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Impact of Gum acacia on oxidative stress and apoptosis in Adenine-induced CKD animal model

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ABSTRACT

Objective: This study was conducted in order to investigate the protective effect of *Gum acacia* (GA) on adenine-induced chronic kidney disease (CKD) on anti-inflammatory and apoptotic markers. **Materials and methods:** Sprague-Dawley rats (n = 30) were divided into three groups at random and given the following treatments for four weeks straight: group 1: Control: continued on the same diet without treatment until the study's conclusion; group 2: Adenine: changed to a powder diet containing adenine (0.75 % w/w in feed); group 3: Adenine + GA: adenine was supplied in the feed as in the second group for 4 weeks, then gum acacia was added to the drinking water at a concentration of 15 %t w/v. Adenine feeding was used to cause CKD in rats, and gum acacia, GA, was used to cure it concurrently (15 % in drinking water). **Results:** Adenine increased kidney function, lipid peroxidation, P53, caspase-3, Bax, and transforming growth factor (TGF-ß) compared to normal controls. While some antioxidants and B-cell lymphoma 2 (Bcl-2) were on the decline.Concurrent GA therapy considerably lowered these negative effects. When combined, GA lowers oxidative stress and inflammation in CKD-affected rats. **Conclusions:** We come to the conclusion that the induction of CKD in rats by the administration of adenine is accompanied with oxidative stress, apoptosis, and inflammation. The benefits of GA in adenine-induced CKD are linked to the reduction of adenine-induced oxidative stress, apoptosis, and inflammation.

1. Introduction

The clinical condition known as chronic kidney disease (CKD), which is characterised by gradual renal failure, is a severe and expanding global health concern [1].Diabetes, stroke, cardiovascular disease, and other disorders, as well as urinary tractemia and full loss of renal function, can all contribute to kidney failure. The development and

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pathophysiology of CKDare studied using rodent models, as well as to develop therapeutic methods [2]. CKD affects 500 million people worldwide (1 in every 10 adults), and nearly half of those over 75 have a significant risk of morbidity and mortality. Inflammation and oxidative stress are two significant features of the disease's pathophysiological underpinnings and human and animal impacts. Inflammatory mediators with high plasma concentrations, such as C-reactive proteins and tumour necrosis factor, various cytokines, and different indicators of oxidative stress were detected in CKD patients and experimental animals [3]. This findings indicate that apoptosis plays a critical role in CKD progression [4].

According to the Joint Expert Committee for Food Additives of the Food and Agriculture Organization and World Health Organization, Gum Acacia (also known as gum arabic, GA) is "a dried exudate derived from the stems of Acacia Senegal tree or closely similar species of Acacia (family Leguminosae)" (JECFA). It has been suggested that GA may play a role in altering how mammalian cells function physiologically. For instance, in adenine-induced CKD, GA has shown favorable benefits on physiological, biochemical, and behavioral impacts [5]. In rats with CRF brought on by adenine, GA therapy has been demonstrated to alleviate several biochemical, physiological, and behavioral consequences and to modify immunity in mice [6].

In the current work, using several parameters, including TGFß, Bax, BCl-2, as well as the generation of reactive oxygen species, we investigate the anti-inflammatory and apoptotic markers to study the protective effect of GA on adenine-induced CKD. This is done to determine the effects of GA treatment on rats with adenine-induced CKD. in order to study the protective effect of GA on adenine-induced CKD, using several parameters such as TGFß, Bax, BCl-2, as well as the generation of reactive oxygen species, to study the effects of GA treatment on rats with adenine-induced CKD. The findings of our study will help to clarify how GA's advantageous effects work.

2. Material and Methods

2.1. Animals

Thirty male Sprague-Dawley rats (9-10 weeks old, initial weight 230± 7 g) were bred for 7 days before being used in tests under normal conditions (temperature 25°C, humidity 50-60%, humidity 12/12). (24-hour light/dark cycle) received from Cairo, Egypt's Serum and Vaccine Laboratory. All animal-related procedures and care had been done in compliance with international laws and policies (Mansoura University Guide for the Care and Use of Laboratory Animals).

2.2. Experimental design

Three groups of ten rats each were formed by randomly dividing the animals. The first group was provided the same food with no therapy (control), until the end of the research.

For four weeks, Adenine, Sigma, St. Louis, Missouri, USA, 0.75 % w/w in feed was added to the second group's powdered diet.

Adenine was given for 4 weeks before Gum acacia to third group (treatment group) and Gum acacia was given to rats in the drinking water at doses of 15% w/v for four weeks (6).

Rats were individually placed in metabolic cages following the duration of the treatment; urine was collected after 24 hours. After that, blood was drawn from the vena cava and divided into three tubes after the rat was given intraperitoneal injections of xylazine (5 mg/kg) and ketamine (75 mg/kg) to induce anaesthesia. heparin, EDTA,

and a plain tube. For 10 minutes, blood and urine were centrifuged at 1500 rpm. The serum and plasma was then frozen at -80°C until it could be analysed with urine samples.
Until biochemical analysis, the right kidney was submerged in liquid nitrogen and preserved at -80°C.

2.3. Plasma, urine, and kidney homogenate biochemical testing

Several classic and new measures in plasma and urine had been evaluated to verify the kidney's response to adenine feeding. These in plasma include creatinine, blood urea nitrogen using a fully automated system, blood superoxide dismutase (SOD), catalase, malondialdehyde (MDA), reduced glutathione (GSH) according to [7] [8] [9] [10] [11] [12], and urine includes, protein and urinary creatinin [13]. The activity of n-acetylbeta D glucosaminidase (NAG) was measured in urine by Using Enzyme-Binding Immunoadsorption Measurement Kit (R & D Systems Inc.) obtained from the Egyptian American Company for Laboratory Services, Egypt , all biomarker levels were standardised to urine creatinine concentration, which was determined using an automated spectrophotometer, as per the manufacturer's recommendations.

2.4. Apoptotic and inflammatory markers:

Bax, caspase3, Bcl2 and P53, as well as transforming growth factor ß1 (TGF ß1) protein from kidney tissue homogenates were created using a phosphate buffer saline (PBS)/bovine serum albumin (BSA) buffer. The cells were treated for 30 minutes at room temperature with; mouse anti-FITC Bax (Cat. No. 633603); rabbit anti-active caspase-3 (cat. No. 559341); mouse anti-FITC p53 and mouse anti-FITC bcl-2 are available from BD Biosciences in Bicton, Dickinson, California, USA (Cat. No. 554218 & 554221 respectively) and mouse anti- PE TGF-ß (Cat. No. 562339). The cells were cleaned and resuspended in PBS/PSA containing 0.5 percent paraformaldehyde before being analysed using flow cytometry. Fluorescent histograms were produced using gated events with live cell side and front light scattering properties. The BD FACScanTM system was used for flow cytometry (Becton Dickinson, SanJose, CA) according to [14].

2.5. Analytical statistics: All data was presented as mean ±S.E. The data was analysed using one-way analysis of variance (ANOVA). A value of p 0.05 was used as the statistical significance criteria. SPSS Version 21 was used to conduct statistical analysis.

3. Results

3.1.Biochemical measurements:

Kidney function (creatinine and BUN) levels were elevated associated with loss of renal function in adenine-treated animals (Table 1). In rats treated with adenine, the renal injury parameter NAG, a biomarker of proximal tubule injury in urine, was significantly higher than in healthy control rats (Table 1). Gum acacia reduced all these markers of kidney damage. In addition, the urinary protein / creatinine ratio was significantly higher in rats treated with adenine than in the control group and the group treated with *Gum acacia*.

3.2. Hematological parameters:

To determine whether CKD animals had anemia, measurements of Hgb, hematocrit, and RBCs levels were measured Table 2. Administered adenine caused a significant reduction in hematological parameters as compared to controls and those treated with Gum acacia. The values of hematological parameters were improved after treatment with the Gum acacia when compared to the adenine group.

3.3. Effect of Gum acacia and adenine in antioxidant:

Rat SOD activity, GSH levels, and catalase levels were revealed to be impacted by adenine and gum acacia treatments in (Fig 1a). *Gum acacia* produced a slight but not statistically significant increase in antioxidant levels, while adenine produced a significant decrease in there levels. Treatment with *Gum acacia* greatly reduced these declines. Adenine, on the other hand, caused much higher MDA levels than controls and *Gum acacia* groups (Fig 1b).

3.4. Analysis of apoptotic and inflammatory markers:

The effect of adenine in feed with or without gum, renal apoptotic and inflammatory markers are shown in Fig 2. The effect of adenine on renal Bcl-2 rats showed a significant reduction as compared to control group (13.5±0.87) & (43.7±1.04) respectively ,($P<0.001$). while, kidney anti-apoptotic marker Bcl-2 percent count showed a substantial improvement in adenine-GA treated group(25.9±0.89) as compared to adenine group ($P<0.001$) (Fig 2a). On other hand, renal P53% counts significantly increased in the adenine-treated group compared to the control group (51.9±1.12 *vs* 13.5±0.76, respectively; P 0.001), while, after GA treatment, renal P53 % counts significantly reduced in adenine-GA treated group (32.7±as compared to adenine group($P<0.001$)(Fig 2b).

Caspase-3 in kidney tissue was significantly increased in the adenine-treated group (46.69%±0.97) compared to control (17.69±0.93) and after treated with GA significantly decreased(28.28±0.63) ($P<0.01$) (Fig 2c). The Bax in kidney tissue was significantly increased in the adenine-treated group (49.02±0.73) compared to control group (14.45±1.02), and rats treated with both adenine and GA considerably and noticeably reduced this rise (32.13±0.93) ($P<0.001$) (Fig2d).

The concentrations of the anti-inflammatory TGFß1 were significantly elevated in adenine-treated group compared to the control (50.6±0.87 & 13.9±0.65) respectively ($P<0.001$). However, the concentration of this cytokine was significantly reduced after GA-treatment (32.9±0.91) ($P<0.001$) compared to the adenine-treated rats (Fig.2e).

Discussion

Both developed and undeveloped nations, chronic kidney disease are regarded as an aggravating issue. The present guidelines are quite complex because the current approach to treating CKD focuses more on limiting its effects than on curing the illness itself [15].

The findings of the present study demonstrated that *Gum acacia* treatment improved the measured kidney function parameters, according to biochemical findings and hematological results. This is comparable to the previously described finding lung [6] [16] .

It is widely known that oxidative stress results from an imbalance between increases in reactive oxygen species (ROS) generation a subsequent decrease in the body's natural antioxidant defence mechanisms causes oxidative stress [17]. The image is connected to more and more disease problems [18]. Excessive production of free radicals and oxidant agents leads to oxidative stress, which harms genetic material, proteins, lipids, and lipoproteins as well as cell membranes (DNA). We were able to assess various markers of oxidative stress (antioxidants and antioxidant enzymes) and lipid peroxidation in plasma in order to learn more about the potential pathways directly related to the

detrimental effects of adenine on the kidney, both before and after treatment with GA. The measured data demonstrated that adenine administration dramatically increased oxidative stress and lipid peroxidation, followed by a considerable drop in antioxidant enzymes. In previous experiments, research team found that lipid peroxidation levels rose in the kidney [19] , lung [6] and cecum [16]. Homogenates of mice treated with adenine, followed by a decline in the antioxidant enzymes.

It is recognized that apoptosis and inflammation are common in CKD and could be the main cause of the resulting hypertension and other side effects [20] [21]. In this study, the measured data demonstrated that adenine administration dramatically increased p53, caspase-3 and Bax along with a decreased in the anti-apoptotic markers Bcl-2. Contrarily, rats given GA showed reduced levels of p53, Bax, and caspase-3 along with an increase in the anti-apoptotic markers Bcl-2.

Previous research revealed that the pro-apoptotic protein Bax can be directly activated by p53 to start apoptosis through the mitochondrial route [22]**,**, which significantly aids in the development of CKD [23] [24]**.** The mitochondrial cell death pathway is caused by malfunctioning mitochondria, which show membrane depolarization and fragmentation, high levels of ROS production, and the release of apoptogenic proteins (such as caspase-3) in response to stimuli [24]. Cell death is delayed as a result of the anti-apoptotic B-cell lymphoma-2 (Bcl-2) protein's ability to stop the activation of apoptotic caspases in the mitochondria[25]. It was discovered that Bcl-2 may be downregulated in kidney disorders, which would therefore increase a Bax-induced apoptotic effect. Here, GA raised kidney Bcl-2 levels while lowering caspase-3 levels. Numerous studies indicated that caspase dependent apoptosis played a significant role in the emergence of tubular damage and renal failure [26] [15]. Therefore, GA has the capacity to control apoptotic pathways, stop renal tissue injury, and then stop the evolution of fibrosis.

Inflammation has a proven role in the aetiology of CKD. It has been established that renal illness is influenced by TGF- β 1, a strong cytokine that promotes fibrosis and apoptosis [27]. After the onset of obstruction, it has been demonstrated that renal TGF- β 1 mRNA expression increases significantly, and patients with ureteric calculi have higher plasma levels of TGF- β 1 [28]. This anti-inflammatory cytokine's concentration was somewhat decreased when GA and adenine were administered together [16]. Directly proof that GA has anti-inflammatory properties [29][30]. Results from the current study showed that TGF- β1 levels in adenine-treated group were elevated than that in control group. Our results showed that the renal tissue's TGF- β1levels significantly decreased after a GA treatment.

Conclusions: We studied the role of *Gum acacia* (GA) on avoiding CKD in rats fed adenine and found that it was effective in minimising a number of biochemical and physiological changes brought on by adenine. It was conclusion that the induction of CKD in rats by the administration of adenine is accompanied with oxidative stress, apoptosis, and inflammation. The benefits of GA in adenine-induced CKD sare associated with a decrease in oxidative stress, apoptosis, and inflammation.

Participant consent

not required.

Not applicable Consent for Publication

Contribution of the author

All authors had input to the study's conception and design. The material preparation, data collection, and analysis were handled by Safaa A. Derbala and Mona S. Gouida. The manuscript's first draught was written by Safaa A. Derbala, and all of the writers gave input on earlier draughts. All authors read and gave their approval to the final draught.

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Contrary Interests

The writers have not disclosed any financial or professional conflicts of interest.

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Availability of the code: Not applicable

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Table 1: Biomarkers parameters

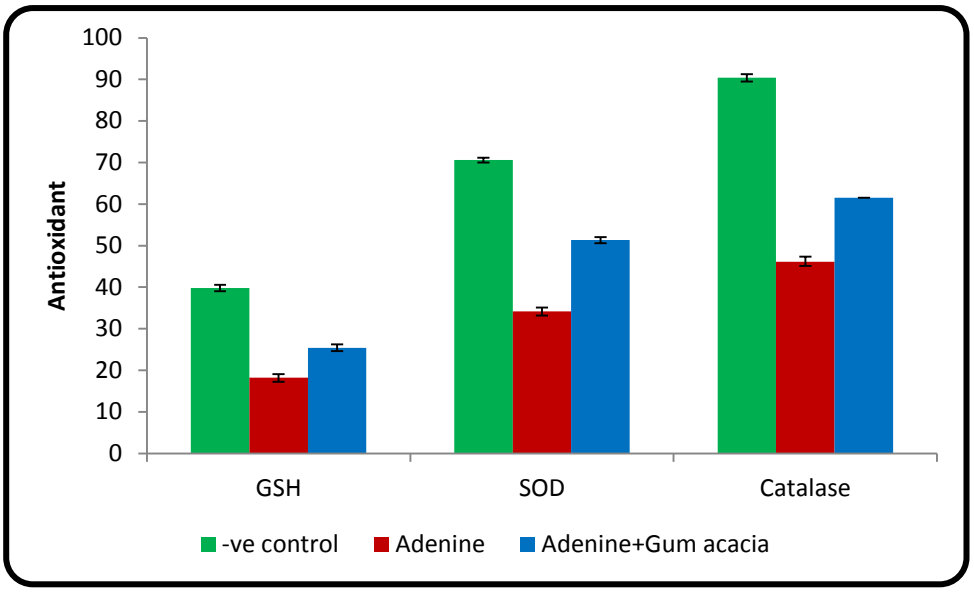
Parameter	-ve control	Adenine	Adenine+ Gum acacia	p
Creatinine **mg/dl**	0.55±0.011[a]	2.35±0.25[b]	1.07±0.026[c]	0.001
BUN **mg/dl**	23.2±0.61[a]	80.0±1.77[b]	40.3±0.87[c]	0.001
U prot /creat. Ratio	0.04±0.003[a]	1.23±0.06[b]	0.7±0.026[c]	0.001
NAG activity **IU/L**	5.7±0.09[a]	22.8±1.6[b]	14.3±0.81[c]	0.0011

Each value represents the mean ± SD, values superscripts with different letters were significantly different at p≤0.05.

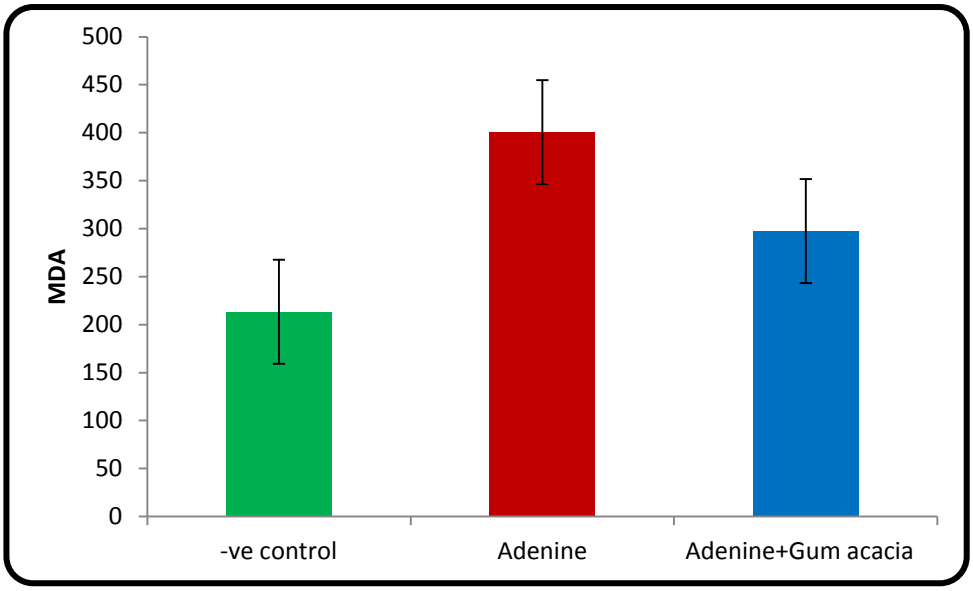
Table2: hematological parameters

Parameter	-ve control	Adenine	Adenine+ Gum acacia	p
Hgb **mg/dl**	16.87±0.10[a]	11.46±0.16[b]	13..27±0.14[c]	0.001
HCT %	46.5±0.94[a]	32.78±0.65[b]	40.99±0.33[c]	0.001
RBCs **10^6/µL**	6.76±0.11[a]	4.54±0.06[b]	5.40±0.1[c]	0.001

Each value represents the mean ± SD, values superscripts with different letters were significantly different at p≤0.05.

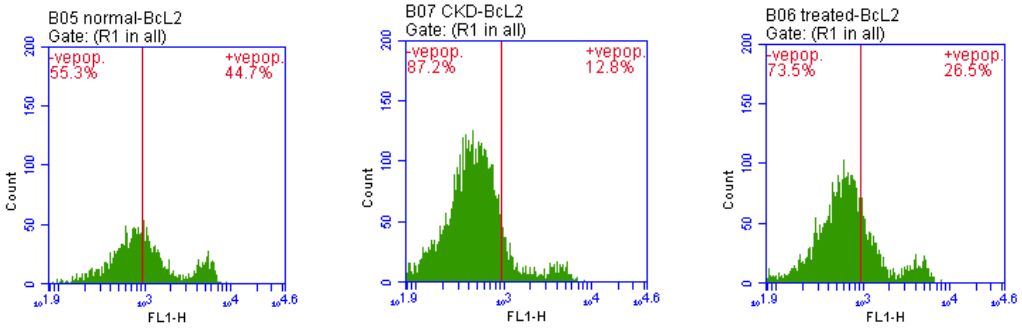


A

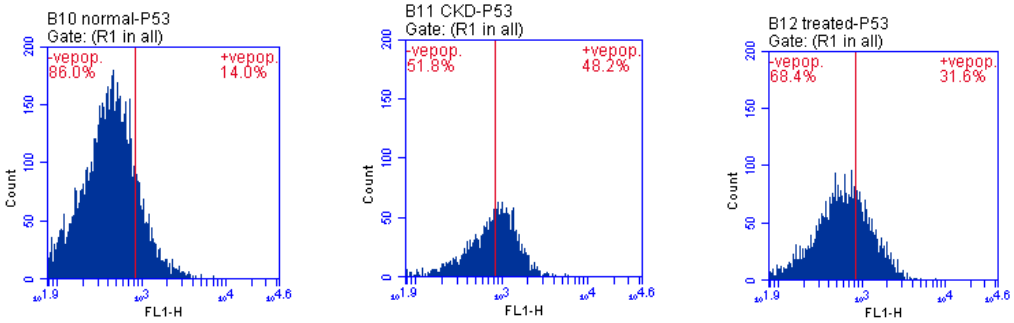


B

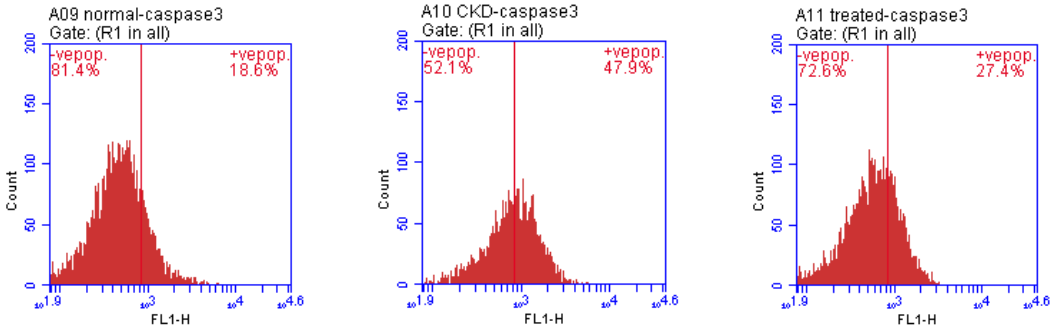
Fig(1): Effect of adenine in feed (0.75 percent w/w), with or without gum arabic (15 percent w/v in drinking water), on parameters of (A) oxidative stress and (B) lipid peroxdation in plasma.


B05 normal-BcL2
Gate: (R1 in all)
-vepop.
55.3%
+vepop.
44.7%
B07 CKD-BcL2
Gate: (R1 in all)
-vepop.
87.2%
+vepop.
12.8%
B06 treated-BcL2
Gate: (R1 in all)
-vepop.
73.5%
+vepop.
26.5%
Count
FL1-H

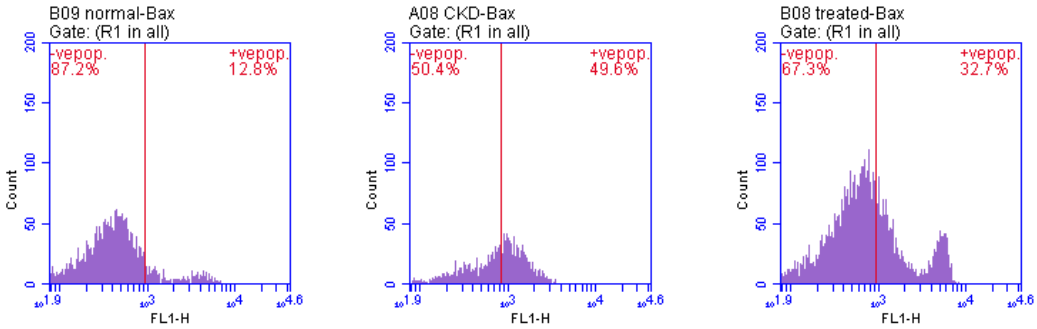
(a)


B10 normal-P53
Gate: (R1 in all)
-vepop.
86.0%
+vepop.
14.0%
B11 CKD-P53
Gate: (R1 in all)
-vepop.
51.8%
+vepop.
48.2%
B12 treated-P53
Gate: (R1 in all)
-vepop.
68.4%
+vepop.
31.6%
Count
FL1-H

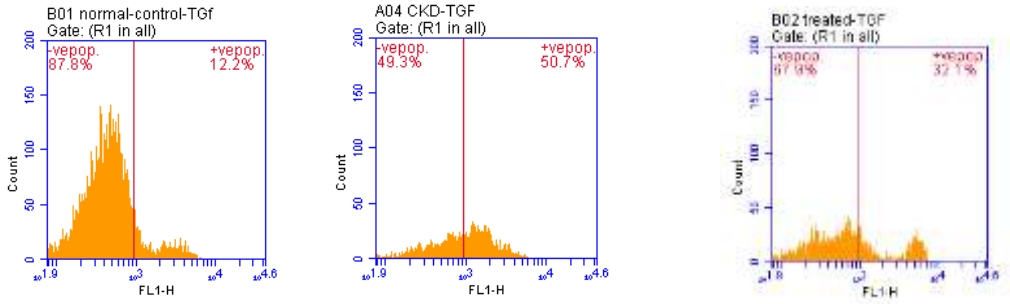
(b)


A09 normal-caspase3
Gate: (R1 in all)
-vepop.
81.4%
+vepop.
18.6%
A10 CKD-caspase3
Gate: (R1 in all)
-vepop.
52.1%
+vepop.
47.9%
A11 treated-caspase3
Gate: (R1 in all)
-vepop.
72.6%
+vepop.
27.4%
Count
FL1-H

(c)


B09 normal-Bax
Gate: (R1 in all)
-vepop.
87.2%
+vepop.
12.8%
A08 CKD-Bax
Gate: (R1 in all)
-vepop.
50.4%
+vepop.
49.6%
B08 treated-Bax
Gate: (R1 in all)
-vepop.
67.3%
+vepop.
32.7%
Count
FL1-H

(d)



(e)

Control group **Adenine-treated group** **Adenine+GA treated group**

Figure 2: Flow cytometric analysis of (a) Bcl-2, (b) p53, (c) caspase-3, (d) Bax and (e) TGF-ß in the kidney of control , adenine-treated and adenine+ *Gum acacia* rat groups.