Therapeutic Effects of Fermented and Dried Corn Silk (*Zea mays* L.) on Induced Hyperuricemia and Hepatic Cell Injury in Wrister Rats

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Abstract

A rare investigation has been made into the prevailing Hyperuricemia rate in developing countries. In Hyperuricemia (inclined serum uric acid) monosodium urate crystals deposit in joints and tissues. The goal of this research was to access the therapeutic effects of fermented corn silk tea and corn silk powder on hyperuricemia and their action to reduce hepatotoxicity in vivo, 80% ethanol extraction of fermented corn silk alleviated serum uric acid levels in an induced hyperuricemia Wrister rat model by Gentamycin. The efficacy study was for 45 days, containing 30 animals (male and female rats) divided into 6 groups (5 Wister rats in each group) with an average weight of 180 g/kg. G0 and G01 were control groups, groups 1 or 2 were induced and groups 3 and 4 were without induced hyperuricemia. Group 1 (induced hyperuricemia) and Group 3 (without induction) were given the extract of fermented corn silk tea and group 2 and 4 were given the corn silk powder in feed. In the hyperuricemia-induced group, the mean value of uric acid, creatinine, Alanine transaminase, and Aspartate transaminase showed a significant decrease for those who were treated with fermented corn silk tea. Dried corn silk powder showed mixed results of the mean value of uric acid, creatinine, Alanine transaminase, and Aspartate transaminase. Fermented corn silk had the best effect in reducing the raised serum uric acid. The results manifested a remarkable ($p<0.05$) decrease in serum uric acid, creatinine, Alanine transaminase, and Aspartate transaminase levels. The findings suggest that using fermented corn silk could be an effective strategy to alleviate hyperuricemia in the population predisposed to the development of gout and kidney stones in later stages and can lower the aggregated levels of liver cell injury.

Keywords

Corn Silk, Creatinine, Hyperuricemia, Fermentation, Hepatocytic Injury

1. Introduction

The therapeutic effects of corn silk for treating various afflictions proved beneficial in ancient times in China, Africa, and several other countries. Corn silk (*Stigma maydis*) is an inexpensive plant component that is usually dissipated during corn cultivation. Carbohydrates, lipids, proteins, minerals, vitamins, volatile oils, and several other potentially bioactive compounds like flavon, phenolic compounds, and high antioxidant activity are present in corn silk. The first classic medicinal use of corn silk was recorded back during the Ming Dynasty, a Chinese physician named Lan Mao, wrote a book on the *Materia Medica of South Yunnan* (1397-1470). Sweet corn silk is non-toxic. Its wonders are tracked down in "The Dictionary of Medicinal Plants". In traditional Chinese medicine (TCM) theory, corn silk is considered to be an effective diuretic, helping in alleviating the syndrome of internal stagnation of fluid dampness, having atherapeutic effect in treating diabetes, cancer, cardiovascular diseases, microbiological infections and several other cited literature on corn silk affirms that it exhibits properties of anti-hypertensive, anti-inflammatory, reducing obesity and edema [1].

In humans, during purine metabolism, purine nucleotides undergo an oxidation process and after a series of reactions, they convert into hypoxanthine and xanthine, uric acid (UA). Xanthine oxidase (XO) is responsible for the production of these molecules. Alleviated serum uric acid levels, more than 6.8 mg/dl are considered hyperuricemia (HUA). A gradual and consistent buildup of uric acid over a few decades in the joint vicinity of the human body leads to the chronic condition of hyperuricemia resulting in the condition of gout and several other diseases. In previous research, corn silk showed a therapeutic effect on induced liver fat and lipid profile in rats [2]. They found significant results by using corn silk extract in rats, the hepatoprotective effects of corn silk were investigated. Hydro-alcoholic extract of corn silk was evaluated. Histopathological changes were seen when the subjects were treated with 100 mg/kg doses of corn silk.

The effects of Arabic gum and corn silk on nephron-hepatic toxicity induced by Gentamycin in experimental rats were investigated. The results demonstrated the effective use of Arabic gum and corn silk as their combination is an excellent source of antioxidants that destroys free radicals during the in-vivo study.

Considering the literature review of corn silk's therapeutic effects, in the current study corn silk was fermented, or dried (two different treatments), and then fermented corn silk extract was prepared and dried form of corn silk was mixed with the feed. The effects of fermented corn silk and CS dried powder were evaluated by analyzing the

values of serum uric acid, creatinine, SGPT, and SGOT. This study was designed to ascertain corn silk's effectiveness in hyperuricemia and its efficacy in lowering the values of liver biomarkers due to its high profile of phenolic, flavonoids, and antioxidant content [3].

2. Materials and Methods

2.1 Corn Silk Procurement and Preparation of Fermented and Dried Corn Silk

Fresh corn silk, from the local market of Faisalabad, was collected. After cleaning, corn silk was withered for 12-16 hours, and then mechanical maceration was applied with gentle hands. At low-temperature silk was pre-conditioned until the corn silk turned brown from yellow color during processing [4]. After thorough drying, fermented corn silk was packed, and its extract was prepared with 80% ethanol to be used in a clinical rat trial (1.3 g/kg). After sorting and cleaning, corn silk was dried under sunshade for 12 to 14 hours. Sun dried corn silk was milled and filtered using sieve of the size 0.075 mm in diameter. Teabags of dried corn silk were prepared which later were used in Wrister rat feed. Figure 1 explains the flowchart of experiment.

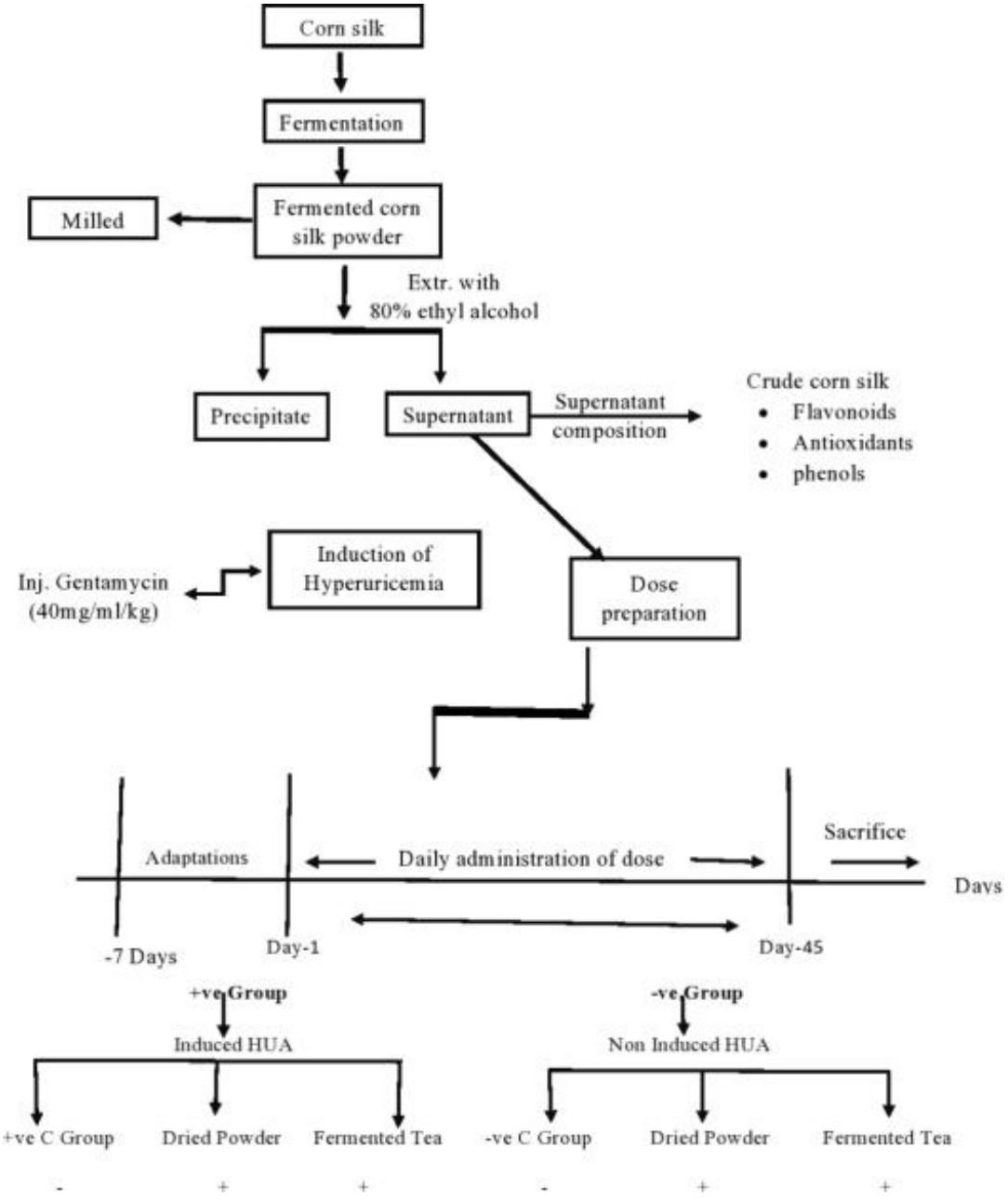

Figure 1. Animal experiment design for the effect of fermented corn silk on hyperuricemia (HUA)

2.2 Preparation of Extract of Corn Silk

The fermented corn silk extract was made against 80% ethanol by maceration process for at least 3 and a maximum of 8 days at room temperature. The sample was stirred intermittently and then with Whatman paper, was filtered out.

The sample was dried overnight, ethanol evaporated from the sample and remaining plant extract was collected from a petri dish and kept safe in an opaque plastic test tube which later was used for experimental purposes in the rat feed [5].

2.3 Analysis of Corn Silk Chemical Composition

The procedure of AOAC 2008 and AACC 2010 were utilized to ascertain the protein level, fats, ash, crude fiber, moisture, and total carbohydrates. Below is given the used formula of difference.

$$Total\ carbohydrates = 100 - (weight\ in\ grams\ [moisture + fat + protein + ash + fiber]$$

2.4 Analysis of Corn Silk Mineral Contents

Atomic absorption spectrophotometer (Hitachi polarized Zeeman AAS, Z-8200, Japan) was used in the determination of the values of Ca, Mg, Fe, and Zn in corn silk samples using the procedural protocols of AACC (2010).

2.5 DPPH Scavenging Activity of Corn Silk

The content of DPPH scavenging activity in corn silk crude extract was determined by the method with little modifications [6]. Crushed corn silk powder was filtered using a 100-mesh sieve. For the DPPH scavenging activity, 0.004% DPPH was mixed with 3 mL corn silk extract in methanol solution at varying concentrations, and mixture was kept in the dark area for 30 minutes. Absorbance for the scavenging activity of corn silk was noted at 517nm on atomic absorption spectrophotometer. A control sample without corn silk extract was analyzed as well. The test was carried out three times for accurate results [7].

The percentage calculation of inhibition of DPPH radical:

$$DPPH\ Inhibition\ (\%) = \frac{Absorbance\ of\ blank - absorbance\ of sample}{Absorbance\ of\ blank} \times 100$$

2.6 Total Flavonoids Content (TFC)

For this analysis 2 mL distilled water was, 0.5 mL corn silk extract, 0.15 mL of 5% $NaNO_2$ solution was mixed then the sample was incubated for 6 minutes, and after that 4% NaOH was also added to the solution [8]. Methanol was added up to the volume of 5 mL. by adding methanol. After mixing the mixture, the absorbance was assessed at 510nm.

2.7 Total Phenolic Content (TPC)

In a test tube, a sample of corn silk extract of 200 µL was added in test tubes; mixed the sample well with 1.0 mL of Folin-Ciocalteu's reagent and 0.8 mL of sodium carbonate (7.5%) in test tube and placed to rest for 30 minutes. The spectrophotometer was used to access the content of the extract at 765 nm, absorption was measured through a spectrophotometer [9].

2.8 Efficacy Study in Hyperuricemia Rats

Table 1. The groups of biological experiments

G0	Negative control (diet)
G01	Positive control (Gentamycin* +diet)
Group 1	HUA induced + fermented corn silk extract +feed
Group 2	HUA induced + dried corn silk powder in feed
Group 3	Non-induced HUA + fermented corn silk extract + feed
Group 4	Non-induced HUA + dried corn silk powder in feed

*Gentamycin at dose rate of 40mg/kg body weight given interaparitonialy foe one week to induce kidney injury i.e. hyperuricemia for group 1 and 2.

Table 1 mention Animal house of the Government College University Faisalabad, Pakistan provided 30adult Wrister rats (both male and female), weighing about 170 ± 5g. For one week, animals were fed on the normal basal diet and water to adapt before starting the experiment. The rat model of hyperuricemia was prepared through induction of Gentamycin injection (Punjab pharmacy, Faisalabad, Pakistan) via the intraperitoneal route. Ethical approval for animal study was obtained from the Office of Research, Innovation and Commercialization of University

of Agriculture, Faisalabad (ORIC-UAF) [10]. An administered dose rate was 40 mg/mL/kg for a week. 30 animals were divided into 6 groups (n=5 each): positive and negative control G0 and G01, groups 1 or 2 (induced HUA), and groups 3 and 4 (Non-Induced HUA) for experimentation. After inducing the HUA, the HUA-induced group was kept on rest for a week before the start of the therapeutic trial. Extract of fermented corn silk was given to Group 1 (induced HUA) & group 3 (non-Induced HUA) and Group 2 (induced HUA) and 4 (non-induced HUA) were fed on feed mixed with corn silk powder. The trial duration was 45 days. The volume of treatment was

set according to the weight of the rats. The weight of the rats was measured every week. The blood sample drawn on day-0, day- day-20 and day 45 was used for laboratory analysis. Detection kits for uric acid, creatinine, SGPT, and SGOT were provided by the Mubasher Laboratory, Faisakabad, Pakistan [11]. All the materials and chemicals used for the experiment were analytical and of HPLC grade. When the trial ended, the rats were sacrificed to collect the sample for further laboratory tests (uric acid, creatinine, SGPT, and SGOT) and analysis.

2.9 Statistical Analysis

Collected data of the research, had been subjected to the analysis of findings to the SPSS computer software. A two-way analysis of variance (ANOVA) was used to access the data significance statistically. Values of $P<0.5$ were considered statistically significant. Graphical representation of data was done using a box plot treatment. QQ plots were developed to check the normality of data, all data was analyzed under the confidence interval of 95% [12].

3. Results and Discussion

3.1 Chemical and Compositional Analysis of Fresh Corn Silk

Table 2. Analysis of fresh corn silk chemical composition and nutritional value (n=3)

Parameters	Fresh corn silk %
Moisture	75.33±0.01
Protein	2.95±0.16
Fat	1.26±0.15
Ash	5.1±0.10
Fiber	6.12±0.21
Carbohydrates	9.24±0.13
Mineral analysis of corn silk powder	
Ca	2950±5.60 mg/100g
Mg	1760±4.08 mg/100g
Fe	36.274±0.05 mg/100g
Zn	7.4594±0.05 mg/100g

In t able 2, the chemical compositional analysis of fresh corn silk shows the lowest moisture content, making it ideal for storage, extending its shelf life, and easy to use, protein, fat, ash, crude fiber, carbohydrates [13]. When compared with the other contents of corn silk, a significant amount of carbohydrates constitutes most of the part in its composition. In another study, it's chemical composition was investigated and the amount of Moisture 4.33±0.0717 in dried corn silk, Protein value 13.0±1.0031, lipids value 1.13±0.0815, crude fiber value 21.01±1.0210, ash value 6.43±0.0512 and carbohydrates value 54.11±0.6341% was noted. In another study, investigated the amount of carbohydrates was 51.37g/100g, protein 17.94g/100g, crude fiber 16.11g/100g, moisture 9.06g/100g, ash 4.60g/100g, and fat 0.91g/100g. Potassium and sodium content of mature corn silk was 35671.67, and 266.67 l g/g, respectively [14].

In table 2, Major elements i.e., the mineral content of corn silk like Ca, Mg, and minor elements such as Fe, and Zn were measured. A study by (Rahman & Wan Rosli) showed that corn silk is high in minerals like value of Calcium 1087.08±105 µg/g, Magnesium content was 1219.17 ± 143.07 µg/g, potassium was 26281.67 ± 1379.7 µg/g, and sodium was 190.67 ± 22.61 µg/g. In another study, recorded amount of minerals content was (Zinc 2.066 ± 0.0577 µg/g, Mangnese, 1.65±0.0371, iron 1.93 ± 0.04567 µg/g and Copper 45.6±0.1346 µg/g, Calcium 877.30±0.3340 µg/g, Magnesium 711.00±1.0112 µg/g, sodium 216.44±0.1210 µg/g and potassium 30262±1.0208 µg/g of both macro or micro mineral in dried corn silk [15].

Table 3. Analysis of TPC, TFC and antioxidant activity of corn silk (n=3)

Parameters	Value	Absorbance
TPC	77.6 mg GAE/g	0.486
TFC	120.568 mg RE/g	0.487
DPPH	74.8186 (inhibition)	0.694

In table 3, findings showed the detected values of total polyphenols, total flavonoids, and DPPH scavenging activity of antioxidants in the extract of corn silk. This data was similar to the findings of (El Kewawy) the TFC and TPC contents in CS ranging from 80.8 mg GAE/g to 117.1 mg GAE/g or 30.1 mg RE/g to 88.8 mg RE/g respectively [16]. The antioxidant activity possessed by corn silk (ethanol extract) which was determined by DPPH Radical-Scavenging activity, noted to be 85.301±0.1210.

3.2 Effects of Fermented and Dried Corn Silk on Hyperuricemia and Kidney Markers and Liver Injury

Abnormal upregulation of serum uric acid results in hyperuricemia and is documented to be the prominent indicator of induced hyperuricemia in rat models. Levels of serum uric acid were measured (in vivo) to investigate the hypouricemic effects of dried and fermented corn silk. In this study, a rat model of hyperuricemia was developed using gentamycin, owing to its effect on renal and hepatic injury. After completing the trial of 45 days, a blood sample was taken for testing, and values of uric acid, creatinine, alanine transaminase (SGPT), and aspartate transaminase (SGOT) were measured. A significant reduction in kidney markers (uric acid and creatinine) was noticed.

Table 4. Therapeutic effects of fermented corn silk extract or dried corn silk powder on kidney markers (uric acid and creatinine) and liver markers (SGPT, SGOT) in Wrister rats

Treatment	Uric acid	Creatinine	SGPT	SGOT
-ve	2.22 ± 0.055	0.44 ± 0.055	50.61 ± 1.14	43.2 ± 2.59
+ve	2.96 ± 0.055	1.64 ± 0.055	64.2 ± 1.48	36.6 ± 1.14
Dried (I)	2.72 ± 0.084	1.56 ± 0.084	53.8 ± 1.30	36.2 ± 1.92
Fermented	2.42 ± 0.084	1.1 ± 0.1	44.2 ± 1.30	29 ± 1.73
Dried (N-I)	2.12 ± 0.084	0.56 ± 0.089	49 ± 1.23	40.6 ± 2.88
Fermented	2.04 ± 0.089	0.36 ± 0.055	46.8 ± 1.10	49.8 ± 1.30

Table 4 shows the significant reduction ($P < 0.05$) in uric acid (2.42±0.084) in the induced rat model (positive group) and creatinine (1.1±0.1) when treated with treatment-2 (fermented corn silk) compared to the treatment-1 (2.72±0.84), (1.56±0.084) respectively, which is dried corn silk mixed with their feed. Creatinine and uric acid were assessed to measure the level of toxicity caused by gentamicin in the positive (induced) group [17]. Gentamycin 40mg/ml/kg was given to cause renal damage and noted by the values of creatinine, uric acid, reduced hypo cellularity of kidney structure; glomeruli, presence of a substantial tubular cat, moderate brush border loss [18].

Being a powerhouse of diuretics, corn silk helps in reducing the kidney problems and possesses anti-inflammatory properties [19]. When compared with the control group and dried corn silk group, subjects who were treated with fermented corn silk extract showed low levels of uric acid and creatinine. In another study, investigated the role of polysaccharides of corn silk in reducing kidney injuries and also studied the effect on promoting the excretion of serum uric acid [20].

Kidney damaging compounds could be inhaled, injected, and may be consumed which damages the structural and functional role of kidneys, making them unable to function. Studied the effectiveness of corn silk and used more than 500 mg/kg corn silk to reduce nephrotoxicity. In another study, used a rat model for the study of nephrotoxicity. The study showed the effectiveness of the corn silk methanol extract in reducing urea (35.15 2.29) and creatinine (0.38 0.45) [21].

The study of Amjad also showed the highly significant results of corn silk in reducing serum urea and creatinine which highly correlates with the findings of this study which demonstrate the medicinal effects of fermented corn silk to reduce the uric acid and serum creatinine level in a rat model of hyperuricemia caused by the toxicity of gentamycin [22].

3.3 Effects of Fermented and Dried Corn Silk Liver Enzymes (SGPT, SGOT)

The therapeutic effects of fermented corn silk or dried corn silk against hepatic enzymes (SGPT, SGOT) on the gentamycin-induced rat model were checked and results are given in table 4. Rats were treated with two treatments, corn silk powder and extract of fermented corn silk to lower the liver enzymes showed significant reducing effects on serum SGPT, SGOT (44.2 ± 1.30, 29 ± 1.73) respectively with fermented corn silk and (53.8 ± 1.30, 36.2 ± 1.92) respectively with dried corn silk. Treatment with extract of fermented corn silk showed more prominent results when compared with dried corn silk powder or the control group. Corn silk is a potent source of flavonoids with high antioxidant properties that help prevent liver cells from damage and help in improving liver functions [23]. Another study by investigated the effect of corn silk on glucose level improvement, kidney functions and liver functions in a rat model. They used higher concentrations of corn silk to assess the increased therapeutic effects. The higher concentrations of phytochemical content which are potent antioxidants, play a great role in hepatoprotective activities in wrister rats. Corn silk was reported to reduce the levels of serum Alkaline phosphatase (18.74%) [24].

According to the findings of (Thirugnanasampandan) only 0.36g fat was found in the composition of corn silk which makes it more suitable to be used to treat liver illnesses. worked on the corn silk and suggested that whether it is water extract or ethanol extract of corn silk, it helps to treat kidney and liver biomarkers due to its high profile of antioxidant agents (phenols), vitamins, and minerals [25]. In another study, the effect of both corn silk and Arabic gum, in different concentrations, was assessed to analyze their impact on renal and hepatic toxicity induced by gentamycin 80mg/kg for one week. A significant reduction in kidney markers and liver enzymes was noticed [26].

4. Conclusion

In the study, the therapeutic or medicinal effects of fermented and dried corn silk were assessed on hyperuricemia and its effect on liver enzymes. The study indicated the high concentration of phytonutrients, highly active antioxidants, presence of macro, and micro minerals in corn silk extract. The corn silk effects in reducing gentamycin-induced hyperuricemia and liver cell injury were investigated. Both the fermented and dried corn silk alleviated the raised levels of serum uric acid and hepatic enzymes, but the results of fermented corn silk were more prominent. Analysis of corn silk indicated the high profile of phenols, antioxidants, and flavonoids which not only exhibit the anti hyperuricemic effects but also have liver enzyme lowering capacity preventing liver cell injury.

Data Availability Statement

All the derived data supporting the findings of this study are used in this manuscript.

Conflicts of Interest

The authors declare that they have no conflict of interest.

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Animal Study

Institutional Biosafety and Bioethics Committee, the human efficacy trial was carried out in the NIFSAT, UAF and local community of Faisalabad with the approval of the Institutional Biosafety and Bioethics Committee (D#1503/ORIC) UAF, Pakistan.

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