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Rhubarb root extract alleviates $HgCl_2$-induced acute kidney injury by downregulating nuclear factor-ƙB and neutrophil-associated gelatinase lipocalin expression in renal tissues

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ABSTRACT

Background/Aim: Rhubarb is a Chinese herb with a nephroprotective effect against $HgCl_2$-induced acute kidney injury (AKI). Nuclear factor-ƙB (NF-ƙB) and neutrophil gelatinase- associated lipocalin (NAGL) are inflammatory biomarkers markedly increased in AKI. Downregulation of NF-ƙB and NAGL genes was found to blunt the pathophysiological consequences of renal damage. Developing a strategy to downregulate their expression might represent an efficient treatment for AKI. **Aim:** to elucidate whether rhubarb root extract can downregulate NF-ƙB and NAGL gene expression in $HgCl_2$-induced AKI.

Materials and Methods: Sixteen Wistar rats were equally divided into four groups: control group, extract group (12g rhubarb root extract /kg b.w., twice a week), positive control group (0.5 mg $HgCl_2$/Kg b.w., twice a week.), treatment group (0.5mg $HgCl_2$/Kg b.w. + 12 g rhubarb root extract /Kg b.w.) twice a week. By the end of the 8th week, all animals were sacrificed after ether anaesthesia, blood was collected for biochemical investigations (blood urea, serum creatinine, total protein, and albumin), and kidneys were removed for measurement of NF-ƙB and NAGL gene expression by RT-PCR, along with malondialdehyde (MDA) and total antioxidant capacity (TAC) levels in kidney tissue.

Results revealed significant ($p<0.01$) recovery in kidney weight and functions, MDA and TAC in the treatment group compared to positive control group. A highly significant ($p<0.01$) decrease was also observed in NF-ƙB and NAGL gene expression in the rhubarb root extract-treated group compared to the positive control group.

Conclusion: Rhubarb root extract ameliorates $HgCl_2$-induced AKI in rats through downregulation of NF-ƙB, and NAGL gene expression.

1. Introduction

Heavy metals are naturally occurring elements in earth's crust, however, due to their uncontrolled anthropogenic activity, their natural biogeochemical cycles have been greatly impacted. Since they are not degradable, heavy metals persist in the environment, exert their toxic effects on humans, and on almost all living creatures. Therefore, heavy metal pollution has become one of the major environmental challenges [1].

Mercury is one of most hazardous heavy metals, and because of its increased industrial and medicinal uses, mercury concentration is continuously increasing. In the environment, coal combustion in thermal power plants is the major anthropogenic mercury source [2]. The presence of mercury in the environment is a worldwide concern. Inorganic mercury is present in industrial materials, used in medical devices and employed in batteries. In addition, mercury is an essential constituent in fluorescent light bulbs, and has been associated with human poisoning in gold mining areas [3].

Through contaminated soil, mercury is absorbed by plant roots, and thereafter, it accumulates in the plant system and penetrates into the food chain, causing various health and environmental risks [2]. In addition, in developing countries, health problems and environmental risks are greatly exacerbating due to the increased exposure to mercury in small-scale mining [4]. Since kidneys are greatly involved in the detoxification and excretion functions, most environmental toxins, unfortunately, accumulate in kidneys, which makes kidneys highly susceptible to environmental contaminants. The most nephrotoxic mercury species are the inorganic species [5, 6]. Following exposure to mercury compounds, inorganic mercury takes a nonuniform distribution, accumulates mainly in renal proximal tubules, ultimately leading to acute renal injury (ARI) [7] and inflammation, which further exacerbates kidney damage. Controlling inflammation has been shown to mitigate further deterioration of kidney injury and promote slow recovery [8].

Nuclear factor- kappa B (NF-ƙB) is a crucial regulator of inflammation and cell survival [9, 10], that is known to play an essential role in the pathogenesis of acute kidney injury (AKI) [9]. Hyperactive NF-ƙB causes activation and recruitment of immune cells, which, in turn, triggers oxidative stress, ultimately leading to organ injury through apoptosis, necrosis, and fibrosis [11].

Several previous studies have shown that downregulating or blocking the NF-ƙB signalling pathway, could attenuate the inflammatory response and oxidative stress in the kidney tissues of rats with AKI [12, 13]. Therefore, developing a strategy to target NF-ƙB signalling pathway is important to achieve effective treatment of AKI [11]. Another protein that is produced in response to inflammation is neutrophil-associated gelatinase lipocalin (NAGL) [14]. NGAL is a 25kDa protein [15, 16] that is found in normal traces in renal epithelial tissues, however, serum and urine NAGL levels dramatically increase in cases of renal tubular injury [17]. NAGL has been highlighted in several studies as a diagnostic biomarker in different aspects of kidney disease [18], in addition, NGAL gene inactivation has been revealed to ameliorate the deleterious consequences of renal damage [19]. Studies have shown that patients with acute renal failure (ARF) induced by acute mercury poisoning should be given dialysis, however, the potential toxicity and side effects of synthetic reagents have been a challenge for a long time [20], therefore, alternative mercury intoxication treatment methods using natural substances like herbs with high efficiency and low side effects, are mandatory [21]. Rhubarb (Polygonaceae) is a perennial stout herb [22] that has long been used in traditional Chinese medicine (TCM) in the treatment of both chronic and acute kidney disease. Rhubarb extract was also found to ameliorate $HgCl_2$-induced AKI [21] in addition to possessing a good nephroprotective effect, which primarily protects the kidneys from fibrosis, oxidation, inflammation, autophagy, and apoptosis [23].

The aim of the present work was to investigate whether the ameliorating effect of rhubarb root extract against $HgCl_2$-induced AKI in rats is mediated through downregulation of NF-ƙB and NAGL gene expression.

2. Materials and methods

2.1. Chemicals

Mercuric chloride: was purchased from Sigma Aldrich, USA (product number 215465).

Rhubarb: Dry cut roots were obtained from Agora Market (7184), Egypt, and were identified by a botanist in the botany department, faculty of science, Ain Shams University.

2.2. Preparation of Rhubarb root extract:

Dry rhubarb roots were grinded and powdered, and then the extract was prepared as previously mentioned [24] by soaking the ground powder into boiling water for one hour. The ratio of rhubarb to water during the extraction procedure was 1:20 (weight: volume). The extract was then filtered and used.

2.3. Animals

Adult male Wistar albino rats weighing (150 -200 g) were obtained from the Veterinary Serum and Vaccine Research Institute (Cairo, Egypt). The animals were housed in plastic cages with well-aerated covers at 25 ± 2°C, along with natural light/dark cycles. Animals were allowed free access to water and were supplied daily with a standard diet.

2.4. Experimental Design

Sixty adult male Wistar albino rats were randomly divided into four groups, each containing 15 rats:

1-Control group, fed with standard diet.

2-Extract group, receiving oral rhubarb root extract twice a week for 8 weeks at a dose of 12g/kg body weight (b.w.) [25].

3-Positive control group, receiving intraperitoneal $HgCl_2$ at a dose of 0.5mg /Kg b.w. twice a week for eight weeks [26].

4-Treatment group, receiving both intraperitoneal $HgCl_2$ at a dose of 0.5 mg /Kg b.w. + oral rhubarb root extract at a dose of 12 g/Kg b.w. twice a week for eight weeks. By the end of the eighth week, all animals were sacrificed after ether anaesthesia, and then blood was collected by ocular vein puncture and kidneys were removed for biochemical, haematological and molecular investigations. Throughout the experiment, all the procedures and experimental protocols were approved by the ethical committee of the Zoology Department, Ain Shams University, and were carried out according to the Guide for the Care and Use of Experimental Animals.

2.5. Biochemical analyses

These included determination of:

- Blood urea nitrogen (BUN) [27] (Spectrum, Egypt, Catalogue Number 318 001)
-Serum creatinine [28] (Spectrum, Egypt, Catalogue Number 235 001).
-Serum total protein, and albumin [29, 30] (Spectrum, Egypt, Catalogue Numbers 310001 and 210001, respectively).
- Malondialdehyde (MDA) (Sigma Aldrich, Catalogue Number MAK085) and total antioxidant capacity (TAC) [31] (Sigma Aldrich, USA, Catalogue Number MAK187) in kidney tissue homogenate.

2.6. Molecular studies

These included measurement of NF-kB and NAGL gene expression in kidney tissue by quantitative real-time PCR (qRT-PCR) as following:

1-RNA Isolation: Total RNA was extracted from renal tissue of the respective animal in each group using TRIzol ™ reagent. RNA Isolation kit was supplied by Thermo Fisher Scientist, USA (Catalogue Number 12183018A).

2-Reverse Transcription: Synthesis of cDNA from total RNA samples was the first step in the gene expression quantification experiment. The High-Capacity cDNA reverse transcription Kit was supplied by Thermo Fisher Scientist, USA.

3-Syber green RT-PCR analysis: The SYBR Green PCR Master Mix was used for gene expression quantification experiment. The SYBR Green PCR Master Mix kit was supplied by Thermo Fisher Scientist, USA. The products obtained by the reverse transcription (cDNA) were amplified by using the primers for NF-kB and NGAL genes.

4-Quantitative real-time PCR analysis: PCR amplification was performed using primers as previously reported [32, 33] (table 1).

Table 1. Primer sequences for qRT- PCR analysis of NAGL and NF-kB gene expression

genes	Primer sequence
NGAL	forward: 5'- GATGTTGTTATCCTTGAGGCCC -3' reverse: 5'-CACTGACTCACGACCAGTTTGCC -3'
NF-kB	forward: 5'- CCTAGCTTTCTCTGAACTGCAAA-3' reverse: 5'-GGGTCAGAGGCCAATAGAGA-3'
β-actin	forward: 5'-ACCCACACTGTGCCCATCTA -3' reverse: 5'- CGTCACACTTCATGATG -3'

5- PCR amplification: This included initial denaturation at 95 °C for 10 minutes followed by 45 cycles of amplification, each cycle consisted of: melting at 95°C for one minute, annealing at 60°C for 30 seconds and extension at 72°C for one minute. Human β-actin was amplified for normalizing the quantities of transcripts of each of the above genes. The result of real-time PCR was expressed as the threshold cycle (C**T**) which was calculated using the real time cycle software. Relative expression for NGAL and NF-kB genes was calculated by 2^-ΔCT method and the differences in gene expression were compared to the healthy group and expressed as fold change (FC).

2.6. HistopathologyPreparation of paraffin sections

The kidney tissues were

- Fixated in 10% formalin
- Dehydrated through ascending grades of alcohol:
  70% alcohol: 1.5 hours
  90% alcohol: 1.5 hours
  Absolute alcohol: 3 hours
- Cleared in xylene for 4 hours
- Infiltrated by impregnating in soft pure paraffin through three different grades (each one for one hour) at 56 ^{o}C.
- Embedded in hard paraffin wax at 58 ^{o}C and oriented in blocks.

- Cut into sections of 5-6 micrometer thickness.
- Stained by using hematoxylin and Eosin (H & E)
- Mounted in DPX and covered.
- The slides were observed using optical microscope (model LX 500) and photographed using a camera iVm 5000 through the ProgRes program capture Pro 2.7.

3. Results

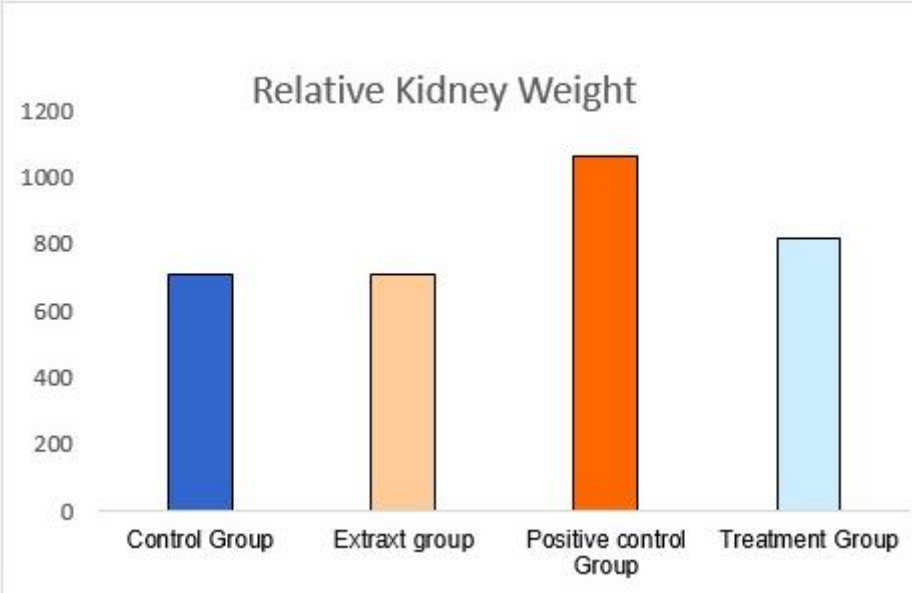


Figure. 1 Comparison between the studied groups regarding kidney weight (mg).

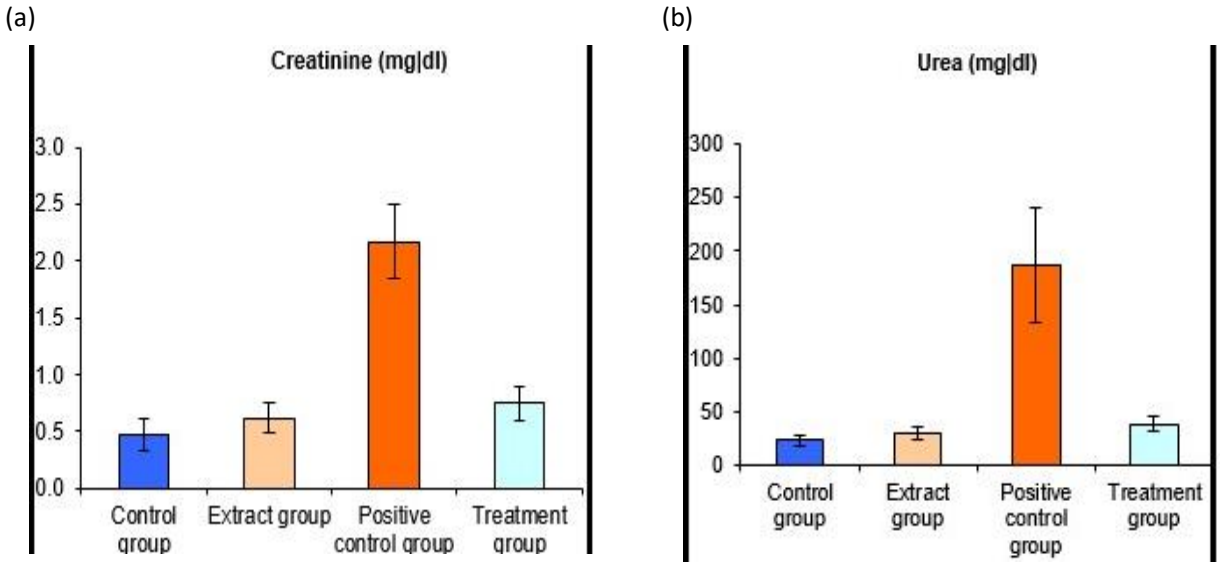


Figure. 2 a & b Comparison between the studied groups regarding serum creatinine (a) and blood urea (b) (mg/dl).

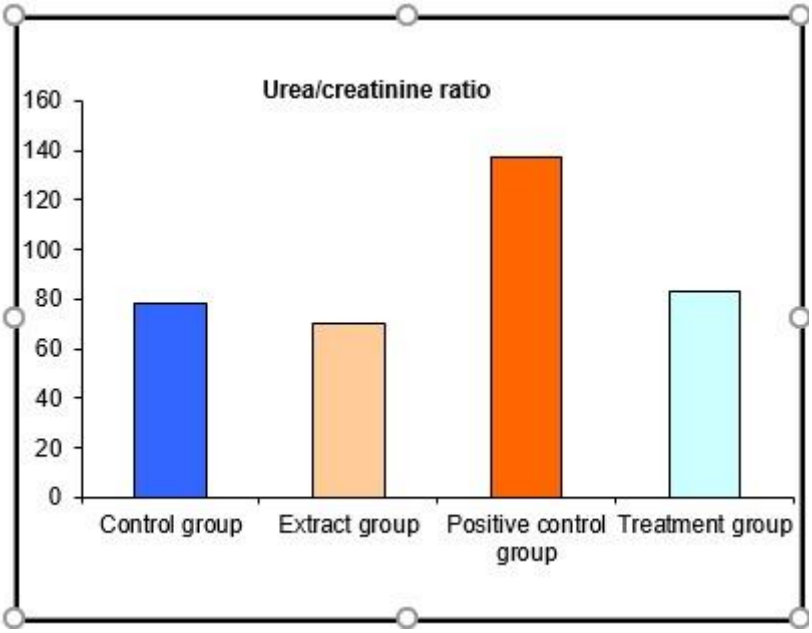


Figure. 3 Comparison between the studied groups regarding serum urea/creatinine ratio.

(a) (b)

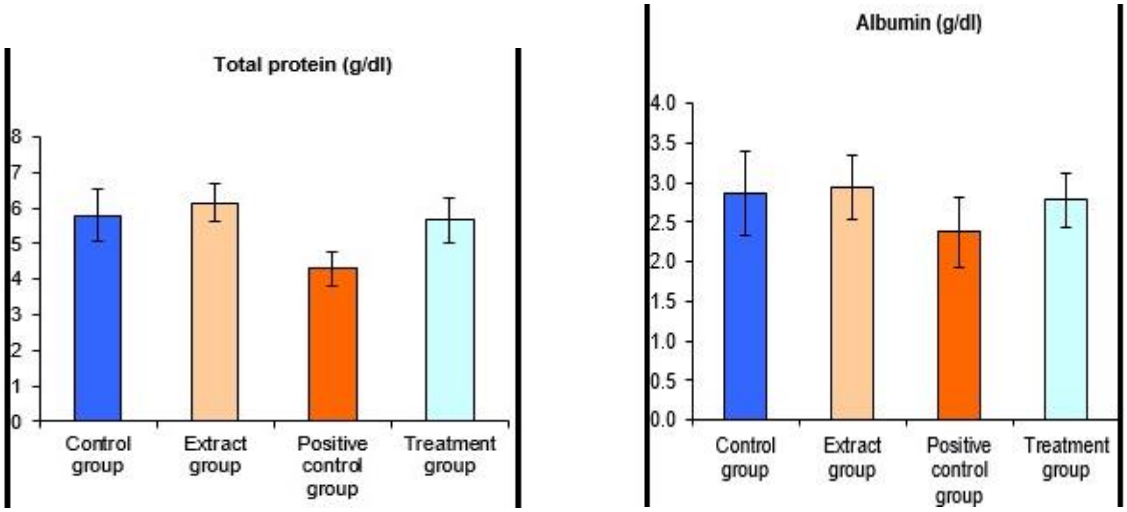


Figure. 4 a & b Comparison between the studied groups regarding total protein (a) and albumin (b) (g/dl).

(a) (b)

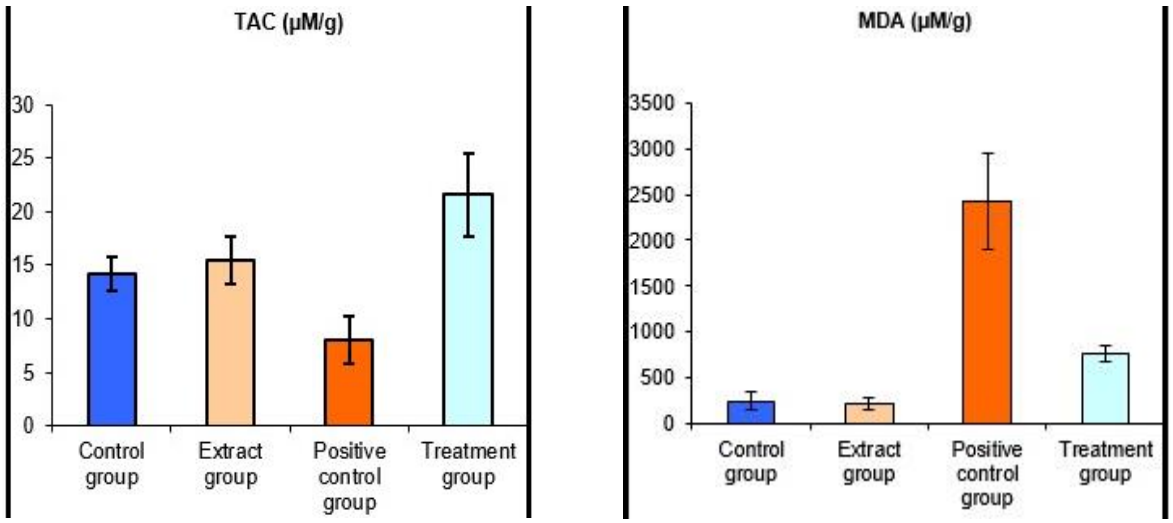


Figure. 5 a & b Comparison between the studied groups regarding MDA (a) and TAC (b) (µM/g).

(a) (b)

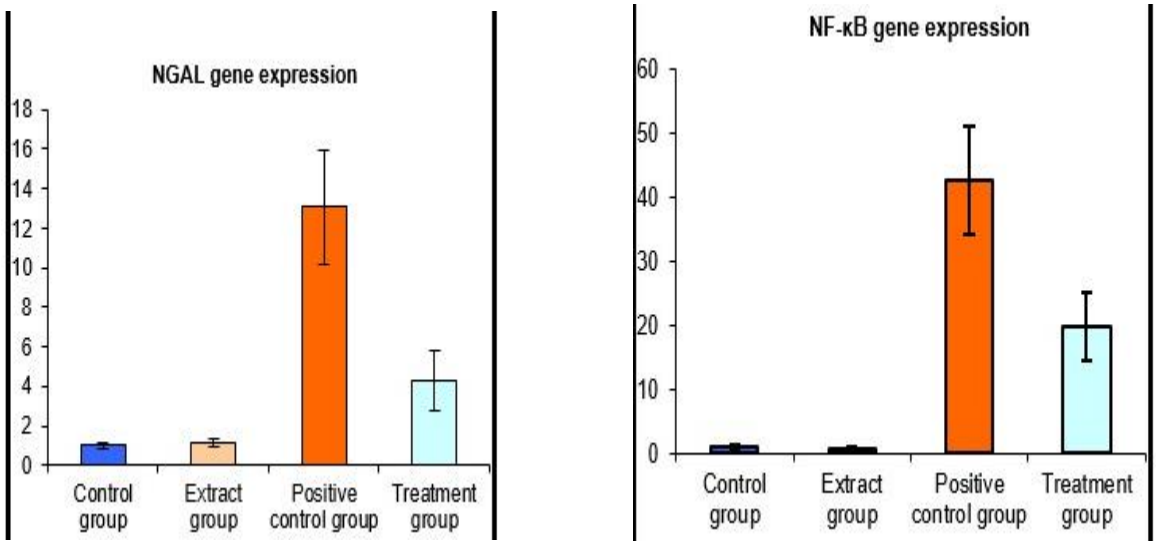


Figure. 6 a & b Comparison between the studied groups regarding NGAL (a) and NF-ƙB (b) gene expression levels.

As illustrated in fig. 1, comparing initial and final rat wright between the studied groups did not reveal any significant differences. However, a highly significant difference ($p < 0.01$) was observed comparing kidney weight among the studied groups. Furthermore, post hoc analysis by LSD revealed a highly significant difference ($p < 0.01$) in kidney weight comparing the positive control group to the control group, extract group and treatment group, in addition to a significant difference ($p < 0.05$) comparing the treatment group to the extract group

As shown in fig. 2, highly significant differences ($p < 0.01$) in both serum creatinine (fig. 2a) and blood urea (fig. 2b) levels were observed comparing the studied groups. Post hoc analysis by LSD revealed a highly significant difference ($p < 0.01$) in serum creatinine comparing the positive control group to the control group, extract group and treatment group, in addition to a highly significant difference in the treatment group compared to the control group. Post hoc analysis has also revealed a highly significant difference ($p < 0.01$) in blood urea levels in the positive control group compared to the control group, extract group and treatment group.

As revealed in fig. 3, highly significant differences ($p < 0.01$) in urea/creatinine ratio were observed comparing the studied groups. Post hoc analysis by LSD revealed a highly significant difference ($p < 0.01$) in urea/creatinine ratio comparing the positive control group to the control group, extract group and treatment group.

As shown in fig. 4, highly significant differences ($p < 0.01$) were observed in both total protein (fig. 4a) and albumin (fig. 4b) levels comparing the studied groups. Post hoc analysis by LSD revealed a highly significant difference in both total protein and albumin comparing the positive control group to the control group, extract group ($p < 0.01$) and treatment group ($p < 0.01$ and $p=0.014$, respectively), in addition to a significant difference ($p = 0.033$) in total protein in the treatment group compared to the extract group.

As illustrated in fig. 5**,** highly significant differences ($p < 0.01$) were observed in both MDA (fig. 5a) and TAC (fig. 5b) levels comparing the studied groups. Post hoc analysis by LSD revealed highly significant differences ($p < 0.01$) in both MDA and TAC comparing the positive control group to the control group, extract group and treatment group, in addition to a highly significant difference ($p < 0.01$) in the treatment group compared to both the control group and the extract group.

As shown in fig. 6, highly significant differences ($p < 0.01$) were observed in both NGAL (fig. 6a) and NF-ƙB (fig. 5b) expression levels comparing the studied groups. Post hoc analysis by LSD revealed highly significant differences ($p < 0.01$) in both NGAL and NF-ƙB gene expression comparing the positive control group to the control group, extract group and treatment group, in addition to a highly significant difference ($p < 0.01$) in the treatment group compared to both the control group and the extract group.

Table 2. Correlation between NGAL and NF-ƙB gene expression with body weight, relative kidney weight and the studied biochemical markers.

	FC (NGAL)		**FC (NF-ƙB)**	
	Positive control group			
	r	**P-value**	**r**	**P-value**
Initial rat weight (g)	0.093	0.742	-0.264	0.341
Final rat weight (g)	0.011	0.970	-0.250	0.369
Relative Kidney weight	-0.121	0.624	-0.271	0.440
	Treatment group			
	r	**P-value**	**r**	**P-value**
Initial rat weight (g)	0.207	0.459	0.243	0.383
Final rat weight (g)	0.039	0.889	0.063	0.825
Relative Kidney weight	-0.262	0.372	-0.266	0.351
	Positive control group			
	r	**P-value**	**r**	**P-value**
Creatinine (mg/dl)	**0.824****	**0.000****	**0.631***	**0.012***
Urea (mg/dl)	**0.933****	**0.000****	**0.717****	**0.003****
Urea/Creatinine ratio	**0.872****	**0.000****	**0.685****	**0.000****
	Treatment group			
	r	**P-value**	**r**	**P-value**
Creatinine (mg/dl)	**0.559***	**0.030***	**0.580***	**0.024***
Urea (mg/dl)	**0.550***	**0.033***	**0.612***	**0.015***
Urea/Creatinine ratio	**0.524***	**0.000****	**0.601***	**0.031***
	Positive control group			
	r	**P-value**	**r**	**P-value**
Total protein (g/dl)	**-0.643***	**0.010***	**-0.911****	**0.000****
Albumin (g/dl)	**-0.672****	**0.006****	**-0.820****	**0.000****
	Treatment group			
	r	**P-value**	**r**	**P-value**
Total protein (g/dl)	**-0.639***	**0.010***	**-0.668****	**0.006****
Albumin (g/dl)	**-0.818****	**0.000****	**-0.836****	**0.000****
	Positive control group			
	r	**P-value**	**r**	**P-value**
MDA (µM/g)	**0.868****	**0.000****	**0.600***	**0.018***
TAC (µM/g)	**-0.618***	**0.014***	**-0.671****	**0.006****

	Treatment group			
	r	**P-value**	**r**	**P-value**
MDA (µM/g)	**0.671****	**0.006****	**0.624***	**0.013***
TAC (µM/g)	**-0.627***	**0.014***	**-0.592***	**0.020***
	Positive control group			
	r	**P-value**	**r**	**P-value**
FC (NGAL)	–	–	**0.732****	**0.002****
FC (NF-ƙB)	**0.732****	**0.002****	–	–
	Treatment group			
	r	**P-value**	**r**	**P-value**
FC (NGAL)	–	–	**0.990****	**0.000****
FC (NF-ƙB)	**0.990****	**0.000****	–	–

p-value > 0.05: Non-significant; *: *p*-value < 0.05: Significant; **: *p*-value < 0.01: Highly significant (HS); r: Spearman correlation coefficient.

A significant ($p < 0.05$) positive correlation was observed between serum creatinine and both NGAL and NF-ƙB gene expression levels in the positive control group (r=0.824 and 0.631, respectively), and the treatment group (r=0.559, 0.580, respectively). In addition, a significant ($p < 0.01$) positive correlation was observed between blood urea and both NGAL and NF-ƙB gene expression in the positive control group (r=0.933and 0.717, respectively), and treatment group (r=0.550, 0.612, respectively). A significant ($p < 0.01$) positive correlation was also observed between urea/creatinine ratio with both NGAL and NF-ƙB gene expression in the positive control group (r=0.872 and 0.685, respectively), and treatment group (r=0.524, 0.601, respectively). On the other hand, a significant ($p < 0.05$) negative correlation was observed between total protein and both NGAL and NF-ƙB gene expression in the positive control group (r= -0.643, -0.911, respectively) and treatment group (r= -0.639, -0.668, respectively). In addition, a significant ($p < 0.01$) negative correlation was observed between albumin levels and both NGAL and NF-ƙB expression levels in the positive control group (r= -0.672 and -0.820, respectively), and treatment group (r= -0.818, -0.836, respectively).

Another significant ($p < 0.05$) negative correlation was observed between total protein and both NGAL and NF-ƙB gene expression in the positive control group (r= -0.643, -0.911, respectively) and treatment group (r= -0.639, -0.668, respectively). In addition, a significant ($p < 0.01$) negative correlation was observed between albumin levels and both NGAL and NF-ƙB gene expression in the positive control group (r= -0.672 and -0.820, respectively) and treatment group (r= -0.818, -0.836, respectively). However, a significant ($p < 0.05$) positive correlation was observed between MDA and both NGAL and NF-ƙB gene expression in the positive control group (r= 0.868, 0.600, respectively) and treatment group (r= 0.671, 0.624, respectively). On the other hand, a significant ($p < 0.05$) negative correlation was observed between TAC levels and both NGAL and NF-ƙB expression levels in the positive control group (r= -0.618and -0.671, respectively) and treatment group (r= -0.627*, -0.592, respectively). Finally, spearman correlation analysis has also revealed a highly significant ($p < 0.01$) positive correlation between NGAL and NF-ƙB gene expression levels in the positive control group (r=0.732) and treatment group (r= 990).

3.1. Histopathological results

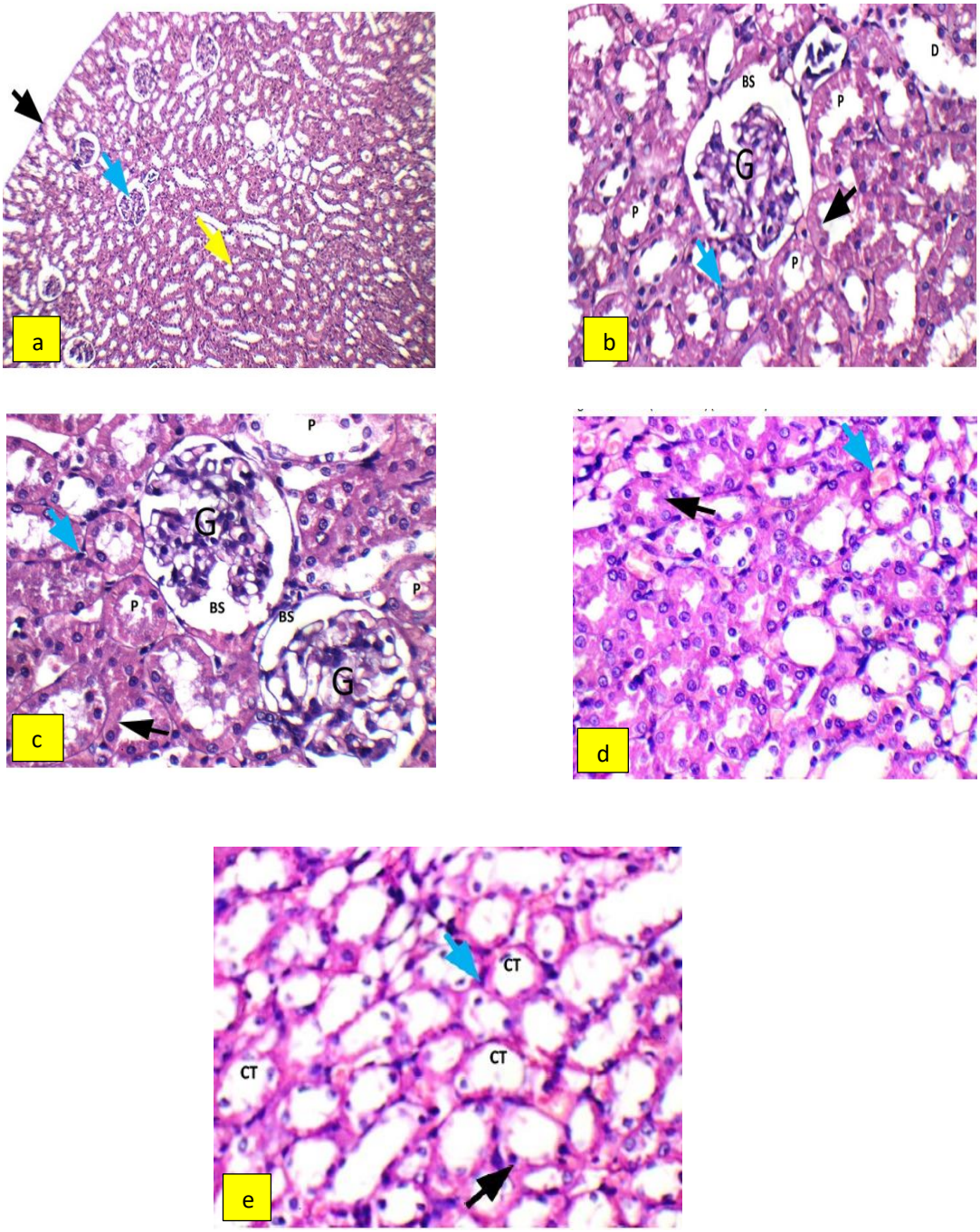
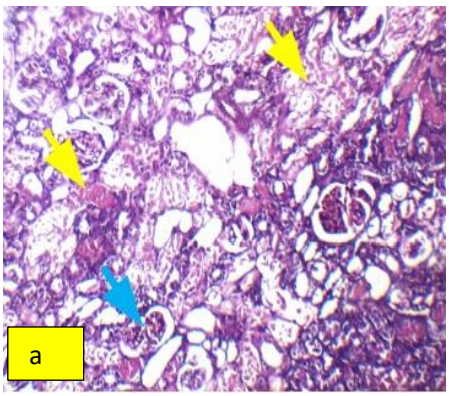


Figure. 7 (a-e) Histopathological study for kidney tissues of the control group **a: Control:** kidney showing average renal capsule (black arrow), average glomeruli (blue arrow), and average tubules (yellow arrow) (H&E X 200). **b: Control:** high power view showing average glomeruli (G) with average Bowman's spaces (BS), average proximal tubules (P) with preserved brush borders (black arrow), average distal tubules (D), and average interstitium (blue arrow) (H&E X 400). **c: Control:** another view showing average glomeruli (G) with average Bowman's spaces (BS), average proximal tubules (P) with preserved brush borders (black arrow), average distal tubules (D), and average interstitium (blue arrow) (H&E X 400. **d: Control:** another view in cortico-medullary area showing average tubules (black arrow), and average blood vessels (blue arrow) (H&E X 400). **e: Control:** another view in renal medulla showing average collecting tubules (CT) with average epithelial lining (black arrow) and average interstitium (blue arrow) (H&E X 400).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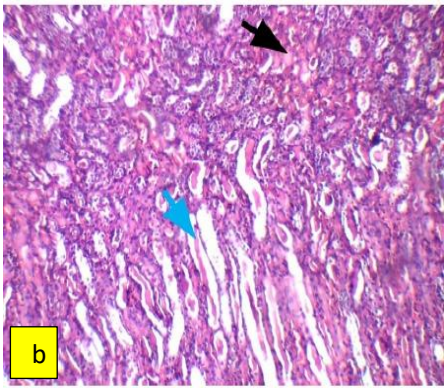
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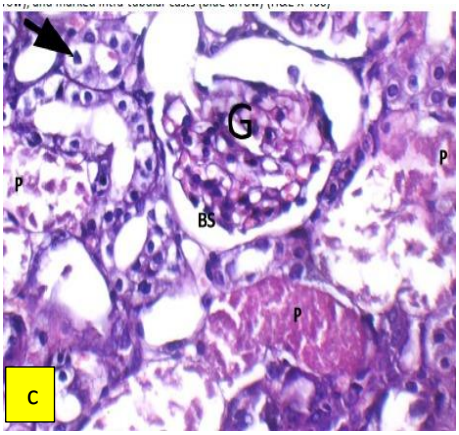
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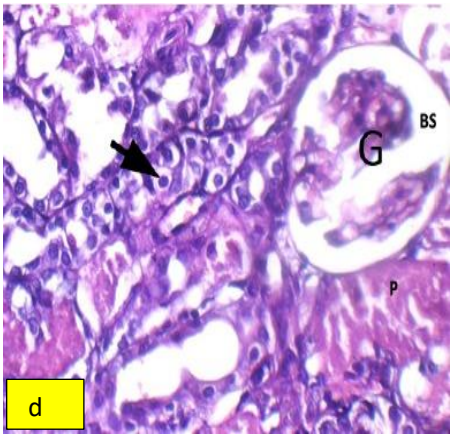
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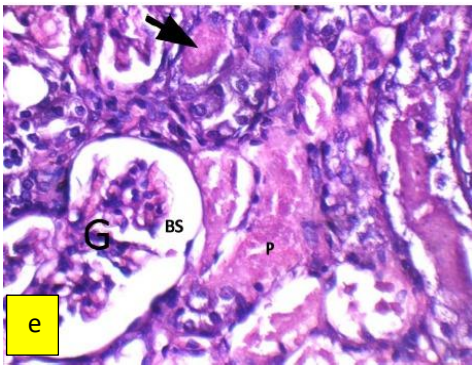
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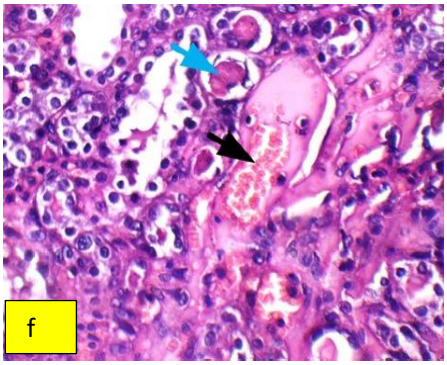
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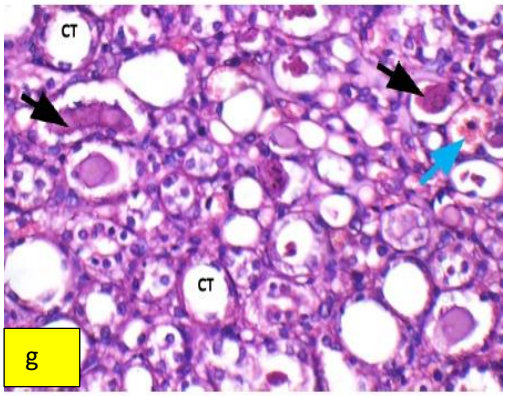
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CT
g

Figure. 8 (a-g) Histopathological study for kidney tissues of the positive control group. **a: positive control:** kidney showing average renal capsule (black arrow), scattered small-sized glomeruli (blue arrow), and marked tubular necrosis (yellow arrow) (H&E X 200). **b: positive control:** another view in cortico-medullary area showing marked tubular necrosis (black arrow), and marked intra-tubular casts (blue arrow) (H&E X 400). **c: positive control:** high power view showing average glomeruli (G) with average Bowman's spaces (BS), and markedly necrotic proximal tubules (P), and others with markedly oedematous epithelial lining (black arrow) (H&E X 400). **d: positive control:** another view showing atrophied glomerulus (G) with widened Bowman's spaces (BS), and markedly necrotic proximal tubules (P), and others with markedly edematous epithelial lining (black arrow) (H&E X 400). **e: positive control:** another view showing small-sized glomerulus (G) with widened Bowman's spaces (BS), and markedly necrotic proximal tubules (P), and others with intra-tubular casts (black arrow) (H&E X 400). **f: positive control:** another view in cortico-medullary area showing markedly dilated congested blood vessels (black arrow), and marked intra-tubular casts (blue arrow) (H&E X 400).

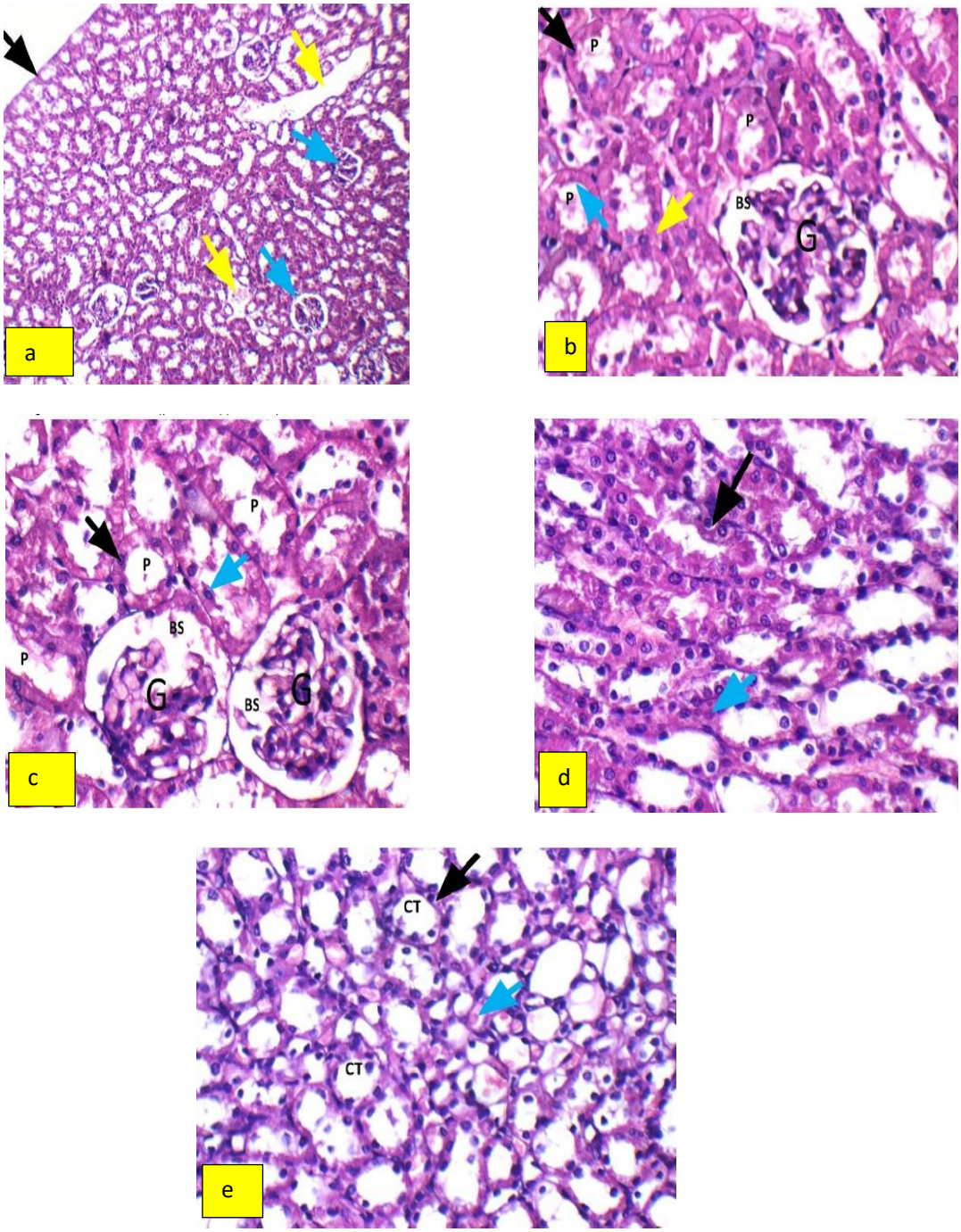

Figure. 9 (a-e) Histopathological study for kidney tissues of the extract group. **a: Extract:** kidney showing average renal capsule (black arrow), average glomeruli (blue arrow), and mildly dilated congested blood vessels (yellow arrow) (H&E X 200). **b: Extract:** high power view showing average glomeruli (G) with average Bowman's spaces (BS), proximal tubules (P) with average epithelial lining (black arrow) and preserved brush borders (blue arrow), and average interstitial blood vessels (yellow arrow) (H&E X 400). **c: Extract:** another view showing average glomeruli (G) with average Bowman's spaces (BS), proximal tubules (P) with average epithelial lining (black arrow) and partial loss of brush borders (blue arrow) (H&E X 400). **d: Extract:** another view in cortico-medullary area showing average tubules (black arrow), and average interstitium (blue arrow) (H&E X 400). **e: Extract:** another view in renal medulla showing average collecting tubules (CT) with average epithelial lining (black arrow) and average interstitium (blue arrow) (H&E X 400).

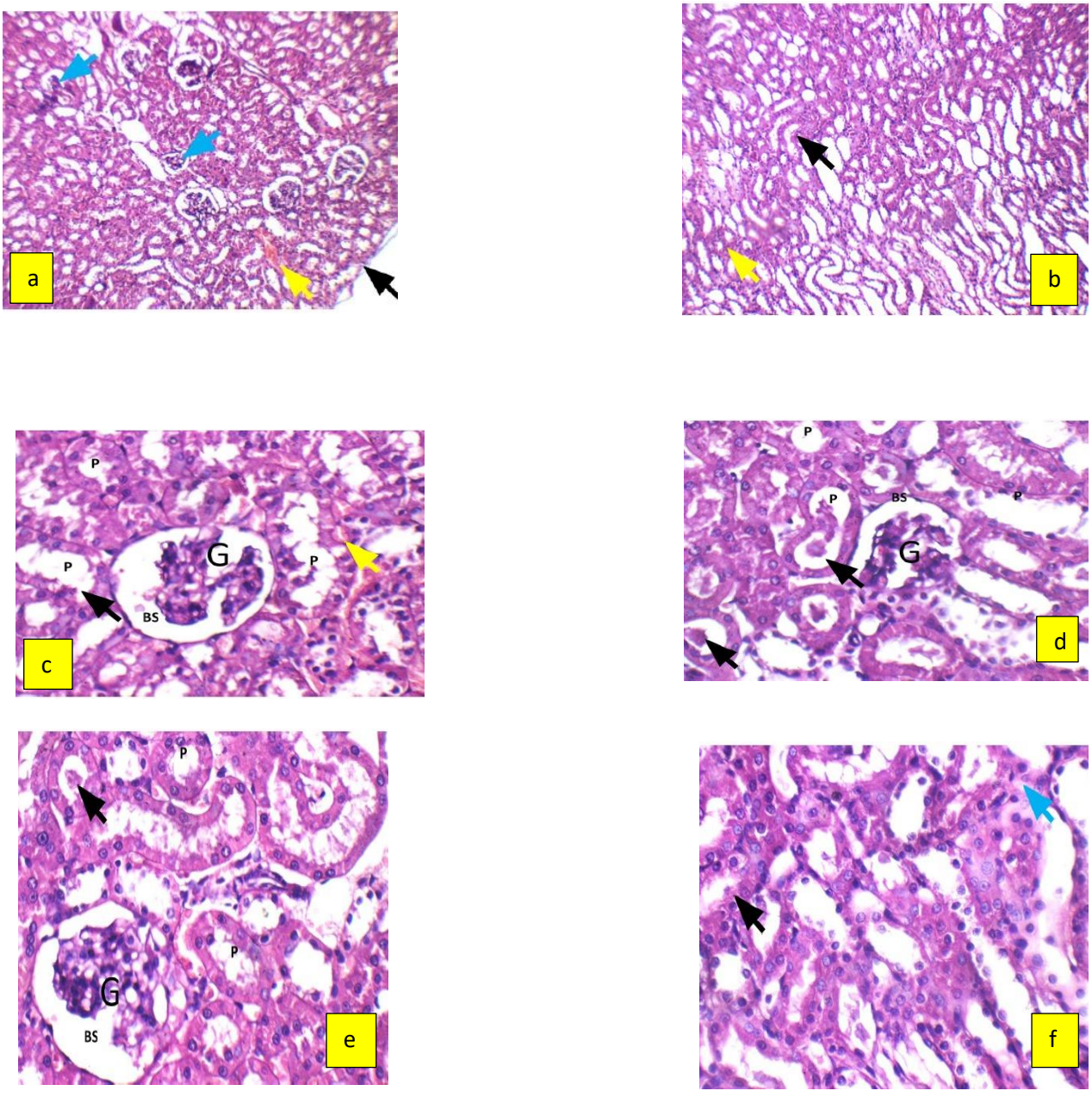

Figure. 10 (a-g) Histopathological study for kidney tissues of the treatment group. **a: Treatment:** kidney showing average renal capsule (black arrow), scattered small-sized glomeruli (blue arrow), and mildly dilated congested blood vessels (yellow arrow) (H&E X 200). **b: Treatment:** another view in cortico-medullary area showing average tubules (black arrow), and average interstitium (blue arrow) (H&E X 200).**c: Treatment:** high power view showing small-sized glomeruli (G) with widened Bowman's spaces (BS), scattered proximal tubules (P) with partial loss of brush borders (black arrow), and mildly congested interstitial blood vessels (yellow arrow) (H&E X 400).
d: Treatment: another view showing small-sized glomeruli (G) with average Bowman's spaces (BS), proximal tubules (P) with intra-tubular casts (black arrow) (H&E X 400). **e: Treatment:** another view showing small-sized glomeruli (G) with widened Bowman's spaces (BS), few proximal tubules (P) with intra-tubular casts (black arrow) (H&E X 400). **f: Treatment:** another view in cortico-medullary area showing average tubules (black arrow), and average interstitium (blue arrow) (H&E X 400). **g: Treatment:** another view in renal medulla showing average collecting tubules (CT) with average epithelial lining (black arrow) and mildly congested peri-tubular capillaries (blue arrow) (H&E X 400).

As seen in fig. 7, kidney showed average renal capsule, average glomeruli with average Bowman's spaces, average proximal tubules with preserved brush borders, average distal tubules, average cortico-medullary area, and renal medulla showed average collecting tubules with average interstitium (Fig. 7 a, b, c, d, e).

As seen in fig. 8, kidney showed average renal capsule, scattered small-sized and atrophied glomeruli with widened Bowman's spaces, markedly necrotic proximal tubules, and others with oedematous epithelial lining and intra-tubular casts, cortico-medullary area showed markedly necrotic tubules with marked intra-tubular casts and markedly dilated congested blood vessels, and renal medulla showed collecting tubules with marked intra-tubular casts and mildly congested peri-tubular capillaries (Fig. 8 a, b, c, d, e, f, g).

As seen in fig. 9 kidney showed average renal capsule, average glomeruli with average Bowman's spaces, scattered proximal tubules with partial loss of brush borders, average distal tubules, mildly dilated congested blood vessels, average cortico-medullary area, and renal medulla showed average collecting tubules with average interstitium (Fig. 9 a, b, c, d, e).

As seen in fig. 10 kidney showed average renal capsule, scattered small-sized glomeruli with widened Bowman's spaces, scattered proximal tubules with partial loss of brush borders and intra-tubular casts, mildly congested interstitial blood vessels, average cortico-medullary area, and renal medulla showed average collecting tubules with mildly congested peri-tubular capillaries (Fig. 10 a, b, c, d, e, f, g).

4. Discussion

Inorganic mercury poisoning causes AKI [7] which leads to various deleterious impacts, such as proteinuria and accumulation of end products such as creatinine and urea [10] In the present study, assessing the lethality of $HgCl_2$ in kidneys revealed a highly significant increase in kidney weight in the positive control group compared to the control group, extract group and treatment group. These results are in consistence with several previous studies which indicated that $HgCl_2$ poisoning results in a reliable increase in rat kidney weight [34, 35, 36], however, in the present study, kidney weight has significantly decreased and almost recovered in the treatment group compared to the positive control group (fig. 1). On the other hand, the current results revealed an almost 4.5-fold, and an 8-fold increase in serum creatinine and blood urea, respectively, along with a significant increase in urea/creatinine ratio in the positive control group compared to the initial levels in the control group, however, a remarkable highly significant decrease in each of them was observed in the rhubarb extract-treated group compared to the positive control group (fig. 2 a, b and fig. 3).

Total protein and albumin levels have shown significant declines in the positive control group compared to the initial levels in the control group. Again, these declines were ameliorated and almost recovered in the rhubarb extract- treated group (fig. 4 a, b). The current findings are in agreement with previous studies which stated that patients with AKI presented a better renal recovery rate as well as better parameters of renal function following treatment with rhubarb root extract [37].

The current results are concomitant with previous studies which revealed that rhubarb extract could ameliorate $HgCl_2$-induced ARF and that its anthraquinones in particular are the effective components responsible for this activity in rhubarb extract [21]. Anthraquinones are the main characteristic as well as pharmacodynamic ingredients of rhubarb extract [38, 39]. The proportion of anthraquinones ranges from 3 to 5% in different species [40]. More than 30 anthraquinones have been isolated and identified from rhubarb extract [41], of which free anthraquinones, such as Rhein, possess the ability of protecting kidney [42] and inhibiting the formation of renal fibrosis [43]. In addition, rhubarb tannins have also been documented to have nephroprotective effects in animal models of AKI [44], in fact, since the 1880s, rhubarb tannins have been discovered to reduce blood urea nitrogen and possess a nephroprotective effect [45].

Since oxidative stress plays a well-established role in the pathogenesis of $HgCl_2$- induced renal intoxication [46], MDA and TAC were measured in kidney tissue homogenate, and the results revealed a 10-fold increase in MDA levels in the positive control group compared to both the control and the extract groups, however, MDA levels have shown a parallel dramatic decrease in the treatment group compared to the positive control group. Meanwhile, TAC levels have shown a highly significant decrease in the positive control group compared to both the control and the extract groups, which was accompanied by a parallel significant increase in the treatment group compared to all of the studied groups, and exceeding even its initial level in the control group (fig. 5 a, b), which reflects the beneficial effects of rhubarb root extract as a powerful antioxidant.

The current results are in accordance with previous studies which revealed that rhubarb extract possesses antioxidant and anti-inflammatory activities, efficiency to treat various inflammation-related diseases, and powerful therapeutic potential for kidney injury [47]. The current results are also in agreement with other studies [23, 48] which revealed that rhubarb extract could efficiently increase the levels of antioxidant and anti-inflammatory agents in rats, and thus could further alleviate renal damage caused by oxidative stress and inflammation.

In fact, in the context of kidney damage, oxidative stress and inflammation constitute a vicious cycle in which oxidative stress induces inflammation through various underlying molecular mechanisms [49], one of which is the NF-ƙB - driven inflammation [10]. In addition, it has been revealed that $HgCl_2$ administration results in a cascade reaction that ends with free radical production and increased rate of NF-ƙB expression, leading to inflammation, and tissue damage [50].

In the current study, measuring the expression of NF-ƙB gene in kidney tissues revealed a 42-fold increase in the levels of NF-ƙB gene expression in the positive control group compared to both the control and the extract groups, which was accompanied by a significant parallel dramatic decrease in the treatment group to reach almost half its level in the positive control group (fig. 6 a). A significant positive correlation was observed between NF-ƙB gene expression along with creatinine, urea, and MDA levels, which was obvious in the positive control group and the treatment group. On the other hand, a significant negative correlation was observed between NF-ƙB gene expression along with total protein, albumin, and TAC levels, and this was also obvious in the positive control group and the treatment group (table 2).

The current results are in accordance with several previous studies which suggested that inhibiting or blocking the NF-ƙB pathway could attenuate the inflammatory response and oxidative stress in kidney tissues of rats with experimentally-induced AKI [12]. The current results are also in agreement with other studies [51, 52] which reported that rhubarb extract prevents AKI by inhibiting NF-ƙB gene expression.

The current results about the efficiency of rhubarb root extract in down regulating NF-ƙB gene expression are also supported by previous studies which demonstrated that three species of rhubarb extract could inhibit the experimentally-induced activation of the NF-ƙB pathway, thereby reducing the induced inflammatory response [53] Several studies have tried to elucidate the mechanism through which rhubarb extract could attenuate NF-ƙB gene expression. It has been, suggested that the efficiency of rhubarb extract to downregulate NF-ƙB gene expression is mediated through upregulating SIRT1 gene [54], and since SIRT1 acts as an inhibitor of NF-ƙB activation [55], so that, reducing or inhibiting the activity of SIRT1, results in an inflammatory response, triggered by the NF-ƙB-dependent activation of genes for cytokines.

Conversely, compounds increasing SIRT1 level, such as those contained in rhubarb extract, are able to reduce the inflammatory response mediated by NF-*κ*B [56]. On the other and, based on the fact that NF-ƙB is switched from resting to active state by nuclear translocation with subsequent activation of gene transcription [57], it has been proposed that the anti-inflammatory effect of rhubarb extract is mediated through regulating NF-ƙB nuclear transloation [58].

Considering the changes observed in the expression of NAGL gene in the current studied animal groups, a 13-fold increase in the NAGL gene expression was observed in the positive control group compared to both the control and the extract groups. This dramatic increase, however, was accompanied by a highly significant parallel recovery in the treatment group, where NAGL gene expression decreased to almost one third its level in the positive control group (fig. **6 b**), which is in accordance with a previous study which revealed that rhubarb extract can efficiently reduce the expression of NGAL in rabbits with kidney injury, and could successfully prevent kidney injury [59].

Spearman correlation analysis revealed a significant positive correlation between NAGL gene expression along with creatinine, urea, and MDA levels. This was obvious in the positive control group and the treatment group. Moreover, a significant negative correlation was observed between NAGL gene expression along with total protein, albumin, and TAC levels which was also obvious in the positive control group and the treatment group (table **2**).

The current results are in accordance with previous studies which stated that NAGL is an acute phase protein whose expression is upregulated in human epithelial cells under different inflammatory conditions [60] and is one of the top upregulated genes in damaged kidneys [61] The current results are also in agreement with other studies which revealed a significant upregulation in the levels of NGAL mRNA expression in animals administrating $HgCl_2$ compared to the control group [1]. The current results are also in accordance with previous studies which revealed that NGAL plays an important role in systemic inflammation and oxidative stress [62]. In addition, NAGL has been proven to prevent the inflammation-induced kidney damage [63].

The efficiency of rhubarb extract to downregulate NAGL gene expression in the present study might be understood in light of the spearman correlation analysis which revealed a significant positive correlation between NAGL gene expression and NF-ƙB gene expression in both the positive control and treatment groups (table **2**). Several studies have revealed that NAGL gene expression is upregulated by activation of NF-ƙB [60, 64], and since rhubarb extract has been proven to be an efficient down-regulator of NF-ƙB, so that, it can be proposed that rhubarb extract downregulates NAGL through downregulation of NF-ƙB gene expression.

5. Conclusion

In conclusion, based on the provided observational evidences of the current study, it can be proposed that the ameliorating effect of aqueous rhubarb root extract against $HgCl_2$-induced AKI is mediated through downregulation of NF-ƙB and NGAL gene expression in kidney tissues, bringing them back to near normal levels. Since downregulation of both genes is an essential goal in the amelioration and/or treatment of AKI in general, therefore, further studies concerning the potential therapeutic role of rhubarb extract in other models of AKI, are urgently needed, hoping that aqueous rhubarb root extract can be developed as an antidote or chelating pharmaceutical agent in the treatment of different aspects of AKI.

6. Abbreviations

ISN: International Society of Nephrology; AKD: acute kidney disease; $HgCl_2$: mercuric chloride; ARI: acute renal injury; NF-ĸB: Nuclear factor- kappa B; AKI: acute kidney injury; NAGL: neutrophil-associated gelatinase lipocalin; ARF: acute renal failure; TCM: traditional Chinese medicine; b.w.: body weight; BUN: Blood urea nitrogen; MDA: Malondialdehyde; TAC: total antioxidant capacity; qRT-PCR: quantitative real-time PCR; TC: threshold cycle; FC: fold change.

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