesterol was significantly increased by week 1; however, by weeks 2 and 3, there was no significant difference in diabetic control rats when compared to the normal control group. Treatment with bitter yam sapogenin extract resulted in a significant increase in faecal excretion of HDL-cholesterol by weeks 2 and 3 but did not significantly alter faecal cholesterol output by week 1 compared to the diabetic control group. Supplementation with commercial diosgenin resulted in a significant increase in faecal excretion of HDL-cholesterol by week 3 but did not significantly alter faecal HDL-cholesterol output by weeks 1 and 2 compared to the diabetic control group.

Table 5: Effect of bitter yam sapogenin extract on intestinal lipids (mg/g intestine)

Lipids	Normal control	Diabetic control	Sapogenin extract	Commercial diosgenin
Total Lipid				
Proximal	8.3 ± 0.8	10.1 ± 0.8†	7.2 ± 0.7†‡	6.9 ± 0.5†‡
Mid	7.5 ± 0.6	8.5 ± 0.5†	6.7 ± 0.6† ‡	5.8 ± 0.5†‡
Distal	6.0 ± 0.4	7.4 ± 0.3†	4.2 ± 0.4† ‡	4.5 ± 0.4 †‡
Total Cholesterol ($x10^{-1}$)				
Proximal	9.7 ± 0.4	11.2 ± 0.4 †	6.3 ± 0.3 †‡	6.4 ± 0.5†‡
Mid	6.7 ± 0.3	8.0 ± 0.3 †	4.2 ± 0.1†‡	4.1 ±0.2 †‡
Distal	5.1 ± 0.2	6.1 ± 0.2 †	3.5 ± 0.1†‡	3.1 ± 0.2†‡
Total Phospholipids ($x10^{-2}$)				
Proximal	6.1 ± 0.4†	7.7 ± 0.5	6.7 ± 0.4†	6.2 ± 0.4†
Mid	4.4 ± 0.3	5.6 ± 0.3†	2.9 ± 0.2†‡	2.8 ± 0.3†‡
Distal	2.4 ± 0.1†	3.8 ± 0.3	2.2 ± 0.2 †	1.7 ± 0.1†

Values expressed as mean ± SEM.
Total lipid and cholesterol: †denotes significant differences (P<0.05) from normal control group whereas ‡ denotes significant differences (P<0.05) from diabetic control group.
Total phospholipids (Proximal & Distal): †denotes significant differences (P<0.05) from diabetic control group.
Total phospholipids (Mid): †denotes significant differences (P<0.05) from normal control group whereas ‡ denotes significant differences (P<0.05) from diabetic control group.

Table 5 shows the effect of yam sapogenin extract on intestinal lipids. Intestinal total lipid and cholesterol levels were significantly increased in diabetic rats compared to the normal control group. Supplementation of the diet with bitter yam sapogenin extract or commercial diosgenin resulted in a significant decrease in total lipid and cholesterol in all three regions of the intestine compared to the diabetic and normal control groups.

Intestinal phospholipid level was significantly increased in diabetic control rats compared to the normal control group. Supplementation of the diet with bitter yam sapogenin extract or commercial diosgenin resulted in a significant decrease in phospholipids compared to the diabetic and normal control groups.

Figure 1 shows faecal lipid output and mass of faeces excreted on a weekly basis. There was no significant difference in the weekly faecal lipid output of diabetic rats compared to normal control rats. Total faecal lipid excreted was significantly higher in diabetic rats fed bitter yam sapogenin extract or commercial diosgenin for the three weeks when compared to the diabetic control group.

The mass of faeces excreted was significantly reduced in the diabetic control group compared to the normal control rats for the three weeks. Supplementation of the diet with bitter yam sapogenin extract did not significantly alter the mass of faeces excreted after one week, however by weeks 2 and 3, faecal output was significantly increased compared to the diabetic control group. Supplementation with commercial diosgenin significantly increased the mass of faeces excreted by week 2; however, it did not significantly alter the mass of faeces excreted by weeks 1 and 3.

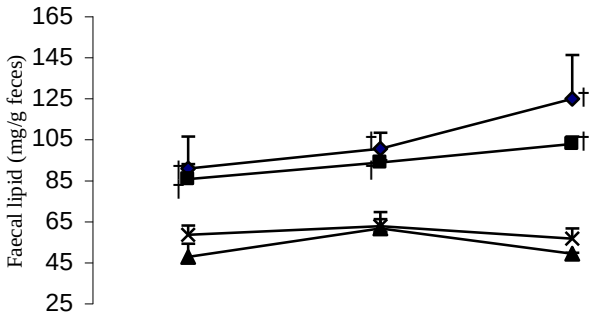

Fig. 1: Weekly faecal output.

DISCUSSION

Several researchers have reported on the effect of saponins on the availability of minerals but the results have been variable. Southon *et al*. (1988) and West *et al*. (1978) reported that saponins at high concentrations formed complexes with minerals which resulted in decreased availability of minerals for absorption or metabolism. Morgan *et al*. (1972) and Peterson (1950) in contrast, reported that digitonin and quillaja saponins did not complex minerals. The ability of saponin to complex minerals is extremely variable and depends upon the source of the saponin present (Peterson 1950). Our data indicate that the supplementation of the diet with bitter yam sapogenin extract or commercial diosgenin did not

significantly alter faecal magnesium, calcium, and zinc excretion but significantly decreased faecal sodium and potassium excretion. The lowered levels of sodium and potassium in the faeces of rats fed diets supplemented with bitter yam sapogenin extract or commercial diosgenin indicate that saponins may increase intestinal absorption of monovalent minerals leading to a decrease in their faecal excretion. Ewart (1931) showed that saponins may increase the absorption of some minerals. The lack of significant alterations in excretion of calcium, magnesium and zinc and the increased excretion of sodium and potassium indicates that saponin induced-changes in mucosal function affect intestinal absorption of minerals in different ways. We have earlier reported that bitter yam sapogenin extract or commercial diosgenin supplement significantly reduced Na+-K+-ATPase activity in all three regions of the intestine compared to the diabetic control group (McAnuff *et al*. 2005). Results from this study however did not show any direct correlation between the decrease in excretion of mono-valent cations (sodium and potassium) and the activity of intestinal Na^+/K^+ ATPase.

Our results also show that iron absorption may be impaired by the supplementation of the diet with bitter yam sapogenin extract during the first week of feeding. This is consistent with reports from previous works that indicated that saponins may interfere with iron metabolism by rendering it unavailable for absorption or produce changes in the intestinal mucosal function leading to reduced efficiency of nutrient absorption (Southon *et al*, 1988). The presence of saponins in yam which is a staple food in the Caribbean may partly be responsible for the reported (Simmons 1990) cases of iron deficiency in the region.

It has been reported (Oakenfull and Sidhu, 1983) that saponins form complexes with membrane cholesterol or extract cholesterol from the membranes. In the present study, the significant decrease in intestinal lipids towards normal may be due to the binding of intestinal lipids by bitter yam sapogenin extract or commercial diosgenin for excretion in the faeces. This may cause a shift in the intestinal membrane permeability and may account for the reported (Cayen and Dvornik, 1979; Morehouse *et al*., 1999; Sidhu *et al*., 1987; Southon *et al*., 1988) decrease in the intestinal absorption of carbohydrates and lipids which is beneficial in the management of diabetes. The observed increase in the excretion of cholesterol and phospholipids in the groups fed bitter yam sapogenin extract or commercial diosgenin are the results of their cholesterol inhibition in the intestine. Morehouse *et al*. (1999) in an earlier study attributed the increase in the excretion of faecal neutral sterols to saponin consumption.

In conclusion, the consumption of bitter yam sapogenin extract or commercial diosgenin has varied effects on mineral excretion. The supplements significantly increased faecal output and the significant increase in lipid excretion may partly account for the hypolipidaemic properties of saponins. Our data did not show any direct correlation between the decrease in excretion of mono-valent cations and the activity of intestinal Na^+/K^+ ATPase.

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DEVELOPMENT AND VALIDATION OF AN HPLC-UV METHOD FOR THE DETERMINATION OF GATIFLOXACIN IN BULK MATERIAL, PHARMACEUTICAL FORMULATIONS, HUMAN PLASMA AND METAL COMPLEXES

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ABSTRACT

A simple reversed phase HPLC method was developed for the quantitative determination of gatifloxacin (GTX) in the bulk material, pharmaceutical formulations and human serum using Mediterranea C18 (25 x 0.46mm, 5μm) column. The mobile phase, acetonitrile, methanol and water (40:40:20 v/v pH 2.7 adjusted by phosphoric acid), was delivered at a flow rate of 1.0 ml/min. The eluent was monitored using spectrophotometric detection at 286 nm. The method is specific to GTX and able to resolve the drug peak from formulation excipients and metal impurities. The method is accurate (99.18-101.87%), precise (intra-day variation 0.14-1.67% and inter-day variation 0.32-1.80%) and linear within the range 0.1-25μg/ml (R^2=0.999) concentration and was successfully used in monitoring left over drug in drug-metal complexes. The detection limit of GTX at a signal-to-noise ratio of 3 was 1.73 ng/ml in human plasma while quantification limit in human serum was 5.77 ng/ml. The proposed method is applicable to routine analysis of GTX in pharmaceutical formulations as well as in human plasma samples.

Keywords: Gatifloxacin; metals interaction; HPLC-UV analysis; magnesium; calcium; chromium; manganese; iron; cobalt; nickel; copper; zinc; cadmium.

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