

Possible Effect of Corn Silk Extracts on Selected Liver Markers and Plasma Glucose in Rabbit

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Abstract Corn silk contains phytochemicals of medical benefits such as proteins, vitamins, carbohydrates, Ca, K, Mg and Na salts, fixed and volatile oils, steroids such as sitosterol and stigmasterol, alkaloids, saponins, tannins, and flavonoids. Extract of corn silk is being applied traditionally in the treatment of some medical conditions. This study therefore, aimed at the evaluation of the effect of corn silk extract on liver markers and plasma glucose. Fifteen rabbits of either sex divided into three experimental groups of 5 rabbits each were studied. The extract of the corn silk was obtained using in methanol and water. The control group (A) with average weight of 758 g were not ingested with the extract throughout the period of study. Group B with average weight of 1040 g were ingested with the water extract for 3 weeks while group C with average weight of 984 g were ingested with the methanolic extract for 3 weeks. Plasma LDH, GGT and Glucose were estimated in the rabbits biochemically by spectrophotometry. The rabbits were well kept and placed on normal diet throughout the period of study. There was a significantly lower mean value of plasma glucose in the rabbits ingested with methanol corn silk extract compared with the control subjects after one week of administration ($p < 0.05$). There was also a significantly lower plasma glucose and a significantly higher LDH level in the rabbits administered with the methanolic extract than the given aqueous extract of corn silk ($p < 0.05$). The results obtained also showed a significantly lower plasma GGT in the rabbits administered with the aqueous and methanolic extract of corn silk than the results obtained in the rabbits studied as control ($p < 0.05$) after 3 weeks of administration. The result obtained showed a significantly lower mean value of glucose in the rabbit administered with the methanolic extract compared with those that were given aqueous extract for 4 weeks with $p < 0.05$. The administration of methanolic and aqueous extract of corn silk has an hypoglycaemic and hepatoprotective effects on the rabbits. The methanolic extract was also found to increase plasma LDH. These parameters should therefore be estimated in the patients undergoing treatment with corn silk extract for effective clinical management. The results obtained also show a significant increase in weights of rabbits administered with the aqueous and methanolic extract of corn silk than the results obtained in the rabbits studied as control ' $p < 0.005$ '.

Keywords: Corn silk, methanolic extract, aqueous extract, LDH, Glucose, GGT

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1. Introduction

Corn silk (*Zea mays* L.) refers to the stigmas from the female flowers of maize. Fresh corn silk resembles soft silk threads 10-20 cm long that are either light green or yellow-brown in color. Corn silk contains proteins, vitamins, carbohydrates, Ca, K, Mg and Na salts, fixed and volatile oils, steroids such as sitosterol and stigmasterol, alkaloids, saponins, tannins, and flavonoids [1]. There have been many reports on the biological activities of corn silk constituents. Methanol extracts of corn silk showed an antioxidative activity on the level of lipid peroxidation [2,3]. Volatiles from corn silk inhibited the growth of *Aspergillus flavus*, indicating that it has an antifungal activity [4].

Corn silk has been used in many parts of the world for the treatment of edema as well as for cystitis, gout, kidney stones, nephritis and prostatitis. Base on folk remedies,

corn silk has been used as an oral antidiabetic agent in China for decades. However, in spite of its widespread use, the mechanisms underlying hypoglycemic activity of corn silk was not yet understood. Diuretic and uricosuric effect corn silk have also been reported [5]. The herbal drug *Maydis stigma*, style of female flower of *Zea* has also being used for the treatment of variety of diseases such as in urinary tract diseases, gonorrhea, benign prostatic hyperplasia, hypertension etc [1,2]. Presently, there is scanty report of corn silk extract on the plasma level of lactate dehydrogenase, Gamma Glutamyl Transferase and glucose in the serum. This work was designed to determine the effect of corn (aqueous and methanol extract) on liver markers and plasma glucose.

2. Materials and Methods

2.1. Materials

2.1.1. Subjects

A total twenty (20) subjects were recruited in this study. Five subjects were served as methanolic test, five subjects as aqueous test, five subjects as control and five for emergency cases. The subjects were rabbits (*Oryctolagus cuniculus*) of about 5 weeks old.

2.1.2. Description of the Study Areas

Owo local government area lies on the Northern senatorial district of Ondo States, Nigeria within latitude 70100 N and longitude 70100 E, it is 150 m above sea level and enjoys abundant rainfall of over 1,500 mm annually, Plate 1 shows Owo Local Government Area in Ondo State.

2.1.3. Grouping of experimental animals:

In order to have fair representation of weight categories in all the treatment groups, rabbits in each weight categories were distributed into three groups each, such that, the number of rabbits in each group is equal with different weight.

2.1.4. Relabeling of Rabbits According to Treatment Groups

The rabbits were relabeled according to the treatment group tag in which they belong for example belly red group 2, fore head group 3 etc.

The rabbits were divided into three experimental group of 5 rabbits each.

Group A : five rabbits in which neither corn water extract or methanol extract was administered. This group serves as the control group.

Group B: five rabbits in which aqueous corn silk extract was administered everyday per Kg body weight.

Group C : five rabbits in which methanol extract of corn silk extract was administered everyday per kg body weight.

Blood samples was collected from the rabbits for estimation after one week and two weeks interval after administration of corn silk extract making a total of three weeks, and lactate dehydrogenase, gamma glutamyl transferase and glucose estimation was done for all the samples.

The extract was administered orally at a dose of 400 mg/kg/day for three weeks The control group was left untreated. Body weight was recorded at weekly interval.

2.2. Materials Used

2.2.1. Biological materials

Blood samples.

2.2.2. Reagents

Gamma Glutamyl Transferase, Lactate Dehydrogenase And Glucose Reagents Kits (Randox).

2.3. Methods

2.3.1. Method of Extraction of the Corn Silk

Soxhlet Extraction: Corn silk (dried cut stigmata of *Zea mays*. L.) used for this investigation were collected and were identified and authenticated and the voucher

specimen was dried at room temperature. The aqueous and methanol extract were prepared.

Principle: A solid material containing some of the desired compound is placed inside a thimble made from thick filter paper, which is loaded into the main chamber of the soxhlet extractor is now placed onto this flask. The solvent is heated to reflux. The solvent vapor travels up a distillation arm, and floods into the chamber housing the thimble of solid. The condenser ensures that any solvent vapour cools, and drips down into the chamber housing the solid material. The chamber containing the solid material is slowly filled with warm solvent. Some of the desired compound will then dissolve in the warm solvent. When the soxhlet chamber is almost full, the chamber is automatically emptied by a siphon side arm with the solvent running back down to the distillation flask. The thimble ensures that the solvent does not transport any solid material to the spill pot. This cycle may be allowed to repeat many times over hours or days. During each cycle, a portion of the non volatile compound dissolves in the solvent after many cycles the desired compound is concentrated in the distillation flask.

After the extraction the solvent was removed, typically by means of a rotary evaporator, yielding the extracted compound.

Apparatus: steam bath, clean bucket, electronic weighing balance.

2.3.2. Procedure

218.71 g of the air dried corn silk was weighed, and poured into a clean bucket.

About 7liters of distilled water with a temperature of about 40°C was poured into the bucket with the corn silk.

It was allowed to stay for 72 hours at room temperature.

It was then sieved and distributed into beakers.

The water content was evaporated using a steam bath.

The extract gotten was then weighed.

3. Collection of Blood Samples

Blood samples were collected after two weeks of acclimation. Blood was withdrawn through vein puncture from the earlobes of the rabbits using xylene to dilate the vein. Blood samples was collected into appropriate sample bottles, the blood was mixed, centrifuged, and the supernatant (plasma) was pipette out gently into plain bottles and was estimated instantly.

3.1. Measurement of the Weight of Rabbits

The weight of the rabbits were measured using a weighing balance before administration of extract.

Category one: five rabbits with average weight of 758 g

Category two: five rabbits with average weight of 1040 g

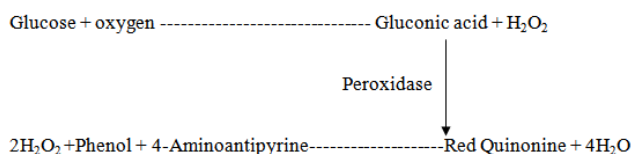
Category three: five rabbits with average weight of 984 g

3.2. Glucose Test

3.2.1. Principle of Glucose Test

Enzymatic colorimetric determination of glucose according to the following reaction.

Method Used: Glucose Oxidase



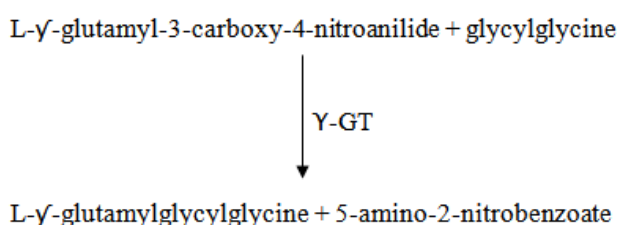
It was read spectrophotometrically at 500nm having the absorbance directly proportional to the concentration of glucose in the sample.

3.3. Determination of Plasma Activities Of GGT

METHOD USED: Colorimetric Method

3.3.1. Principle Of The Test (Colorimetric Method)

The substrate L- γ -glutamyl-3-carboxy-4-nitroanilide, in the presence of glycylglycine is converted by γ -GT in the sample to 5-amino-2-nitrobenzoate. The intensity of the colour complex formed was measured at 405nm.



3.4. Determination of plasma activities of lactate dehydrogenase

Method Used:Enzymatic Method

3.4.1. Principle of Lactate Dehydrogenase

Kinetic determination of lactate dehydrogenase according to the following reaction.



3.4.2. Statistical analysis

All data were analyzed by a one-way analysis of variance using the SPSS package, and the differences between means were established. The data represents

means and standard deviations. The significant level of 5% ($p < 0.05$) was used as the minimum acceptable probability for the difference between the means. The results were expressed as Mean \pm SEM and all procedures were performed at 95% confidence Interval level.

4. Results

The results showed no significant difference in the mean value of plasma LDH, GGT and Glucose obtained from the control group compared with the rabbits administered with the aqueous extract of corn silk for one week ($p > 0.05$). However, there was a significantly lower mean value of plasma glucose in the rabbits ingested with methanol corn silk extract compared with the control subjects after one week of administration ($p < 0.05$). There was also a significantly lower plasma glucose and a significantly higher LDH level in the rabbits administered with the methanolic extract than the given aqueous extract of corn silk ($p < 0.05$) (Table 1 and Table 4).

The results obtained showed a significantly lower plasma GGT in the rabbits administered with the aqueous and methanolic extract of corn silk than the results obtained in the rabbits studied as control ($p < 0.05$) after 3 weeks of administration. (Table 2 and Table 5). However there was no significant difference in the mean value of plasma LDH, GGT in the control compared with aqueous and methanol corn silk extract and also in the rabbits administered with aqueous extract compared with the rabbits administered with methanol extract for weeks three with $p > 0.05$ (Table 2 and Table 5). There was no significant difference in the plasma level of glucose obtained in the control compared with the rabbits administered with both aqueous and methanol extract ($p > 0.05$). The result obtained showed a significantly lower mean value of glucose in the rabbit administered with the aqueous extract compared with those that were given aqueous extract for 4 weeks with $p < 0.05$ (Table 2 and Table 5). The results obtained also show a significant increase in weights of rabbits administered with the aqueous and methanolic extract of corn silk than the results obtained in the rabbits studied as control $p < 0.05$ (Table 1 and Table 2).

Table 1. Below shows the effects of corn silk on GGT, LDH, GLUCOSE and WEIGHTS after one week of administration between test and control groups. (Mean \pm SEM)

SUBJECTS	Glucose (mmol/L)	GGT (IU/L)	LDH(IU/L)	WEIGHT (Grams)
Control Group A(Without Extract)	4.35 \pm 0.2	4.23 \pm 1.6	579.4 \pm 191.94	1013 \pm 100.8
Aqueous Extract Administration Group B	4.26 \pm 0.2	2.3 \pm 0.8	101.6 \pm 14.3	950 \pm 104.1
Methanolic Extract Administration Group C	3.4 \pm 0.2	1.5 \pm 0.2	260.5 \pm 52.05	984 \pm 89.76

Table 2. Below shows the effect of corn silk on GGT, LDH, GLUCOSE and WEIGHTS after three weeks of administration between tests and control. (Mean \pm SEM)

SUBJECTS	Glucose (mmol/L)	GGT (IU/L)	LDH (IU/L)	WEIGHT (Grams)
Control Group A (Without Extract)	3.9 \pm 0.4	3.5 \pm 0	366 \pm 220.68	1025 \pm 125
Aqueous Extract Administration Group B	3.0 \pm 0.2	1.3 \pm 0.1	378.7 \pm 87.4	1213 \pm 132.9
Methanolic Extract Administration Group C	3.5 \pm 0.05	1.5 \pm 0.2	274.1 \pm 66.6	1380 \pm 96.95

Table 3. Below shows the comparative illustration of the effect of corn silk extract (aqueous and methanol), on the weights of the rabbits before and after the extract administration

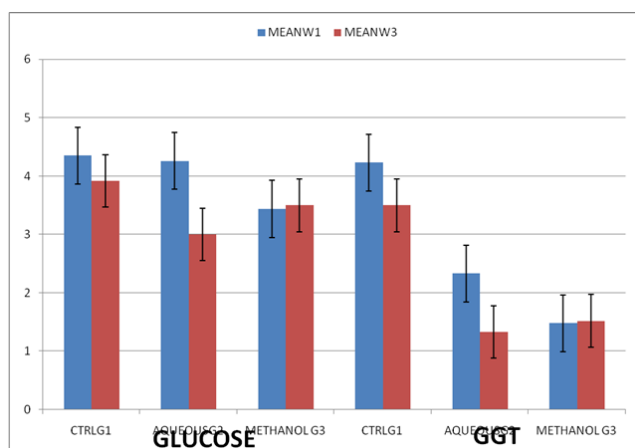
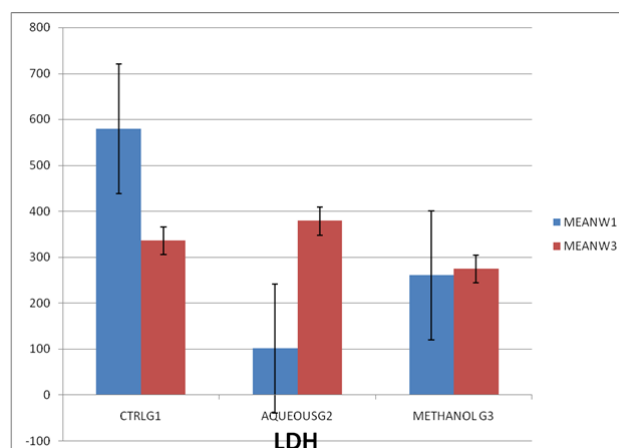
	CONTROL GROUP	AQUEOUS GROUP	METHANOL GROUP
T Value	0.078	1.550	2.997
P Value	0.470	0.085	0.009
Comment	Not Significant	Not Significant	Significant

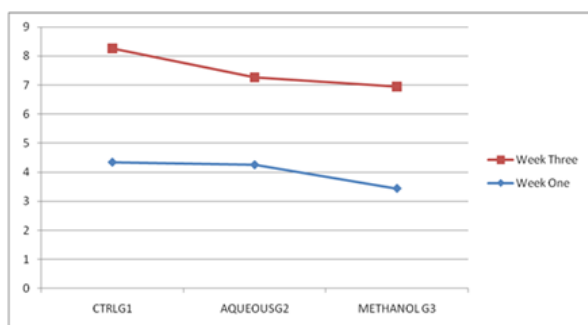
Table 4. Comparative analysis of the effect of corn silk extract on liver markers after one week of administration

WEEK ONE AFTER ADMINISTRATION		LDH (IU/L)	GGT (IU/L)	GLUCOSE (MMOL/L)
CONTROL VS AQUEOUS GROUP	T value	2.031	0.665	1.02
	P value	0.056	0.271	0.174
	Comment	Not significant	Not significant	Not significant
CONTROL VS METHANOLIC GROUP	T value	1.50	1.355	5.36
	P value	0.10	0.12	0.0009
	Comment	Not significant	Not significant	significant
AQUEOUS VS METHANOL GROUP				
	T value	2.33	0.847	2.29
	P value	0.040	0.215	0.026
	Comment	significant	Not significant	significant

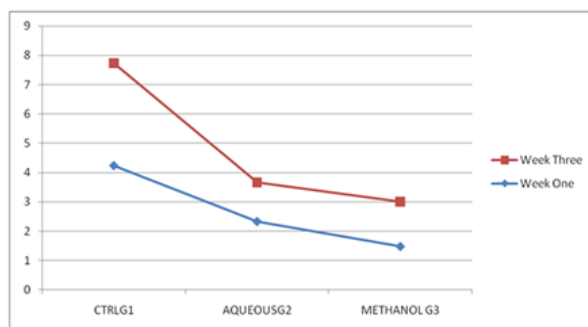
Table 5. Comparative analysis of the effect of corn silk extract on liver markers after three weeks of administration

THREE WEEKS AFTER CONTINUOUS ADMINISTRATION		LDH (IU/L)	GGT (IU/L)	GLUCOSE (MMOL/L)
CONTROL VS AQUEOUS GROUP	T value	0.227	12.8	2.067
	P value	0.416	0.0001	0.005
	Comment	Not Significant	significant	Not significant
CONTROL VS METHANOLIC GROUP	T value	0.746	5.56	1.17
	P value	0.249	0.003	0.15
	Comment	Not Significant	significant	Not significant
AQUEOUS VS METHANOLIC GROUP	T value	1.41	0.950	2.162
	P value	0.104	0.190	0.037
	Comment	Not significant	Not significant	significant

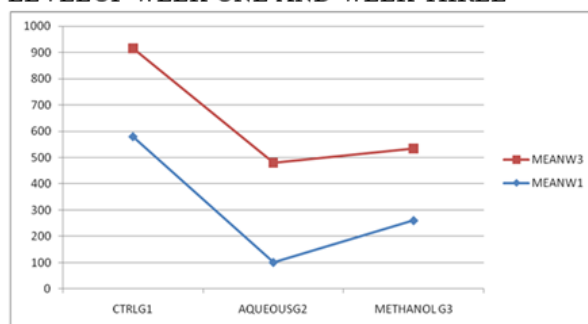
**Figure 1.****Figure 2.**



COMPARATIVE ILLUSTRATION OF THE GLUCOSE LEVEL OF WEEK ONE AND WEEK THREE



COMPARATIVE ILLUSTRATION OF THE GGT LEVEL OF WEEK ONE AND WEEK THREE



COMPARATIVE ILLUSTRATION OF LDH LEVEL OF WEEK ONE AND WEEK THREE

Figure 3.

5. Discussion

A significantly lower mean value of plasma glucose in the rabbits ingested with methanol corn silk extract compared with the control subjects after one week of administration implies that the extract may have an hypoglycaemic effect which is consistent with the reports of Ajali *et al.*, [6] that reported the corn silk extract phytochemicals with significant reduction in blood glucose and Jianyou *et al.*, [4] that found that the corn silk extract markedly reduced hyperglycemia in alloxan-induced diabetic mice. The action of corn silk extract on glycaemic metabolism is not via increasing glycogen and inhibiting gluconeogenesis but through increasing insulin level as well as recovering the injured β -cells. The results of Jianyou *et al.*, [4] suggest that corn silk extract may be used as a hypoglycemic food or medicine for hyperglycemic people in terms of this modern pharmacological study. There was also a significantly lower plasma glucose and a significantly higher LDH level in the rabbits administered with the methanolic extract than the given aqueous extract of corn silk. The

result obtained showed a significantly lower mean value of glucose in the rabbit administered with the methanolic extract compared with those that were given aqueous extract for 3 weeks. The significant difference in the values of glucose and LDH in the rabbits ingested with aqueous and methanolic extract of corn silk could be attributed to the hypoglycaemic effect of alcohol used for the extraction of the extract along with the aqueous extract which was revealed by a significant decrease in glucose level. Alcohol could also cause tissue and cell injury which could raise the LDH plasma level found in rabbits fed with methanolic extract [7]. Ajali *et al.*, [6] also reported reduction in the blood sugar levels of normal and alloxan induced diabetic rats following the administration of methanolic extract of corn silk.

The results obtained showed a significantly lower plasma GGT in the rabbits administered with the aqueous and methanol extract of corn silk than the results obtained in the rabbits studied as control after 3 weeks of administration. This result agrees with the previous findings of Kingsley *et al.*, [8] that reported a significant decrease plasma in ALT, AST, GGT and MDA, and an increase in the activity of SOD and CAT relative to the positive control due to phytochemical constituents of *Zapoteca portoricensis* almost similar to those in corn silk which include proteins, vitamins, carbohydrates, Ca, K, Mg and Na salts, fixed and volatile oils, steroids such as sitosterol and stigmasterol, alkaloids, saponins, tannins, and flavonoids.

The presence of the identified phytochemicals in the extracts partly explains the medicinal applications of corn silk extract. The hepatoprotective potential of this extract may not be unrelated to the antioxidant property of the phytoconstituents [8]. The results obtained also showed a significant increase in the weight of rabbits studied as control. This could be attributed to the nutritive values of the phytochemicals in the methanolic extract of corn silk and the effectiveness of methanol in extracting these phytoconstituents [6].

6. Conclusion

The administration of aqueous and methanolic extract containing proteins, vitamins, carbohydrates, Ca, K, Mg and Na salts, fixed and volatile oils, steroids such as sitosterol and stigma sterol, alkaloids, saponins, tannins, and flavonoids for one to three weeks were found to increase LDH (methanolic extract) and a reduction in blood glucose and GGT due to hepato-protective effects of the phyto-constituents.

Recommendation

Routine estimation of plasma glucose, LDH and GGT in patients undergoing treatment using extract of corn silk is hereby recommended.

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