COMPARATIVE EFFECT OF THE ETHANOLIC EXTRACTS OF ARTICHOKE, CURCUMIN AND GINGER AGAINST INFLAMMATION INDUCED BY ACETIC ACID ON RAT KIDNEY

Journal

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ABSTRACT

In this study, the effect of three ethanolic extracts of Artichoke, Curcumin, and Ginger by the two concentrations "250 & 500 mg/kg b.w" as well as Cleicoxib (100mg/kg b.w) as standard anti-inflammatory on acetic acid – induced inflammation on rat kidney were investigated. Male albino rats were randomly divided into two parts, the first part received the high dose (500 mg/kg b.w.) of each ethanolic extract, and last group received celicoxib (100 mg/kg b.w.) alone, all extracts were taken in tween 20 (1%) daily for 4 weeks. The second part received the low (250mg) and high (500mg) concentrations of each extract daily administration, and the rats were administered a single dose of acetic acid (1.4%) "1ml/rat" to induced-injury and inflammation twice/week, negative and positive control was performed.

At the end of experiment, rats were sacrificed, and kidney was taken to prepare the kidney homogenate and the sub-cellular fractions "Mitochondrial, Microsomal, and Cytosolic fractions". The enzyme activity of Glutathione-S-transferase in each fraction was determined. Also, acid phosphatase (ACP) and β-N-acetyl glucosaminidase (β-NAG) of the lysosomal enzymes of rat kidney were investigated. Total protein content and genomic DNA concentration were determined. DNA isolation on agarose 1% was performed.

Results indicated that the extracts by the two concentrations appeared to decrease the enzyme activities of GST, ACP, and β-NAG in rat treatment by acetic acid. Total protein and genomic DNA were significantly enhanced in toxicated rat kidney as compared to control.

Data indicated that the extracts containing active constituents which have a protective effect against acetic acid-induced inflammation on rat kidney. The activities of lysosomal enzymes and GST were affected by inhibitory effect according to the concentration of each extract. Also the inhibitory effect of GST in each sub-cellular fraction was altered according to the cellular particles.

INTRODUCTION

Extensive studies on the adverse effects of the medicinal plants and establishments of a good correlation between biomarkers and plants are essential for ensuring the efficiency and quality of herbal plants (Sen *et al.,* 2007).

Glacial acetic acid was used to induce acute inflammation and to increase vascular permeability (Hosseinzadeh *et al.,* 2002). It was investigated that some plant extracts exhibited inflammatory effect against acetic acid-induced by acetic acid.

Curcumin (the primary active principle in turmeric (*curcuma longa* L.) has been claimed to represent a potential antioxidant and anti-inflammatory effect with phytonutrient and bioprotective properties. However there are lack of molecular studies to demonstrate its cellular action and potential molecular targets (Balasubramanyam *et al.,* 2003).

Increasing evidence in both experimental and clinical studies suggests that oxidative stress plays a major role in the pathogenesis of many diseases (Rosen *et al.,* 2001). Abnormally high levels of free radicals and the simultaneous decline of antioxidant defense mechanisms can lead to damage of cellular organelles and enzymes increased lipid peroxidation. Therefore, oxidative stress is considered a common endpoint of chronic diseases (Baynes, 1991) and is often characterized by an increase in the steady state concentration of reactive oxygen species (ROS) such as H_2O_2, lipid peroxides, super oxide and hypochlorous acid. The dried rhizome of *Curcuma longa* is a rich source of beneficial phenolic compounds; the curcuminoids (Balasubramanyam *et al.,* 2003).

Glutathione-S-transferase (GST_S) plays an important role in the detoxification of diverse electrophilic chemicals, including anti-cancer drugs. This enzyme catalyzes the conjugation of the abundant intracellular tripeptide glutathione to a broad range of electrophilic drugs and carcinogens (Waxman *et al.,* 1992). GST_S can also inactivate organic peroxides formed by the action of Adriamycin and can quench chloroethylated DNA monoadducts, and DNA hydro-peroxides.

Some pungent constituents present in ginger and other zingiberaceous plants have potent antioxidant, anti-inflammatory and some of them exhibit anti-tumor promotional activity in experimental carcinogenesis (Yance and Sagar, 2006). Ginger was shown to produce a greater inhibition on thromboxane formation and pro-aggregatory prostaglandins. It was also significantly reduced platelet lipid peroxide formation (Kiuchi *et al*., 1992).

The crude extract of *zingibraceae* reduced rat paws and skin edema induced by carragenan or serotonin as demonstrated by Penna *et al.* (2003). The anti-inflammatory activities of flavonols "quercetin, and rutin" and flavanones "hesperetin and hesperidin" were investigated in animal models of acute and chronic inflammation. On neurogenic inflammation induced by xylene, only the flavanones were reported to be effective. The most important compound in reducing paw edema induced by carragenan is quercetin (Rotelli *et al.,* 2003).

Artichoke *"Cynara scolymus L."* is an edible vegetable from the Mediterranean area. Artichoke extracts showed good efficiency in the inhibition *in-vitro* of lipid peroxidation, radical scavenging activity. The *in-vitro* protective acute of artichoke was confirmed by Jimenez-Fscrig *et al.* (2003). The antioxidant properties of some active principles contained in vegetable and aromatic plants, namely, Cynarin from *Cynara Scolymus* were tested (Cervellati *et al.,* 2002).

The purpose of the present study was to investigate the anti-inflammatory effects of the ethanolic extracts of Artichoke, Curcumin and Ginger by determination the activity of glutathione-S-transferase in different sub-cellular particles "Mitochondria, Microsomes, and Cytosolic fractions". Also, the release of lysosomal enzymes "ACP, and β-NAG"; total protein, and genomic DNA in rat kidney in either induced or non-induced treatments were investigated.

MATERIALS AND METHODS

Chemicals: All chemicals and celicoxib standard as anti-inflammatory drug were purchased from Sigma Chemical Company (st. Louis, Mo.).

Animals: Male albino rats (200-250g) were supplied from laboratory Animal house of NODCAR, and allowed free access to the normal standard diet and tap water. Rats were housed eight per cage and kept under controlled conditions of 22±24°C, 50-60% relative humidity and 12h light-dark cycles throughout the experiments. Rats maintained in these facilities for 1 week before the experiment.

Plant preparations: Samples of Artichoke (*Cynara Scolymus*), Curcumin (*Curcuma longa*), and Ginger (*Zingbar officinalis*) were obtained from the medicinal plant station, Faculty of pharmacy, Cairo University, Egypt.

Fruits of Artichoke and the rhizomes of curcumin and ginger were cleaned, air dried, and grinded to fine powder then extracted by 70% ethanol. All extracts were filtered and lyophilized (Leal *et al.,* 2003). Two concentrations of each extract "250-500 mg/kg b.w" were used and given daily intragastrically for 4 weeks.

Celicoxib was suspended in distilled water with tween 20 (1%), it was prepared freshly each time and administered as a single dose 100 mg/kg b.w daily intragastircally for 4 weeks.

Experimental design: Rats were divided into two parts; the first part: received the high dose of each extract "500 mg/lkg b.w.", last group received celicoxib "100 mg/kg b.w." alone, all extracts were taken in tween 20 (1%) daily for 4 weeks. The second part: received the low and high dose of each extract daily administration and the rats were administered a single dose of acetic acid (1.4%) 1 ml/rat for induced-injury and inflammation (Hosseinzadeh *et al.,* 2002) twice/week, negative and positive control was performed.

All concentrations of extracts were taken according to Thomson *et al.* (2000). After four weeks (overnight fasting), rats were sacrificed by decapitation to isolate the kidney for the following investigation:

Tissue homogenates and fractionation of the sub-cellular particles:

Tissue homogenate were prepared in a ratio of 1 gm of wet tissue per 9 ml of 1.15% kCl, mitochondrial and microsomes of rat kidney were prepared as described by (Hogeboom, 1995) (1 mg of protein / 0.1ml suspension).

Kidney was homogenized in cold sucrose (0.25M), following an initial centrifugation of the homogenate at 9000xg / 15min. Then 3.5 ml of 0.1M $Cacl_2$ in sucrose buffer was added. Centrifugation at 37000xg for 15min was performed; the yield (cytosol & microsomal pellet) was placed in liquid nitrogen for -80°C (Cha *et al.,* 1983).

Protein determination: Protein content of kidney homogenate; microsomal; mitochondrial, and cytosolic fractions was determined by the method of Lowry *et al.* (1951).

Determination of glutathione-S-transferase activity: The enzyme activity was measured spectrophotometrically using CDNB as a substrate (Habig *et al.,* 1974).

Preparation of lysosomal enzymes in rat kidney: The whole lysosomal fraction was prepared from rat kidney according to Tanaka and Iizuka (1968).

Determination the lysosomal enzymatic activity: The activities of Acid phosphatase (ACP), and β-N-acetyl glucosaminidase (β-NAG) were measured according to Van Hoof and Hers (1968) with some modification by Younan and Rosleff (1974).

Extraction of genomic DNA for determining the purity and concentrations in rat kidney:

Isolation of genomic DNA was performed according to the method described by Sambrook *et al.* (1989) and Blin and Stafford, (1976). DNA identification was carried out on agarose 1% as described by Heilig *et al.* (1994). DNA detection on the gel was visualized by ethidium bromide bands containing as little as 1-10 µg DNA by UV (Surzycki, 2000).

Statistical analysis: Mean values of each treatment group were compared to the positive control by using non-paired students (t) test. The difference at $P<0.05$ was considered to be statistically significant (Snedecor and Cochram, 1967).

RESULTS AND DISCUSSION

We found that acetic acid treatment was significantly increased the inflammation parameters in rat kidney Table (1).In addition, levels of GST in the sub-cellular fractions were significantly increased in acetic acid-induced acute inflammation of kidney. There is a slightly inhibitions of each extract on the activity of GST in different fractions. No significant effect of the extracts on GST activity in the cytosolic fraction. The inhibitory effect of the plant extracts appeared to be variable according to the sub-cellular fraction, this effect was dose-dependent.

It was found by Bhagwat *et al.* (1998) that the constitutive expression of GSTs was maximum in the liver cytosol as compared to other tissues, also cytosolic GST activity using (CDNB) as a substrate was 2-4 fold higher than that in the microsomal and mitochondrial fractions. It was appeared that, a selective activator of the microsomal GST and there was a selectivity in the effects of different xenobiotics on the expression and catalytic activity of GST iso-enzymes from different sub-cellular compartments of tissues.

In this study, the effect of ethanolic extracts of Artichoke, Curcumin and ginger by two concentrations on the lysosomal enzymatic release of ACP, β-NAG as compared to the positive control was observed in Table (1)

The results indicated that the enzyme release was significantly increased in acetic acid-induced inflammation in rat kidney. Treatment of rats with the ethanolic extracts significantly decreased the elevation in the activities of acid phosphatase and β-N-acetyl glucosaminidase of lysosomes in rat kidney as compared to control ($P<0.05$).

Table (1): Effect of Artichoke, Curcumin, and Ginger by the two concentrations, as well as celecoxib as standard anti-inflammatory drug on glutathione-S- transferase activity (GST) "M/min" in the cellular fractions and lysosomal enzymes of ACP, and β-NAG of rat kidney in acetic acid induced inflammation and non- induced treatments. (n=8)

Treatments		Glutathione-S-transferase activity "GST" (M/min)			Total lysosomal enzymatic release "nmole/ml/hr in rat kidney	
		Mitochondrial F.	Microsomal F.	Cytosolic F.	ACP	β-NAG
Positive control		280.20 ± 5.604	458.96 ± 8.702	546.41 ± 8.196	2249.17 ± 67.96	2205.23 ± 55.13
% Change		------	-----	------	--------	---------
Negative control		218.06 ± 6.361	347.50 ± 6.246	397.19 ± 4.758	1242.50 ± 49.68	1548.69 ± 54.18
% Change		↓22.2%*	↓24.3%*	↓27.3%*	↓44.8%***	↓29.8%*
Artichoke (HD)	Non- induced inflammation	258.90 ± 5.942	420.75 ± 9.108	524.51 ± 7.568	642.50 ± 49.26	364.85 ± 18.20
% Change		↓7.6%*	↓8.3%*	↓4.0%*	↓71.4%***	↓83.5%***
Curcumin (HD)		249.34 ± 3.421	435.69 ± 5.522	527.33 ± 7.610	498.34 ± 19.920	422.33 ± 14.77
% Change		↓11.0%*	↓5.1%*	↓3.5%†	↓77.8%***	↓80.8%***
Ginger (HD)		259.54 ± 3.743	440.17 ± 8.712	535.20 ± 4.637	541.67 ± 16.23	463.66 ± 23.15
% Change		↓7.4%*	↓4.1%*	↓2.1%†	↓75.9%***	↓79.0%***
Celecoxib		261.46 ± 4.515	443.13 ± 5.796	526.60 ± 4.560	472.50 ± 11.80	658.43 ± 40.53
% Change		↓6.7%*	↓3.4%†	↓3.6%†	↓79.0%***	↓70.1%***
Artichoke (HD)	Induced inflammation	271.81 ± 6.273	448.66 ± 5.387	540.42 ± 6.912	1185.00 ± 41.48	2008.77 ± 105.90
% Change		↓3.0%†	↓2.2%†	↓1.1%†	↓47.3%**	↓8.9%*
Artichoke (LD)		250.85 ±5.016	427.50 ± 5.213	530.56 ± 7.821	1042.50 ± 36.47	1671.26 ± 66.84
		↓10.5%*	↓6.9%*	↓2.9%†	↓53.6%**	↓24.2%*
Curcumin (HD)		256.76 ± 5.192	454.03 ± 6.076	516.09 ± 6.931	1745.00 ± 87.25	2020.98 ± 84.81
% Change		↓8.4%*	↓1.1%†	↓5.5%*	↓22.4%*	↓8.4%*
Curcumin (LD)		249.86 ± 6.909	426.11 ± 5.721	520.70 ± 5.721	1531.67 ± 61.24	2000.61 ± 84.26
		↓10.8%*	↓7.2%*	↓4.7%*	↓31.9%*	↓9.3%*
Ginger (HD)		250.11 ± 4.592	429.00 ± 5.863	530.93 ± 9.631	1763.33 ± 70.52	2014.57 ± 70.49
% Change		↓10.7%*	↓6.5%*	↓2.8%†	↓21.6%*	↓9.0%*
Ginger (LD)		245.13 ± 5.643	426.67 ± 5.575	509.79 ± 9.724	1708.34 ± 102.5	1822.96 ± 91.12
		↓12.5%*	↓7.0%*	↓6.7%*	↓22.0%*	↓17.3%*
Celecoxib		255.72 ± 10.15	420.42 ± 8.762	513.38 ± 8.380	1542.50 ± 61.68	1861.92 ± 83.75
% Change		↓8.7%*	↓8.4%*	↓6.0%*	↓31.4%*	↓15.6%*

% change between the treatments and positive control
*: Significant = P<0.0125
** Highly significant = P<0.0025
†: Insignificant = P< 0.2

The enzyme activity of ACP exerted a less inhibitory effect than β- NAG in non-induced group, while the inhibitory effect of ACP intoxicated group appeared to be high than β-NAG. This effect was appeared to be dose-dependent. It has been proposed that the protective effect of the flavonoids on lysosomes subjected to oxygen free radicals dose not only originate from their scavenger and anti-lipoperoxidant properties; a more direct action on lysosomal membrane making it more resistant to oxidative aggression has to be considered. The prevention by some flavonoids of lysosome osmotic disruption in iso-osmotic glucose could be the result of an inhibition of glucose translocation through the lysosomal membrane (Decharneux *et al.,* 1992).

The results revealed that the total protein of rat kidney was significantly decreased in acetic acid-induced groups. Treatment with the ethanolic extracts under investigation exerted an significant increase in the total protein content in the three fractions of cytosolic, microsomal than mitochondrial (Table 2).

It was showed a significant change ($P<0.05$) in DNA in acetic acid induced inflammation by decrease the concentration as compared to normal control Table (2) and Figure (1). Ethanolic extracts administration in rat produced highly significant increase in DNA contents in either induced or non-induced toxicity groups (less damage). This enhancement in DNA concentration after treatment with the plant extracts appeared to be highly percentage in non-induced toxicity than intoxicated group. The effect of these extracts on DNA appeared to be dose-dependent.

The obtained data in the present study demonstrated that Artichoke, Curcumin, and Ginger extracts were considered as a potent anti-inflammatory effect on kidney. They could produce a marked increase in total content of protein levels as compared to control. Additionally, extracts could also significantly inhibit the activity of GST as conformed by the results of Ahmed and Fathia (2004).

Table (2): Effect of Artichoke, Curcumin, and Ginger by the two concentrations, as well as celecoxib as standard anti-inflammatory drug on total protein "mg/ml" in the cellular fractions and genomic DNA concentration of rat kidney in acetic acid induced inflammation and non- induced treatments. (n=8)

Treatments		Total protein mg/ml in rat kidney			DNA	
		Mitochondrial F.	Microsomal F.	Cytosolic F.	Purity A_{260}/A_{280}	Concentration µg / ml
Positive control % Change		33.71 ± 1.686 ------	25.89 ± 8.702 -----	17.84 ± 0.714 ------	1.7	790.1±18.2 --------
Negative control % Change		38.29 ± 1.915 ↑13.59%*	32.50 ± 6.246 ↑25.53%*	25.36 ± 1.014 ↑42.2%***	1.8	1285.6±14.7 ↑62.7%
Artichoke (HD) % Change	Non- induced inflammation	49.42 ± 1.123 ↑46.6%***	44.80 ± 1.644 ↑73.04%***	28.62 ± 1.531 ↑60.4%***	1.8	1135.9±23.5 ↑40.0%
Curcumin (HD) % Change		47.31 ± 1.306 ↑40.3%***	42.05 ± 1.502 ↑62.42%***	26.67 ± 1.384 ↑49.5%***	1.7	1230.4±10.6 ↑55.7%
Ginger (HD) % Change		41.05 ± 2.053 ↑21.8%**	41.36 ± 2.962 ↑59.75%***	25.43 ± 1.350 ↑42.5%***	1.7	1135.6±18.3 ↑43.7%
Celecoxib % Change		43.76 ± 1.750 ↑29.8%**	42.46 ± 1.938 ↑64.00%***	24.77 ± 1.160 ↑38.3%***	1.7	1115.1±20.4 ↑41.1%
Artichoke (HD) % Change	Induced inflammation	38.39 ± 1.816 ↑13.9%*	40.82 ± 2.691 ↑57.67%***	27.88 ±0.06 ↑56.3%***	1.9	975.2±11.80 ↑23.4%
Artichoke (LD)		36.43 ±1.485 ↑8.1%*	38.80 ± 2.466 ↑49.86%***	25.44 ± 0.84 ↑42.6%***	1.8	925.8±40.23 ↑17.2%
Curcumin (HD) % Change		37.40 ± 2.120 ↑10.9%*	37.15 ± 1.926 ↑43.49%***	26.17 ± 1.359 ↑46.7%***	1.9	909.1±66.84 15.1%
Curcumin (LD)		35.05 ± 1.297 ↑4.0%†	34.36 ± 0.083 ↑32.72%**	25.96 ± 1.498 ↑45.5%***	2.0	980.6±38.70 ↑24.1%
Ginger (HD) % Change		36.27 ± 2.214 ↑7.6%*	35.22 ± 2.061 ↑36.04%*	24.19 ± 1.088 ↑35.6%***	2.0	960.7±10.5 ↑21.6%
Ginger (LD)		34.39 ± 1.870 ↑2.0%†	30.29 ± 1.172 ↑16.99%*	22.04 ± 1.162 ↑23.5%***	1.9	901.6±13.5 ↑14.4%
Celecoxib % Change		35.90 ± 2.154 ↑6.5%*	30.58 ± 1.840 ↑18.12%*	20.52 ± 0.706 ↑15.0%**	1.9	896.7±21.5 ↑13.5%

% change between the treatments and positive control ***: Very highly significant =P<0.0005
** Highly significant = P<0.0025 *: Significant = P<0.0125
†: Insignificant = P< 0.25

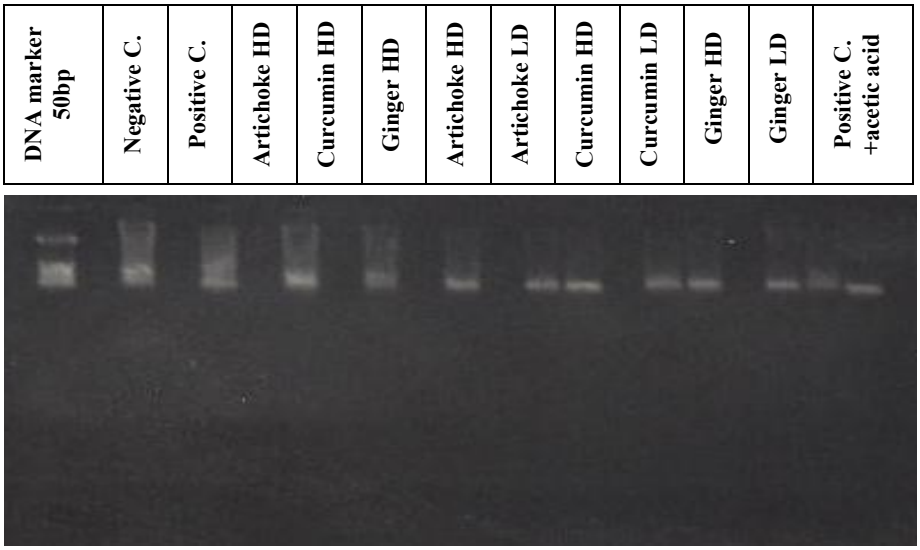

Fig. 1. Genomic DNA of rat kidney isolated from different treatments compared against control and Ladder DNA (50 bp) on agarose 1%. Gel was stained by ethidium bromide and visualized by U.V.

It was reported that Artichoke extract revealed a good efficiency in the inhibition *in-vitro* of lipid peroxidation, and free radical scavenging activity **(**Jimenez-Fscrig *et al.,* 2003). Also, it has been found that ginger attenuated the induction of lipid peroxidation and the oxidative stress in rat induced hepatotoxicity (Ahmed *et al.,* 2000).

Also, Curcumin and other extracts exerted an stabilizing effect on the enzyme activities of ACP and β-NAG of lysosomal enzymes, this effect are in agreement with the results of Redely and Lokesh (1994) who found that curcumin has a membrane stabilizing effect. Moreover, it has been reported that curcumin may exert a cytoprotective effect by inducing liver glutathione-S-transferase (Piper *et al*., 1998).

It was indicated that the free radicals which produced intoxicated processes caused a variety of reactive oxygen species including superoxide radical, hydrogen peroxide and hypochlorous acid (Roos, 1991). These radicals react with biological molecules such as DNA,

protein and phospholipids and eventually damage membranes and other tissues (Vuillume, 1987).

The free radical trapping capacity of curcumin resides mainly due to its phenolic contents besides a β-diketone moiety (Masuda *et al*., 1999). These compounds possibly prevent the reactive oxygen species from acting on DNA (Srinivas *et al.,* 1992), suggesting a possible role of curcumin as a chain breaking antioxidant against lipid peroxidation (Rajakrishnan *et al.*, 1999). The activated oxygen species can induce cellular events such as enzyme inactivation, DNA strands cleavage and also membrane lipid peroxidation (Pratibha *et al*., 2006).

It was investigated that free radicals like superoxide anion and hydroxyl radical exerted their toxic effect by acting on DNA, membrane proteins and lipids (Bhattacharya *et al.,* 2003), while under the effect of plant extracts, DNA was less affected. Also, Ippoushi *et al.* (2003) found that ginger has an inhibitory activity against DNA damage induced by reactive oxygen species (ROS), this effect of ginger may due to the presence of phenolic compounds. It was well known that malondialdehyde from lipid peroxidation reacts with DNA bases and induces mutagenic lesions (Benamira, 1995).

Conclusion In conclusion, this study provides a preliminary data on the anti-inflammatory activity of Artichoke, Curcumin, and Ginger extracts. Also the results supported information that these plant extracts represent a potential antioxidant and anti-inflammatory response on kidney tissue. This effect appeared to be dose-dependent. Further studies are necessary to assess the role of anti-inflammatory active ingredients in defense mechanisms against inflammatory process in kidney tissues.

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التأثير المقارن للمستخلصات الأيثانولية للخرشوف ، الكركم و الزنجبيل ضد الألتهاب المستحدث بواسطة حمض الخليك فى كلى الجرذان

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تم داسة تأثير ثلاث مستخلصات (الخرشوف، الكركم و الزنجبيل) بتركيزين (250 ، 500 ملليجم / كجم وزن الجسم) وأيضا Celicoxib بجرعة واحدة (100 ملليجم / كجم وزن الجسم) كمادة قياسية مضادة للألتهاب ضد الألتهاب الناتج عن استخدام حمض الخليك فى كلى الجرذان، قسمت الجرزان لجزئين رئيسين: الأول أعطى التركيز العالى لكل مستخلص على حده والمجموعة الأخيرة أعطت الـ Cleicoxib بتركيز واحد لمدة أربعة أسابيع ، وتم أعطاء الجزء الجزء الثانى التركيز (المنخفض والعالى) لكل مستخلص يوميا وتم اعطاء الجرزان حمض الخليك بتركيز واحد (1.4%) (1 ملل/ جرز) مرتين فى الأسبوع لحدوث الألتهاب.

فى نهاية التجربة تم ذبح الجرذان وعزل الكلية لفصل الجزيئات تحت الخلوية (متجانس الكلية - ميتوكندريا – ميكروزوم – سيتوزول) وتم تقدير نشاط أنزيمات الجلوتاثيون – أس – ترانسفيرايز (GST) فى كل جزء، وكذلك تقدير النشاط الأنزيمى لجسيمات الليسوسوم وهى الفوسفاتيز الحامضى "ACP" و بيتا-ن-جلوكوزأمينيديز "β-NAG" و تم تقدير محتوى البروتين والحمض النووى DNA وتم عزل الحمض النووى DNA وفصله على الأجاروز 1%.

أوضحت النتائج أن معالجة الجرزان بالمستخلصات الأيثانولية بالتركيزين أدت الى خفض فى نشاط أنزيم الجلوتاثيون – أس – ترانسفيرايز (GST) و النشاط الأنزيمى لأنزيمات الفوسفاتيز الحامضى "ACP" و بيتا-ن-جلوكوزأمينيديز "β-NAG" فى الجرذان التى أعطت حمض الخليك. وقد ارتفع المحتوى من البروتين والحمض النووى فى الجرذان التى حدث لها التهاب مقارنة بالمجموعة الضابطة.

وقد أوضحت النتائج أن المستخلصات تحتوى على مركبات فعالة لها تأثير واقى كمضادات للألتهاب الناتجة عن حمض الخليك فى كلى الجرذان. وقد تأثرالنشاط الأنزيمى لأنزيمات الليسوزوم و الجلوتاثيون – أس – ترانسفيرايز) حسب التركيز لكل مستخلص وكذلك حسب نوع الخلايا المفصولة من ميتوكندريا، سيتوزول، وميكروسوم فى كلى الجرذان.