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Effect of Different Levels of Sorghum Powder on Rats Inflected with Hepatotoxicity

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ABSTRACT

Background: Liver is an important organ in the body as it secrets bile, stores fat-soluble vitamins and handles cholesterol hemostasis. Cereal crops such as sorghum seeds serve as a rich source of dietary fiber and nutrients. These seeds may protect against hepatotoxicity.

Aim: The purpose of this research was to identify the phytochemical components of sorghum powder and evaluate its protective effects against carbon tetrachloride (CCl4)-induced hepatotoxicity in rats.

Materials and methods: Forty-eight adult male albino rats were distributed into six groups (n=8). Group (1) was control (non-treated) and group (2) was injected subcutaneously by carbon tetrachloride CCl4 (2 ml/kg) twice a week for 2 consecutive weeks to induce hepatic toxicity Groups (3), (4), (5) and (6) were administered CCl4 plus 2.5%, 5%, 7.5% & 10% of sorghum powder, respectively. After 12 hours of fasting, blood samples were taken for biochemical examination from the rats in the experiment. Antioxidant activity, ALT and AST levels, lipid profile (HDL-c, LDL-c & VLDL-c), uric acid, and urea nitrogen were all measured with serum samples from sacrificed rats.

Results: Sorghum seeds powder contains a lot of minerals, fatty acids and many bioactive compounds. It has a high antioxidant activity, hepatoprotective, nephroprotective and hypochlosterolemic effects. Sorghum seeds powder ameliorated the histopathological lesion seen in the liver of hepatotoxic male albino rats.

Conclusion: The findings indicated that sorghum powder contains carbohydrates, and many bioactive constituents such as phenolic compounds, carotenoids, chlorophyll, tannins, phytates and dilatory fibers. Sorghum powder decreased body weight gain, increased antioxidant activity but decreased liver enzyme ALT and AST, TC, LDL-c & VLDL-c, while increased HDL-c. Sorghum seeds powder is considered a promising ameliorative agent against CCL4-induced hepatotoxicity in rats. Therefore, sorghum powder may be beneficial for patients suffering from liver toxicity.

Keywords: Sorghum powder- Hepatotoxicity- Phytochemical analysis- Biochemical parameters- Histopathology.

INTRODUCTION:

Liver disease is the leading cause of mortality annually. About 29 million individuals suffer from chronic liver disease and liver disease affects over 30 million people in the USA [(1)].

In England, liver disease ranks fifth in terms of mortality rates, behind cancer, stroke, and respiratory illnesses. Chronic hepatitis B and C, alcohol, and non-alcoholic steatohepatitis related to obesity and metabolic syndrome are among the most prevalent causes of liver disease globally [(2)].

As the country with the highest hepatitis C prevalence worldwide, Egypt will soon see the epidemic's apex. More than half a million people are anticipated to become infected with the virus every year, according to research conducted in 2010. The Egyptian Ministry of Health and Population reports that each year 100,000 Egyptians contract HIV. According to research, Egypt has the highest rate of hepatitis C virus infection in the world, with a prevalence that is ten times higher than in Europe and the USA [(1)].

In India, approximately 10 people are diagnosed with liver cirrhosis each year. The World Health Organization indicates that liver disease is the 10th leading reason for death in India. Every fifth Indian suffers from some form of liver illness. More people die from liver illness than from either diabetes or automobile accidents. Until the disease has progressed and caused significant damage to the liver, there are typically no outward manifestations of liver disease.

There are a variety of liver function tests available to assess the liver's health [(1)].

The liver is the 2nd largest organ in the human body and is responsible for over 5,000 distinct bodily functions. Some of these functions include clotting blood, removing toxins from the blood, converting food into nutrients, controlling hormone levels, warding off infections and illnesses, regenerating after injury, and metabolizing cholesterol, glucose, and iron while also regulating their levels. Most people rarely think about their livers until something goes wrong with them, despite the fact that liver diseases are on the rise and currently affect one out of every ten individuals. Liver illnesses can be hereditary, or they can be caused by a range of conditions that are harmful to the liver.

Sorghum is a cereal that belongs to the Poaceae or Gramineae grass family and was first domesticated among 3,700 & 4,000 years ago [(3)].

It is endemic to northern Africa. After maize, wheat, rice, and barley in terms of cereal crop production in 2017, sorghum ranked fifth with 57.6 million tons produced each year [(4)].

Sorghum is one of the most important cereal crops grown all over the world.

In both the tropics and subtropics, sorghum has become a popular crop. Sorghum is one of the key crops used to manufacture meals like bread and porridge in several under-developed and semiarid parts of Asia and

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Africa, involving India and Nigeria. It provides most of the food and fuel that humans need to survive [3].

Western countries involving the USA, Mexico, and Australia cultivate sorghum for its utilization in animal feed. But due to the natural components present in sorghum, which are excellent for the creation of nutritious and functional meals, there is a growing interest in farming sorghum for biofuel production and food for human consumption [5].

Sorghum is one of the world's most essential but underutilized food crops. The phenolic chemicals and minerals found in sorghum grain are helpful to human health. Sorghum is unlike any other cereal grain in terms of its phenolic profile, which is both more plentiful and diversified. Sorghum's phenolic components are made up mostly of phenolic acids, 3-deoxyanthocyanidins, and condensed tannins. Consuming sorghum whole grain may boost digestive health and lower the chance of developing chronic illnesses, as research has revealed that its phenolic components have powerful antioxidant action in vitro. Sorghum grain has been employed in the creation of functional meals and drinks and as a component in other foods in recent years. As a bonus, the phenolic compounds, 3-deoxyanthocyanidins, and condensed tannins may be extracted and used as a safe, natural, and versatile food ingredient [6].

MATERIALS AND METHODS

Materials

Plant Material

Sorghum is a very old cereal crop. Sorghum was first documented in written form around 8000 B.C. at an archaeological site in northeastern Africa, close to the modern-day boundary between Egypt and Sudan. Sorghum's rapid growth across Africa can be attributed to the fact that it thrives in both humid and dry environments. India is the world's second-largest sorghum producer. The Agriculture Research Center in Giza, Egypt, was where we got our sorghum.

Experimental Animals: Forty-eight adult male albino rats (Sprague Dawley strain) weighting about 120±10 g was purchased from the Animal House of National Research Center, Dokki, Egypt.

Chemicals: El-Gomhoriya Pharm. and Chem. Ind. Co. Cairo, Egypt supplied casein, cellulose, sucrose, choline chloride, D-L methionine vitamin and mineral components.

Methods

Preparation of Plant Material: Sorghum was cleaned then crushed and subjected to chemical analysis (carbohydrates, protein, fatty acids, vitamins and minerals) at National Research Center, Giza, Egypt.

Induction of Hepatotoxicity: After the rats had had enough time to acclimate to the basal diet, they were split up into six separate groups of similar size. The 1st group was given merely a baseline diet in order to serve as a control group, and the second group of rats (n=40) were poisoned by receiving a subcutaneous injection of CCl4 in paraffin oil (1:1 v/v; 2 ml/kg) twice a week for 2 weeks in a row in order to produce liver toxicity [7]. The 2nd group consumed their normal food as a form of positive control. The 3rd group was fed on a basal diet with addition of sorghum powder at 2.5 %. The 4th, 5th and 6th groups were fed basal diet with addition of sorghum powder at 5%, 7.5% and10%, respectively.
The first group is a control group and the second group was injected subcutaneously by carbon tetrachloride (positive control). The 3rd group was fed on a basal diet with addition of sorghum powder at 2.5 %. The 4th, 5th and 6th groups were fed basal diet with addition of sorghum powder at 5%, 7.5% and10%, respectively.

Experimental Design: The animals were kept in clean, well-ventilated cages as part of their housing arrangement. They were provided with a standard casein basal diet in accordance with **Reeves *et al.*** [8], permitted access to tap water on an unrestricted basis, and allowed to acclimate for a period of two weeks prior to their use in the experiment. Following the time of adaption, the animals were categorized into the following six groups, each containing 8 individuals: Group 1: control group, was fed on basal diet only throughout the experimental period. Group 2: control group, rats were fed on basal diet and intoxicated by CCL4. Group 3: Rats were fed basal diet plus 2.5% sorghum powder. Group 4: Rats were fed basal diet plus 5% sorghum powder. Group 5: Rats were fed basal diet plus 7.5 % sorghum powder. Group 6: Rats were fed basal diet plus 10 % sorghum powder.

Biological Evaluation: The animals' food intake (FI) was recorded daily, and they were weighed at the outset of the investigation and two times per week throughout the duration of the research. The body weight gain percentage (BWG %) and the Food Efficiency ratio (FER).

Blood Collection and Serum Separation: Blood samples were withdrawn from each fasted animal and the collected blood samples were centrifuged to obtain the serum for biochemical analysis at National Research Center, Dokki, Egypt.

Biochemical Analysis:

Liver Function: Serum alanine aminotransferase (ALT) levels were measured utilizing the technique described by **Reitman and Frankel,** [9] For this study, we utilized the technique developed by **Ritman and Frankel.** [9] to measure AST levels in serum.

Lipid Profile: Determination of blood total cholesterol (TC) according to **Allain *et al.*** [10] High Density Lipoprotein (HDL) was determined consistent with the method described by **Lopes – Virella *et al.*** [11] Low

Density Lipoprotein (LDL) was estimated, and LDL was measured with respect to **Fiedwald *et al.*** [12] Very low-density lipoprotein (VLDL) was measured consistent with **Fiedwald *et al.*** [12]

Kidney Function: Uric Acid was determined in serum in accordance method describe by **Fossati and Principe,** [13] Urea nitrogen was determined in serum consistent.

Technological Studies: Sorghum was utilized in different product (Pan Bread) and was subjected to panel test at Faculty of Home Economics, Helwan University.

Statistical Analysis

The result that was obtained was provided as a mean ± SD. To analyze the data, a one-way analysis of variance (ANOVA) was performed on them with the statistical software SPSS. According to the findings of **levesque,** [14], the level of significance for the mean variance was ($p < 0.05$).

Ethical Approval: The research project was given approval by the Ethics Board of Helwan University, and the participants in the trial were provided with all the information they need regarding the experiment. Each person who took part in the research was required to give their informed consent in writing. This research was carried out on humans in compliance with The Code of Ethics of the World Medical Association, also known as the Declaration of Helsinki for research involving people.

RESULTS

Chemical Composition of Sorghum Seeds Powder

In the study sorghum seeds powder were analyzed for its content of protein, fatty acid, vitamins minerals and fiber as shown in table (1) The data show that water was 9.54%, protein was 11.35%, fatty acid was 3.40%, fiber 1.42%, ash 1.53% and carbohydrates 72.70% (Table 1).

Table (1): Chemical composition of sorghum seeds powder g/ 100

Chemical Composition (%)	Mean ± SD (g/100g)
Water	9.54± 0.63
Protein	11.35± 0.65
Fat	3.40± 0.51
Fiber	1.42± 0.15
Ash	1.53± 0.13
Carbohydrates	72.70± 0.48

Sorghum powder seeds contain a lot of minerals such as potassium, calcium, magnesium, iron, zinc, Sulphur, copper & phosphorus. The percentage of each of them was 155.11%potasium, 19.72% calcium, 184.20% magnesium, 4.55% iron, 2.32% zinc, 189.90% Sulphur, 0.59% copper and 345.07 phosphorus. (Table 2).

Table (2): Minerals Content of Sorghum Seeds Powder.

Minerals	Mean ± SD (mg/100)
K	155.11± 9.42
Ca	19.72± 1.55
Mg	184.20± 6.44
Fe	4.55± 0.59
Zn	2.32± 0.36
S	189.90± 22.99
Cu	0.59± 0.11
P	325.07± 15.17

Sorghum seeds powder contains many bioactive compounds such as phenolic compounds, carotenoid, chlorophyll, tannins and phytates. The percentages of this compound were 267.70% total phenolic, 7.98% total carotenoid, 4.64% total chlorophyll, 1.66% total tannins and 631.14% total phytates. (Table 3).

Table (3): Bioactive compounds content of sorghum seeds powder

Compound	Mean ± SD
Total phenolic content (mg GAE/100g d.p)	267.70± 27.43
Total carotenoids content (mg/100g d.p)	7.98± 1.23
Total chlorophyll content (mg/100g d.p	4.64± 0.58
Total tannins (mg CAE/100g d.p)	1.66± 0.38
Total phytates (mg/ 100g d.p)	631.14± 45.12

Sorghum seeds powder have antioxidant activity (AA) in percentage 80.37%. (Table 4).

Table (4): Antioxidant activity of sorghum seeds powder

Parameter	Mean ± SD
Antioxidant activity	80.37 ± 2.44%

Table (5) shows the sorghum content of fatty acids it was as follows, 12.0 (0.87%), 14.0 (0.52%), 16.0 (10.34%), 16.1 (2.05%), 18.0 (2.43%), 18.1 (30.65%), 18.2 (45.98%), 18.3 (1.89%), 20.0 (0.75%), 22.1 (1.98%). (Table 5).

Table (5): Fatty Acids of Sorghum Seeds Powder

Fatty acids	Mean ± SD
12.0	0.87± 0.11
14.0	0.52± 0.13
16.0	10.34± 1.25
16.1	2.05± 0.14
18.0	2.43± 0.10
18.1	30.65± 2.98
18.2	45.98± 3.98
18.3	1.89± 0.14
20.0	0.75± 0.09
22.1	1.98± 0.26
Unknowns	2.54± 0.37

The effect of sorghum powder on FI, FER and BWG % of hepatotoxicity rats: The data showed significant decline in FI, BWG & FER comparing with control negative group. (Table 6).

Table (6): Effect of sorghum powder on FI, BWG% and FER of hepatotoxic rats

Groups	FI (g/ day/ rat)	BWG (%) Mean± SD	FER Mean± SD
Negative Control	12.32± 0.28 [a]	55.66± 0.50 [a]	0.10± 0.00 [a]
Positive Control	11.32± 0.60 [ab]	32.65± 0.88 [f]	0.05± 0.00 [b]
2.5% S.P	10.30± 0.68 [bc]	38.51±0.80 [e]	-0.00± 0.00 [c]
5% S.P	10.15± 0.70 [c]	48.33± 0.96 [d]	-0.04± 0.00 [b]
7.5% S.P	9.42± 0.53 [c]	51.36± 0.81 [c]	-0.08± 0.01 [f]
10% S.P	7.36± 0.76 [d]	53.16± 1.10 [b]	-0.02± 0.00 [b]
LSD	1.094	1.542	0.010

(2.5% S.P) 2.5% sorghum powder, (5% S.P) 5%sorghum powder, (7% S.P) 7% sorghum powder, (10% S.P) 10% sorghum powder and (LSD) least significant difference

Data showed significantly reduction in mean value of AST and ALT especially 10% S.P group comparing with control positive group. (Table 7).

Table (7): Effect of sorghum powder on AST and ALT of hepatotoxic rats

Groups	AST	ALT
Negative Control	101.19± 1.27 [c]	37.33± 1.14 [f]
Positive Control	148.16± 1.10 [a]	94.76± 0.45 [a]
2.5% S.P	137.59± 1.37 [b]	68.14± 0.68 [b]
5% S.P	133.45± 0.80 [e]	54.27± 0.91 [c]
7.5% S.P	126.27± 1.04 [c]	51.16± 1.68 [d]
10% S.P	125.98± 2.12 [c]	48.83± 0.88 [e]
LSD	2.407	1.843

The significant decrease in mean value of T-Cholesterol, HDL, LDL and VLDL in blood serum, the high significant decrease scored in 10% S.P group compared with negative control group. (Table 8)

Table (8): Effect of sorghum powder on mean value of total cholesterol (TC), and lipoprotein fractions HDL, LDL and VLDL in hepatotoxicity rats.

Groups	TC	HDL	LDL	VLDL
Positive Control	90.67± 0.99 [f]	47.00± 1.76 [a]	29.43± 0.84 [f]	8.75± 0.28 [d]
Positive Control	142.32± 0.62 [a]	28.29± 1.36 [e]	84.54± 0.90 [a]	17.85± 0.69 [a]
2.5% S.P	135.25± 1.01 [b]	31.05± 0.17 [d]	78.53± 0.69 [b]	12.11± 0.19 [b]
5% S.P	119.29± 0.88 [c]	34.38± 1.20 [c]	44.44± 1.30 [c]	11.58± 0.53 [b]
7.5% S.P	116.33± 1.14 [d]	36.18± 0.84 [c]	45.98± 0.39 [b]	10.31± 0.52 [c]
10% S.P	109.76± 0.92 [e]	41.16± 0.91 [b]	43.55± 0.97 [e]	9.84± 0.24 [c]
LSD	1.680	2.056	1.594	0.804

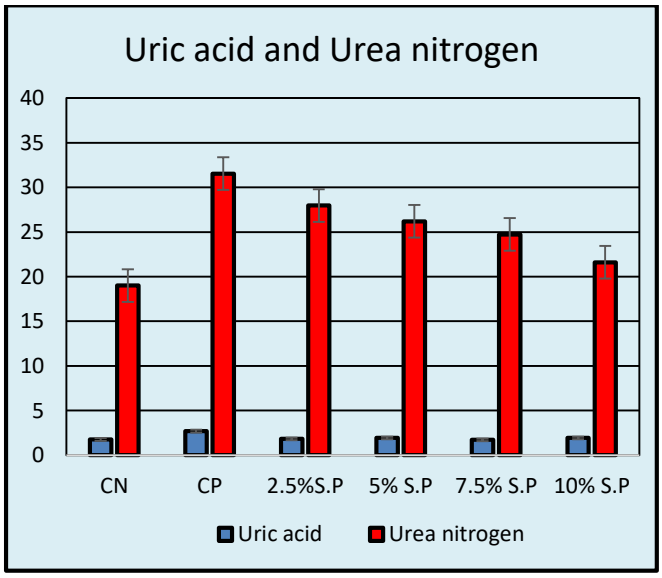

Figure (1): Showed the effect of sorghum powder on mean value of uric acid and urea nitrogen in hepatotoxic rats. The result scored significant decrease in uric acid and urea nitrogen and the lowest score was in 10% S.P group comparing with control positive group

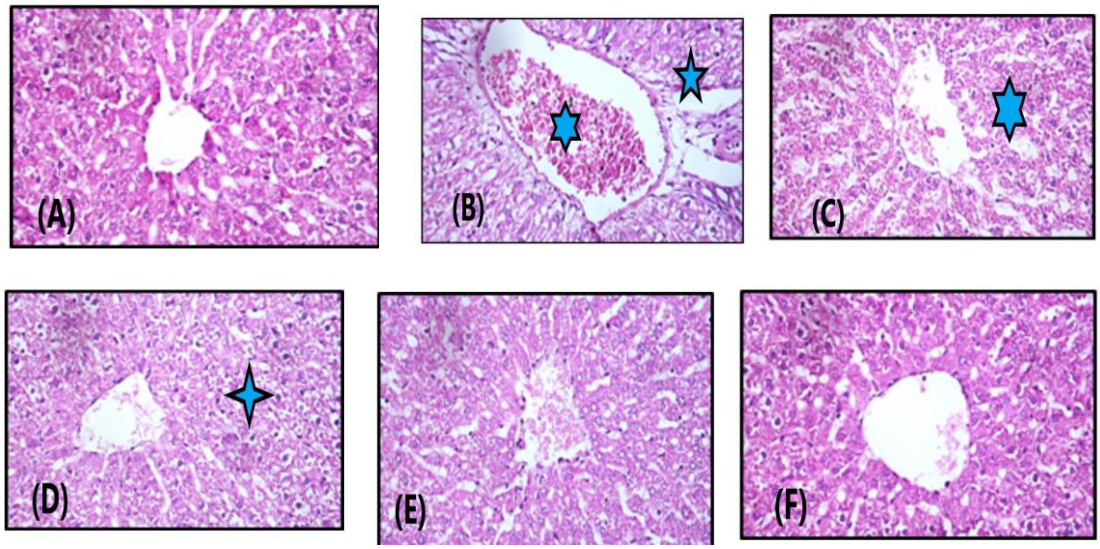

Fig (2): Livers of rats from: (A) photomicrograph of group (1) in liver section showing normal histological structure of hepatic lobules, (H&E, X:400), **(B)** photomicrograph of group (2) in liver section showing massive necrosis (blue star) hepatocytes, together with mononuclear cells infiltration (Blue star), (H&E, X:400), **(C)** photomicrograph of group (3) in intoxicated liver section fed on 2.5% of Sorghum powder showing slight regeneration of hepatocytes slight inflammatory cells infiltration, (blue stars), (H&E, X:400), **(D)** photomicrograph of group (4) in intoxicated liver section fed on 5 % of Sorghum powder showing mild regeneration of hepatocytes and slight inflammatory cells infiltration, (blue stars), (H&E, X:400), **(E)** photomicrograph of group (5) in intoxicated liver section fed on 7.5% of Sorghum powder showing moderate regeneration of hepatocytes, (H&E, X:400), and **(F)** photomicrograph of group (6) in intoxicated liver section fed on 10 % of Sorghum powder showing nearly normal histological architecture of hepatic lobule (H&E, X:400).

DISCUSSION

The current results indicated that sorghum powder decreased FI, BWG%, and FER, resulting in weight loss. Previous researchers came to the conclusion that sorghum powder has potential as a primary care intervention and treatment for obesity, and that it also has the ability to contribute to weight loss. In an animal model, the consumption of sorghum powder dietary fiber and tannins, which are known to have a high antioxidant capacity and they operate in limiting starch digestibility, has been related with reduced body fat in obese men and insulin resistance. This is because sorghum powder dietary fiber and tannins are known to have a high antioxidant capacity [15, 16]. The result cleared that S.P increased antioxidant enzymes and decreased liver enzymes AST and ALT, especially in sorghum powder 10% group comparing with control positive group, which agreement with **Moraes *et al.*** [17] prior findings reported that sorghum powder was associated with diminished inflammatory response and oxidative stress. The data in this study recorded high significant rise in HDL and high significant reduction in the mean value of total cholesterol, LDL & VLDL, in the highest dose group of S.P compared with the control positive group. These results were agreement with **Moraes *et al.*** [15] Research has revealed that the polycosanols in sorghum powder have a powerful hepatoprotective effect by controlling cholesterol absorption, discharge, and synthesis; these are only two of the many bioactive phenolic compounds found in sorghum powder to help prevent dyslipidemia. The result in this study represent the result of determination of kidney function that were a significant reduction in Uric acid and Urea nitrogen comparing with control positive group, the result was agreement with **Moraes *et al.*** [17] **and Blicharz *et al.*** [18] these studies were proved that S.P contains a higher proportion of antioxidants, such as phenolic compounds & vitamin E which protect kidney against inflammatory. It could be concluded that the antioxidant and bioactive components in sorghum powder can protect and treat liver disease.

The histopathological alterations seen in the liver of rats fed on different levels of sorghum powder in the current research agreed to those reported by **Owumi *et al.*** [19] These authors concluded that Sorghum powder induced an improvement of pathological alterations seen in the liver of rats with hepatotoxicity.

Conclusion

It could be concluded that sorghum powder contains carbohydrates and many other bioactive constituents as phenolic compounds, carotenoids, chlorophyll, tannins, phytates and dilatory fibers. Sorghum powder decreased both body weight gain and elevated liver enzymes, improved lipid profile and

increased antioxidant activity. Sorghum powder alleviated histopathological lesions seen in the liver of hepatoxic rats. Therefore, sorghum seeds may be beneficial for patient suffering from liver toxicity.

DECLARATIONS

- **Consent for publication: I attest that all authors have agreed to submit the work.**
- **Availability of data and material: Available**
- **Competing interests: None**
- **Funding: No fund**
- **Conflicts of interest: no conflicts of interest.**

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