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Evaluation of Toxicity Effects on Tissues of Kidney, Heart, and Chromosomes *in vivo* Induced by Sodium Bicarbonate and the Possible Treatment by Ethanolic Extract Solid Dried Tubers for *Cyperus esculentus*

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ABSTRACT

Many fields exist in using rats in the experiment on different materials that have the desired result for the benefit of mankind. The rat is used in laboratories to perform experiments for various sciences such as physiology, pharmacology, cardiology, toxicology, neurosciences, and others. This is since small rodents in size, their life cycle is short, and their genetic traits are numerous, so the desired results appear in short times that do not reach several years like the rest of the animals. Also, the rodents are easy to rise if we provide a comprehensive environment to ensure their continuity. We used sodium bicarbonate in our experiment because of its frequent use today. It should be noted that sodium bicarbonate is considered a dangerous substance because it causes a marked increase in the volume of fluid in the vessels, which leads to acute pulmonary edema. *Cyperus esculentus* or what is known as (Tiger nut) to treat rats, as it is useful and contains some anti-inflammatory. It is used as a heart tonic and has an effective ability to treat diarrhea, and it also reduces colon cancer disorder.

Keywords:
Sodium bicarbonate, *Cyperus esculentus*, Rats, Tissues, Chromosomes.

1. INTRODUCTION

In the areas of laboratory testing, albino rats (*Rattus norvegicus*) were considered a pioneering model of the mammalian system. Sodium bicarbonate is assembled in cosmetic products to manipulate and deal with a lot of diseases [1]. Although the bicarbonate usage, harmful recording was few. The toxicity of oral bicarbonate caused the exhaustion of baking soda effect in the body representing hypochloremia, urine alkalization, hypernatremia, hyporeninemia, metabolic alkalization, intravascular depletion, and hypertension. the occurrence of the inflammation is considered defense efforts the toxins and injuries of bicarbonate by the immune body's response to these damages [2]. After a short-time period of intervention in CKD sufferers with slight acidosis, $NaHCO_3$ supplementation will increase serum bicarbonate in a dose-based way and improves decreased extremity muscle strength. The quantity of sodium bicarbonate ($NaHCO_3$) used to deal with people with organ phosphorous pesticide (OP) publicity now no longer bring about good enough blood alkalization [3].

While nutgrass, *Cyperus esculentus* (Family: *Cyperaceae*), is the supply of treatment, and a colonial, perennial herb concept to have originated in India 2000 years in the past, and extensively utilized in Ayurveda to remedy a whole lot of diseases. It is utilized in several structures of drugs for treating some

ailments, similarly to its prehistoric usage. Anti-parasitic, insecticidal, repellent, antibacterial, antioxidant, anticancer, important nervous, neuroprotective, anti-inflammatory, antipyretic, analgesic, hypolipidemic, weight control, antiplatelet, gastrointestinal, hepatoprotective, anti-diabetic, anti-dysmenorrhea, dermatological, and lots of different consequences had been located with inside the plant. Indigenous peoples of the western Sahel, mainly people who stay in rural regions, consume lots of dried seeds and nuts [4].

2. MATERIALS AND METHODS

2.1 Toxin: Sodium bicarbonate powder ($NaHCO_3$) was obtained from Sigma Aldrich, USA.

2.2 Herbal extraction: The solid dried tubers of tiger nuts (*Cyperus esculentus*) (Fig. 1) that were purchased from the center for herbal plants in Port Said city, cleaned the tiger nuts solid dried tubers by using distilled water and leave to dry for 40 days. Then after this period, the grinder mortar with RM 200 (*Retsch*, Germany) was used for crushing the nuts and converted to powder, this powder was dipped in 2/3 of absolute ethyl alcohol for two days. Then, used qualitative filter paper to filter it. The filtrated sample was evaporated by using the rotary evaporator (*Stuart*, UK) at 45ºC and stored in the sterile vial at 5ºC to use, we collect and prepared the ethanolic solid dried tubers of *Cyperus esculentus* to extract.

Fig (1): *Cyperus esculentus* (Tiger nuts)

2.3 Animal design: 40 male albino rats were obtained from the veterinary animal center in Helwan city, their weight was (120-150 g). The animals were adapted in plastic cages under adequate conditions and allowed water and suitable food (laboratory diet) under normal hygienic conditions, the food and water were daily exchanged and were recorded in any observations. They adapted to laboratory conditions for one week before starting the experiment. Rats were put at room temperature in the animal house of the zoology department, faculty of science of Port Said University, and were divided into randomly four groups (n= 10 rats). The first group (group I) served as negative control (untreated). The second group (group II) was received the extract of (*Cyperus esculentus*) day after day that received 400 mg/kg of body weight oral [6] for 35 consecutive days of treatment, and the third group (group III) consider positive control which intoxicated with 422 mg ($1/10LD_{50}$) of sodium bicarbonate/kg body weight of rat by oral [5] every day in drinking water for 35 days, the fourth group (group IV) was exposed to that received sodium bicarbonate and *Cyperus esculentus* extraction as doses in II and III groups.

Collection of the specimens: At the final of our experiment, the animals fasted overnight. The rats were anesthetized by using suitable anesthetizing agent, and the kidney and heart were taken from an animal used for growth measuring, glucose physiological parameter in serum according to the technique of Petibois *et al.* [7], histological and immunohistochemical explanations by light microscope, Fixation of the kidney and heart were processed by 10% formalin which preserves heart tissue details according to Banchroft, *et al.* and Abu almaaty *et al.* techniques [8,9], according to Misao *et al.* [10] which study of the apoptosis case in kidney tissue, prepare of chromosomes according to Tijo and Whang [11] and techniques, extraction of DNA was extracted from the rat's muscle samples by use DNeasy Mini Kit (Qiagen Santa Clarita, CA), this was done directing to instruction steps of research institute for agricultural genetic engineering at Giza, Egypt. Inter Sample Sequence Repeat "ISSR" analysis is also used A-ISSR-PCR Reactions as ten primers of ISSR were used in the detection of polymorphism as described as the primer sequences in the table (1). According to Hammer *et al.* technique [12], the amplification reaction was executed in 25 µl reaction volume consisting of 3 µl template DNA (10ng), 2.5 µl primer (10pcmol), 12.5 µl Master Mix (*Sigma*), and 7 µl distilled water. And For analyzing ISSR, the bands were appeared that recorded as either present (+) or absent (-) for the four experimental groups and recorded data in table (2). Dice's similarity matrix

coefficients were then calculated between genotypes using the arithmetic averages for unweight pair group method. This matrix was used to perform the phylogenetic tree (dendrogram) performed according to the Euclidean similarity index using the PAST software Version 1.91 [12]. According to Fadda *et al.* and Laemmli techniques [13,14], SDS-PAGE analysis by using proteins of rats' muscles and the molecular weight of protein models was performed according to Weber *et al.* [15].

Primer Codes	Sequences
ISSR-1	5'-AGAGAGAGAGAGAGAGYC-3'
ISSR-2	5'-ACACACACACACACACYT-3'
ISSR-4	5'-ACACACACACACACACYG-3'
ISSR-5	5'-GTGTGTGTGTGTGTGTYG-3'
ISSR-6	5'-CGCGATAGATAGATAGATA-3'
ISSR-7	5'-GACGATAGATAGATAGATA-3'
ISSR-8	5'-AGACAGACAGACAGACGC-3'
ISSR-9	5'-GATAGATAGATAGATAGC-3'
ISSR-10	5'-GACAGACAGACAGACAAT-3'
ISSR-11	5'-ACACACACACACACACYA-3'

Table (1): The ISSR primers' sequences; A: Adenine, C: Cytosine T: Thymine, G: Guanine, and Y: (C or T).

2.4 Analysis for statistical data: Calculation of the bio-statistical data as mean ± SE for all four groups by using the SPSS 16.0 version for performing the differences between values of glucose rates were performed significantly p-value < 0.05 [16].

3. RESULTS AND DISCUSSION

3.1 Growth rate: The results of daily average weight gain/loss for these experimental rats that illustrated in figure (2 A); the growth range for group I was similar to group II, but the range growth of group III was decreased and loss of weight reached to -2.8g in 3rd week. The growth range of group IV was improved caused by *Cyperus esculentus* extract treatment. The effect of sodium chloride (hypertonic 10%) was studied in non-water exposed Wistar albino rats. The weight gain daily ranging was calculated and revealed a significant difference of 16.0, 0.1, 5.3, 3.9, 4.7, and 8.9g [17]. The relative weight for heart rats in the different groups; in group I was 0.0039±0.00029, in group II was similar to the group I (control) equal to 0.0037±0.00025, in group III was 0.0049±0.00032 increased compared to control group, and group IV was 0.0032±0.00014 that showed in figure (2 B). In the different groups, the relative weight of rats' kidneys; was 0.0089±0.00053 in group I, the group II was similar to group I (control) equal to 0.0089±0.00035, 0.01022±0.00025 in group III was increased compared to control group, and 0.0074±0.00016 in group IV that showed in figure (2 C). These results agreed with other studies which caused weight loss, and increased heart and kidney growth rates [17, 18]. And also, this study that detected the results showed that treating sodium bicarbonate exposed to rats with tiger nuts extract in a range of increases in weight gain body and outcomes weights of heart and kidney [19].

3.2 Glucose content in serum: The means of serum glucose level in rats for group III was 130.6±7.53 that highly increased compared with group I, the means of serum glucose level in rats for group II was 89.2±7.92 that decreased compared with group I, the means of serum glucose level in rats for group IV was 96±5.43 that increased compared with group I that explain in figure 3. Significantly represented between positive control and treated control <0.05. The induction of *Cyperus esculentus* extract was affected and reduced the blood glucose levels [20, 21].

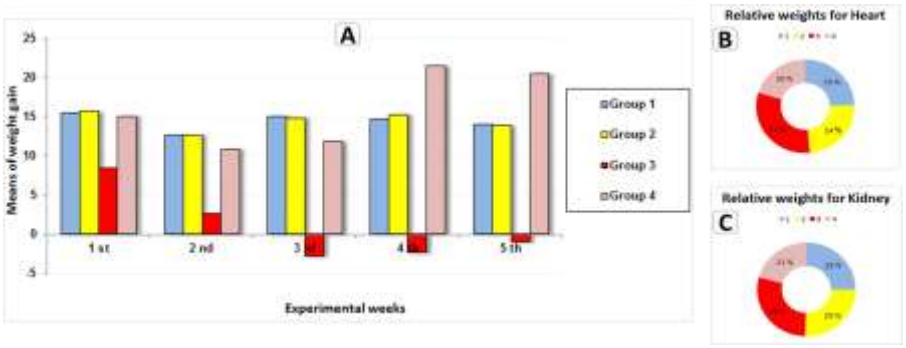

Fig (2): (A) Column chart for means of weight gain in the different four experimental groups, (B and C) Doughnut charts displayed the relative weights for heart and kidney for the different four experimental groups.

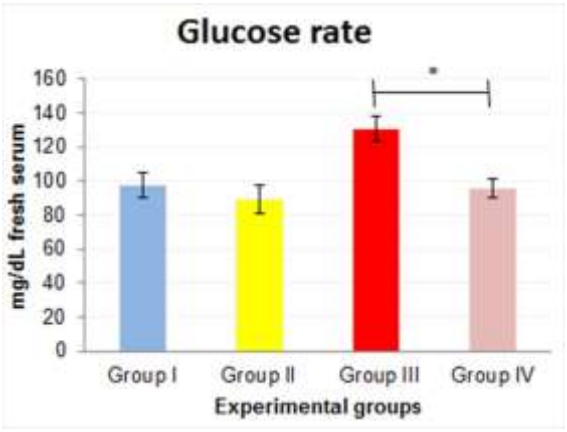

Fig (3): Column chart which displayed the glucose rate for the different four experimental groups, each column meaning the mean for each group, and both cap vertical error bar (I) was representing standard error (SE) for each group, the difference significantly between four groups: * → is indicating to significant P<0.05 for III and IV groups that compared with group I.

3.3 Histological and immunohistochemical explanations for heart and kidney: The normal architecture structure of rat' myocardial from groups I and II that appears normal muscle fiber branched with normal striation, normal nuclei, and normal intercalated discs within cardiac myofibers as shown in figures 4 and 5. In group III, the myocardial that showing as degeneration of branched muscle fibers, breakdown of striation, some normal nuclei, and edema were found. Also, the draining inflammatory cells have appeared within the fibrils of the heart muscles, necrotic nuclei have appeared within muscle fibers branched, and distortion of myofibers (Figure 6). Heart from group IV, mild distortion of muscle fiber branched with improving myo-striation, numerous normal nuclei, slight inflammatory cells, detraction of edema space within myofibers, and mild demolition of muscle fibers (Figure 7).

The Bax and Bcl-2 are proteins in cell cytoplasmic that role in apoptosis occur. The disorder in the representation of these proteins caused defection in the cell cycle and led to apoptosis acceleration. Because of studies regarding anti-Bcl-2 treatment, a good grasp of apoptosis has become even more important [22]. In normal cells, Bax is required for apoptosis. Overexpression of Bcl-2 or Bax that leading to stimulated apoptosis case [23]. And the extraction of Bax, Bcl-2 effect, and apoptotic process was By adding of *Cyperus esculents*, Bax protein level was significantly decreased, Bax and Bax/Bcl-2 ratio decreased and Bcl-2 protein level was increased significantly [24]. The immunohistochemistry micrograph for Bax expression from transverse section of kidney rats for experimental groups in the cytoplasmic epithelium renal tubules showed negative Bax reaction from rats of negative control (group I), mild Bax expression from group II that was similar to Bax reaction in group I, markedly increasing of brown color for Bax reaction from group III that induced with sodium bicarbonate, and moderate Bax expression which from group IV (Figure 8). The immunohistochemistry micrograph for Bcl-2 reaction from transverse section of kidney rats for experimental groups in the cytoplasmic epithelium renal tubules that showed negative Bcl-2

reaction from rats of negative control (group I), mild Bcl-2 reaction from group II that similar appearance of Bcl-2 reaction in group I, markedly increasing of brown color for Bcl-2 reaction from group III that was induced with sodium bicarbonate, and moderate Bcl-2 reaction which from group IV (Figure 9).

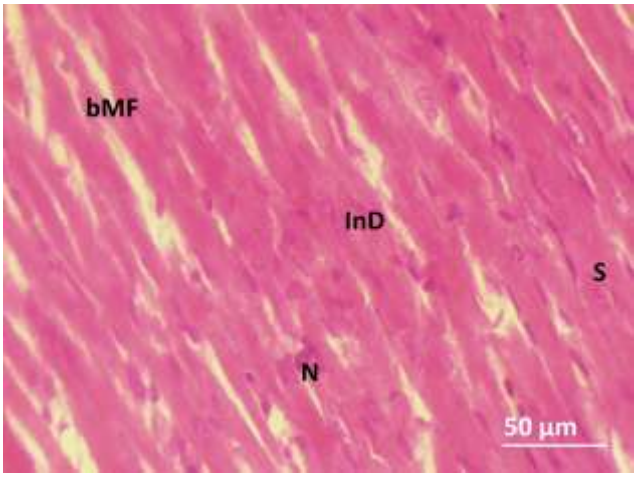

Fig (4): Micrograph of longitudinal section for normal architecture structure of rat' myocardial from group I that appears normal muscle fiber branched (bMF) with striation (S), normal nuclei (N), and normal intercalated discs (InD) within myofibers (H&E, bar 50µm).

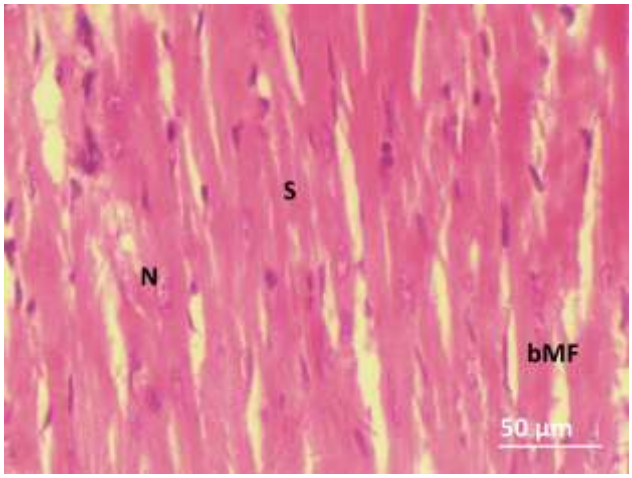

Fig (5): Micrograph of longitudinal section for the normal structure of rat' heart from group II that showing similar to heart tissue of negative control (group I) as expressing normal muscle fiber branched (bMF) with striation (S), and normal nuclei (N) (H&E, bar 50µm).

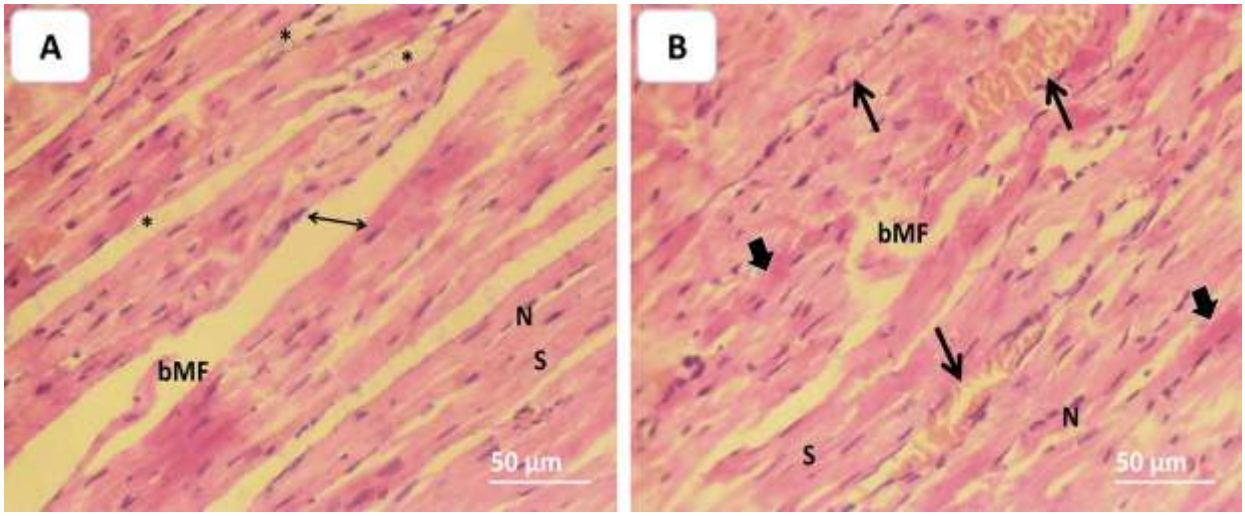

Fig (6): Micrograph of longitudinal section for myocardial from rats of group III that showed (A) degeneration of muscle fibers branched (bMF), striation (S), pyknotic of nuclei (N), and edema were found (left-right arrow ↔). (B) detecting the necrosis of myocardial fibers with draining inflammatory cells have

the appearance (thin arrows) within muscle fibers branched (bMF), striation (S), pyknotic of nuclei (N), and distortion of myofibers (thick short arrows) (H&E, bar 50µm).

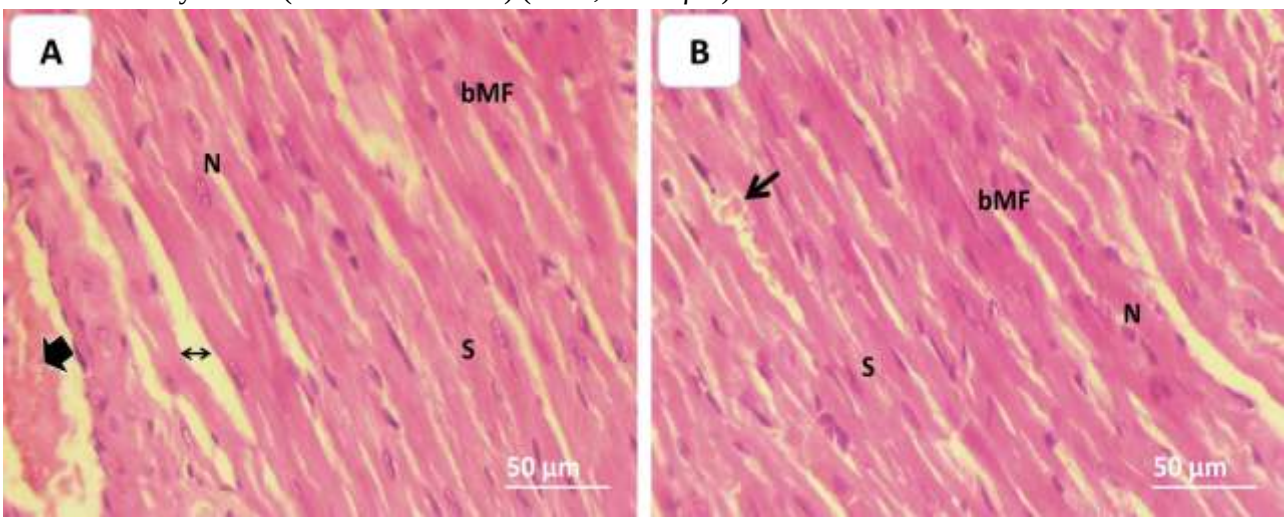

Fig (7): Micrograph of longitudinal section for heart muscle from rats which treated (group IV) (A and B) illuminating mild distortion of branched myofiber (bMF) with muscle striation (S), numerous normal nuclei (N), inflammatory cells appeared (thin arrow), detraction of edema space (left-right arrow ↔) within myofibers, and mild demolition of muscle fibers (thick arrow) (H&E, bar 50µm).

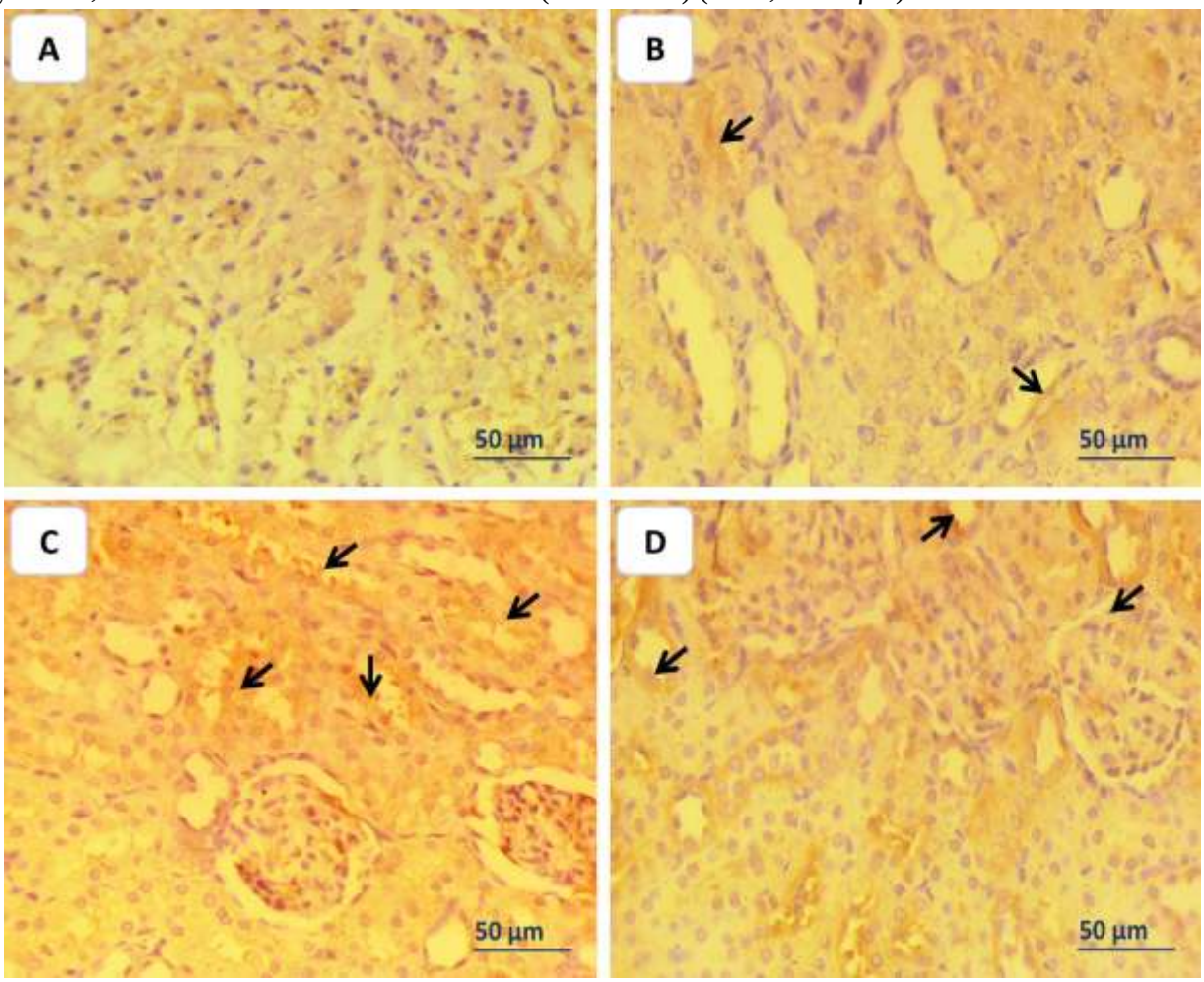

Fig (8): Immunohistochemistry micrograph for Bax expression (arrows) from transverse section of kidney rats for experimental groups in the cytoplasmic epithelium of renal tubules that showed (A) negative Bax reaction from rats of negative control (group I), (B) mild Bax reaction in group II that was similar to the reaction in group I, (C) markedly increasing of brown color as this reaction from group III that induced with sodium bicarbonate, and (D) moderate Bax reaction which from group IV (Bax reaction, bar 50µm).

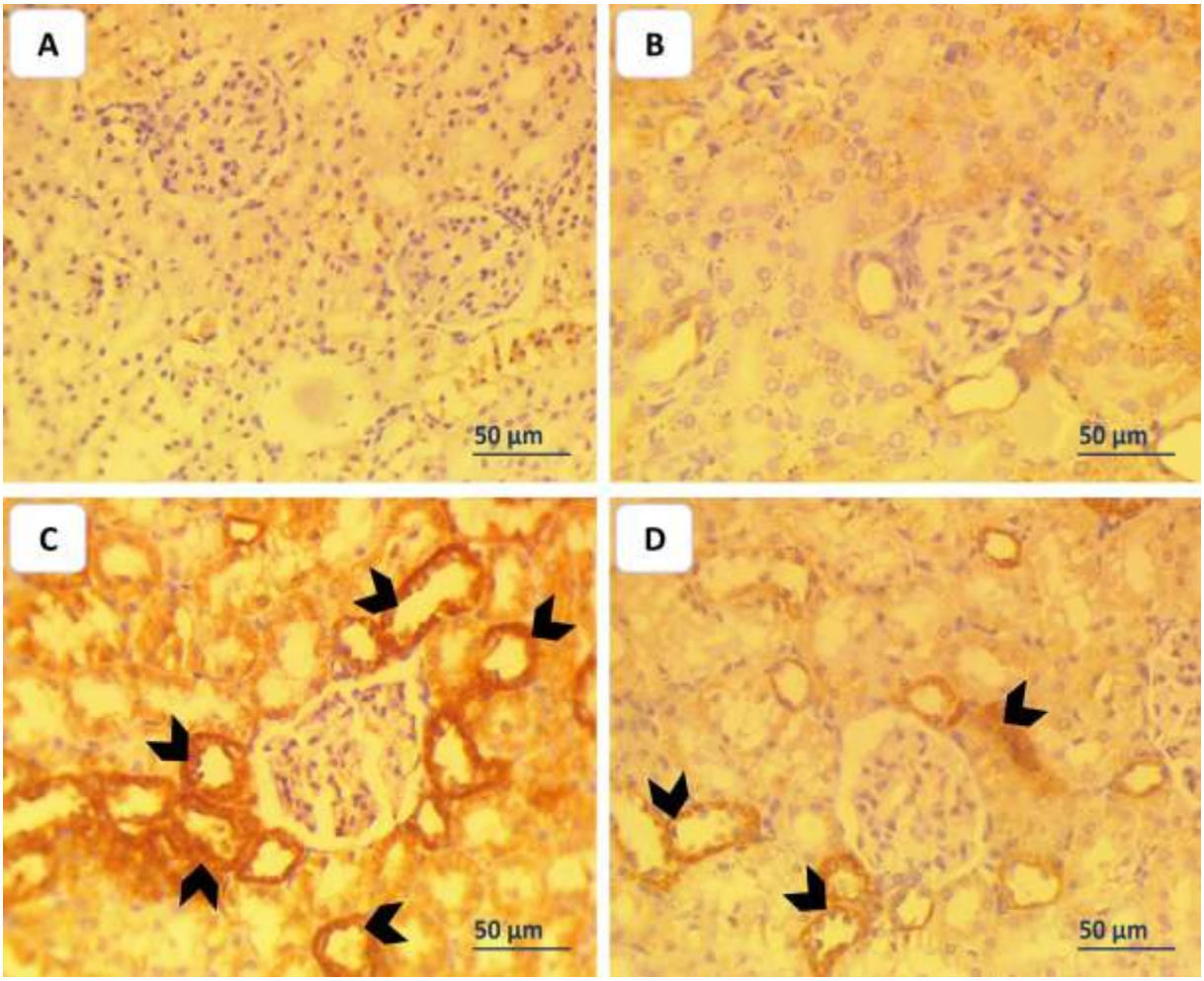

Fig (9): Immunohistochemistry micrograph for Bcl-2 expression (head arrows) from transverse section of kidney rats for experimental groups in the cytoplasmic epithelium of renal tubules that showed (A) negative for Bcl-2 reaction from rats of control (group I), (B) mild of Bcl-2 reaction in group II that was similar to the reaction in group I, (C) markedly increasing brown color for Bcl-2 reaction from group III that induced with sodium bicarbonate, and (D) moderate Bcl-2 reaction that from group IV (Bcl-2 reaction, bar 50µm).

3.4 Examination of chromosomal aberration: The number of chromosomes diploid of a rat (2n) in the I and II groups appeared 42 normal chromosomes as 20 autosomes and 2 sex chromosomes which agrees with Moreno and Jacob [25], the aberration of chromosomes was found in group III and reduced this aberration due to intake of ethanolic extraction of *Cyperus esculentus* solid dried tubers. The chromosomal Aberration depends on the pH value. No clastogenic activity founded over the initial pH range of 7.3–10.9 with the out S9 mix, but the chromosomal aberrations frequency was increased at pH 10.4 with the S9 mix. [26]. The chromosomal aberrations were noted for all experimental groups (Figures 10, 11, 12, and 13).

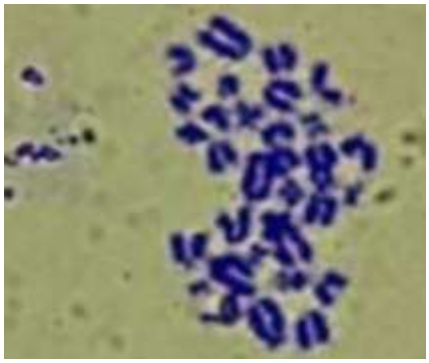

Fig (10): Chromosomal spread of normal rats from group I that expressed as 42 chromosomes (Gemisa, 1000X).

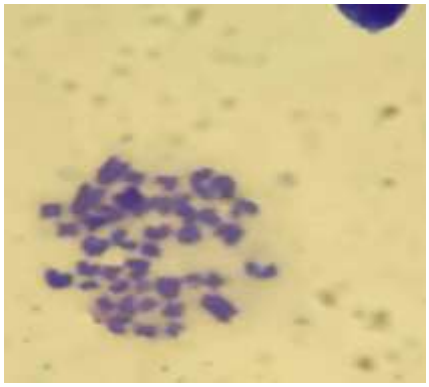

Fig (11): Chromosomal spread from group II which represented normal chromosomes that were similar to chromosomes of group I (Gemisa, 1000X).

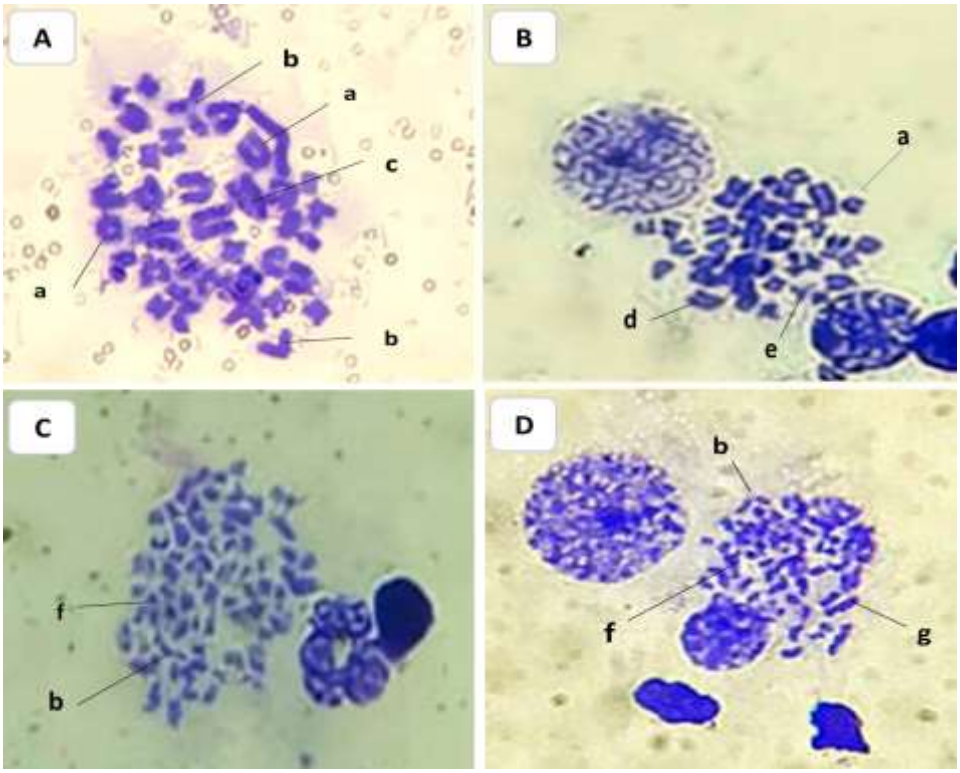

Fig (12): Chromosomal spread of intoxicated rats with sodium bicarbonate (group III): (A) a. chromosomal ring, b. chromosomal deletion, and c. chromosome gap. (B) a. chromosomal ring, d. acentric, and e. translocation. (C) b. deletion, and f. fragment. (D) d. deletion, f. fragment, and g. chromosomal break (Gemisa, 1000X).

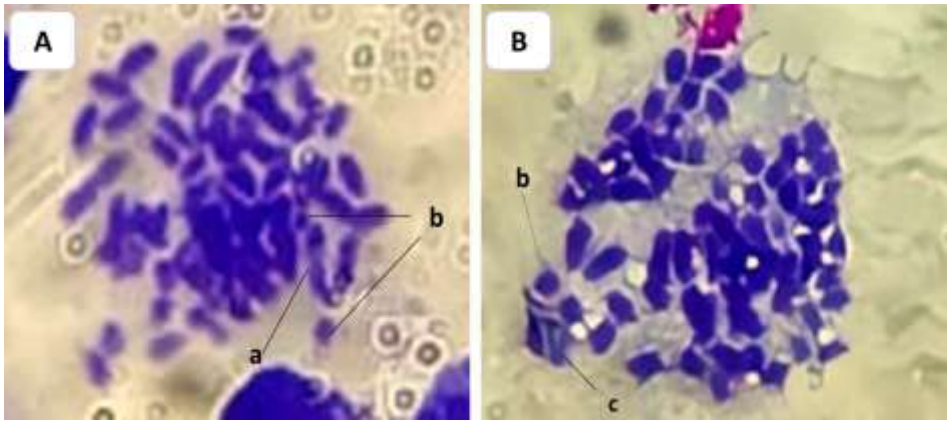

Fig (13): Chromosomal spread of rats exposed to sodium bicarbonate with treated (group IV): (A) a. deletion, and b. fragment. (B) Thickening chromosome; b. fragment, and c. acentric (Gemisa, 1000X).

3.5 ISSR analysis: In our study, by using ten primers for ISSR sequences. Significant profiles amplification of all generated primers with strong bands that detected polymorphism between different four experimental groups (Fig 14). 119 amplified total bands were shown by using ten primers of ISSR (Table 2), for each primer had twelve bands. From 6 (ISSR-5) to 16 (ISSR-9) primers, the ranged total of amplified bands was recorded. The total of monomorphic bands was 95, the total of polymorphic bands without unique bands was 20, unique bands were 5 and the percentage of polymorphism varied from 0% to 44%. The genetic similarity is highest between groups I and II (97.2%), and the genetic similarity is 95% between groups III and IV as shown in figure (15) and table (3).

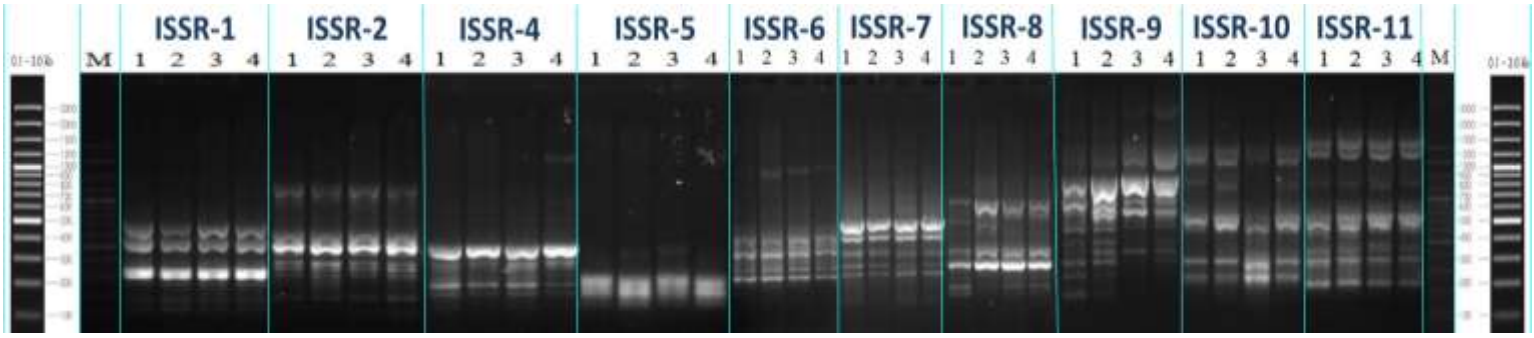

Fig (14): ISSR analysis for the current four groups by using the primers for different ISSRs: (ISSR-1, ISSR-2, ISSR-4, ISSR-6, ISSR-7, ISSR-8, ISSR-9, ISSR-10, and ISSR-11), and the M signify to marker for DNA.

Primer name	Amplified bands' numbers				Amplified bands total	Amplified bands size (bp)	Monomorphic Bands	Polymorphic Bands (without Unique Band)	Unique Bands	Bands of Polymorphic and Unique together	Polymorphism%
	1.	2.	3.	4.							
ISSR-1	13	13	13	12	14	120 bp - 1400 bp	12	1	1	2	14%
ISSR-2	10	10	10	10	10	130 bp - 700 bp	10	0	0	0	0%
ISSR-4	11	10	11	12	13	150 bp - 2000 bp	10	1	2	3	23%
ISSR-5	5	6	6	5	6	180 bp - 500 bp	5	1	0	1	17%
ISSR-6	13	14	13	12	15	250 bp - 1500 bp	11	4	0	4	27%
ISSR-7	10	10	9	9	10	250 bp - 1000 bp	9	1	0	1	10%
ISSR-8	10	10	9	9	10	180 bp - 1000 bp	8	2	1	3	27%
ISSR-9	14	13	11	12	16	180 bp - 2000 bp	9	6	1	7	44%
ISSR-10	10	10	10	11	11	220 bp - 1500 bp	9	2	0	2	18%
ISSR-11	13	13	13	13	14	180 bp - 1600 bp	12	2	0	2	14%
Total	109	109	105	105	119	120 bp - 2000 bp	95	20	5	25	21%

Table (2): Ten primers of ISSR for four experimental groups, the total number for amplified bands, range of molecular weights for showing the size of total bands by base pair (bp), bands for monomorphic, bands for polymorphic bands without unique bands, bands for unique, bands for polymorphic bands with unique bands together, and polymorphism percentage.

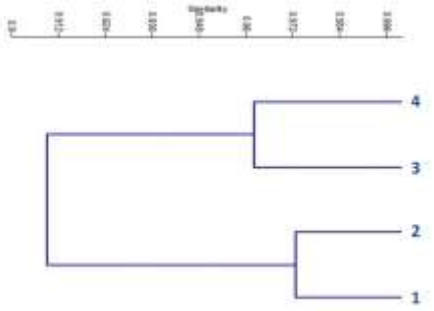

Fig (15): Graph for dendrogram that clarifies a phylogenetic tree analysis for four experimental groups which depended on molecular ten markers for ISSR.

	1	2	3	4
1	100			
2	97.2	100		
3	89.7	91.5	100	
4	90.6	91.5	96.2	100

Table (3): The average for similarities (%) of genetics that assessment was estimated by ten primers of ISSR for four experimental groups.

3.6 Analysis of SDS-PAGE: The molecular weight ranging for bands that were recorded from 11 to 120 KD and 10 bands for four experimental groups by SDS-PAGE analysis represented a total number of resulting bands. The produced monomorphic bands for I, II, and IV groups were 9 bands and 1 band of polymorphic with polymorphism of 4%. And for group III, the 8 bands monomorphic and 2 bands polymorphic resulted in that shown in figure (16) and table (4).

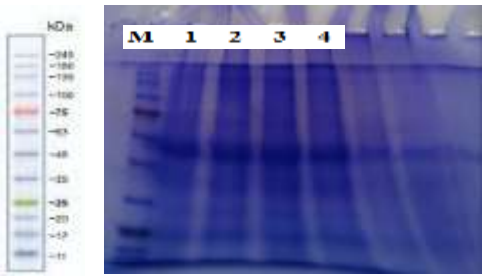

Fig (16): Gel electrophoresis for SDS protein bands for four experimental groups and the M signifies to marker for DNA.

Molecular weights (bp)	**1 Group I**	**2 Group II**	**3 Group III**	**4 Group IV**
120	+	+	+	+
100	+	+	+	+
75	+	+	+	+
63	+	+	+	+
45	+	+	-	-
35	+	+	+	+
25	-	-	-	+
20	+	+	+	+
17	+	+	+	+
11	+	+	+	+

Table (4): The SDS-PAGE protein' bands of four experimental groups.

4. CONCLUSION

After comparing the results, the produced conclusion is that the *Cyperus esculentus* extract improves the growth rates of the body, organs, serum glucose level, enhancement the structure of the heart and kidney, and diminishes the chromosomal aberrations, and mildly improves the tiger nuts on the ISSR and SDS-PAGE analyses.

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