Research Article

Therapeutic efficacy of catechins on systemic complications of rheumatoid arthritis in animal model

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KEY WORDS

Catechins, Complete Freund’s Adjuvant, Rheumatoid arthritis, Methotrexate, Systemic disorder

ABSTRACT

Green tea catechins are polyphenolic compounds that exhibit hallmark anti-inflammatory, antioxidant, and anti-arthritic properties especially epigallocatechin-3-gallate the major and the most abundant green tea catechin. The main goal of this study is to evaluate the role of catechins extracted from green tea on hematological abnormalities and impairments of both liver and kidney functions because of rheumatoid arthritis complications. In this study hot water extraction method was used for high-yield catechin separation, the crude extract was introduced to silica gel column chromatography for purifications, and the obtained catechin was detected by spectrophotometric assaying tests and FT-IR. Complete Freund’s adjuvant was used for in vivo arthritic induction. The results showed that green tea catechins enhanced anemic conditions associated with chronic diseases like rheumatoid arthritis, raised the erythrocyte count, and reduced leukocytosis. Catechins also improved the liver functions of rheumatoid arthritis rats as working by reducing the serum level of liver enzymatic biomarkers alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) from 51.37 ± 3.12, 82.07 ± 4.11 and 37.52 ± 4.06 to 44.65 ± 5.053, 41.7 ± 6.84 and 27.61 ± 5.83 respectively. They also had a good impact on decreasing the abnormal rise of rat’s sera levels of both urea and creatinine as biomarkers of kidney function. These observations indicate good therapeutic efficacy of green tea catechins on systemic effects of rheumatoid arthritis rats.
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Introduction

The bioactive ingredients of green tea are known as catechins. These polyphenolic compounds are extracted from the dry leaves of Camellia sinensis and Camellia assamica which are members of the Theaceae family (Chaudhary and Maurya, 2019). Epigallocatechin -3-gallate (EGCG) is the essential and the major green tea catechin, it presents about 50-80% of its total catechins content (Mak, 2012; Chakrawarti et al.; 2016; Reygaert, 2018; Musial et al., 2020; Farhan, 2022). These naturally occurring compounds exhibit strong antioxidant and anti-inflammatory properties as they have recently been used for the treatment of many inflammatory diseases (Singh et al., 2010; Pae and Wu, 2013; Gabr et al., 2014; Min et al., 2015; Hughes et al., 2017). One of these inflammatory disorders is rheumatoid arthritis, which is an autoimmune disease affecting the synovial membrane lining joint causing arthralgia, synovitis, erosive bone, destructive joints, and cartilage damage (Littlejohn and Monrad, 2018). Long-term lack of treatment of this chronic condition leads to severe systemic effects and extra-articular manifestations including cardiovascular diseases, hematological blood complications, and liver and kidney damage, it also effects on eyes, skin, and lungs and even decreases lifespan and causes premature death (Shams et al., 2021). Methotrexate is a traditional drug used for rheumatoid arthritis treatment. It was successfully helping in reducing arthritic symptoms with good disease outcomes and a low disease activity state (LDAS) but with severe side effects including hematological blood test alterations, and liver and kidney function impairment (Cronstein and Aune, 2020). So, using catechin alone or in combination with methotrexate is a better choice for arthritic treatment than methotrexate alone as it helps in decreasing these unwanted effects as illustrated in this study. In this study, we focused on studying the role of green tea catechins in relieving some of these complications associated with RA disease in Complete Freund’s adjuvant animal model, as CFA was used for arthritic induction in rats causing an inflammatory response (Freund, 1956). For achieving this purpose catechins were extracted, isolated, and purified from green tea leaves, FTIR was performed and the total catechin content in the test compound was spectrophotometrically detected, the isolated catechins were used as anti-arthritic medication in a CFA animal model. The arthritic
symptoms and features of swelling, redness, and increase in rat paw volume were monitored. These symptoms reached a peak on the seventh day of immunization, after that catechin and/or methotrexate treatment strategy began, at the end of the experiment the blood samples were collected for whole blood composition examination, and rat sera levels of all ALT, AST, ALP, urea and creatinine detection.

**Materials and methods**

**Chemicals and drugs**

Green tea leaves were obtained from the local market in Tanta City, Egypt. Chloroform, Ethyl acetate, Vanillin, Ethanol, HCl, Complete Freund's adjuvant (CFA), Stander catechin was purchased from Sigma-Sigma-Aldrich Chemicals Co. (St. Louise, USA), Methotrexate was obtained from EIMC united pharmaceutics Cairo, Egypt. Silica gel (60-120mesh), from EL-Nasser pharmaceutical chemicals com., Egypt. N.S BIO-TEC ALT and AST bio diagnostic commercial kits, urea colorimetric Biomed, and Creatinine Colorimetric Diamonds kits were purchased from Cairo, Egypt.

**Methods**

**Catechins isolation, purification, and their quantitative determination**

**Hot water extraction of catechins from green tea**

Catechin compounds were separated from green tea leaves using the hot water extraction method described by (Price and Spitzer, 1993) to obtain high catechin content. The method depended on dissolving 50g of green tea leaves into 1500 distilled water at 80°C for 40 min, after filtration the solution was treated with chloroform to remove caffeine, and then treated with ethyl acetate for catechins isolation. The organic layer containing catechin was introduced into a rotary evaporator at 75°C to obtain a dark brown concentrated catechin powder that was stored at -20°C for further use.

**Identification of catechin in the test compound**

The Silica gel column chromatography with a mixture of solvents of chloroform, methanol, and water (65:35:10, v/v/v) was used for catechin fractionation into isolated fractions (Tanizawa et al., 1983; Amarowicz et al., 2003), vanillin test for quantitative estimation of catechin (Sun et al., 1998) was performed on each fraction by measuring absorbance at 500nm. High catechin content fractions were pooled, left to dry, and concentrated using a freeze-drying lyophilize to obtain catechin. The isolated catechin underwent several UV spectrophotometric measurements to ensure good catechin separation.
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including dimethyl amino-cinnamaldehyde (DMACA) assay (Ivanova et al., 2010; Csepregi et al., 2013) that considered the specific test for catechin rather than any other flavonoid (Di Stefano et al., 1989), and optical properties measurements of the polyphenolic compounds at wavelengths between 260 and 400 nm using UV-light spectrophotometry (Arnous et al., 2001; Poudel et al., 2008; Csepregi et al., 2013). The JNS-CO FTIR spectrum also confirmed good catechin separation compared with standard catechin.

**In vivo study**

**Experimental design**

The experimental design was performed on 49 male Sprague-Dawley rats that weighed about (110–130 g) and were divided into seven groups each group containing seven rats (n=7), obtained from the animal housing center at the Faculty of Science Alexandria University (Alexandria, Egypt). The experiment was performed under the Ethical Committee and the Institutional Animal Care and Use Committee of the Faculty of Science, Tanta University, Egypt (#IACUC-SCI-TU-0174).

Group I (Normal control): the rats were subcutaneously injected with a single dose of 1.5 ml saline solution, and Group II (Arthritis control) rats were subcutaneously injected with a single dose of 0.05 ml CFA for arthritis induction (Freund, 1956). Group III (Methotrexate control) rats were intraperitoneally (i.p) treated with 0.1 ml methotrexate two times /week for three weeks (Weinblatt et al., 1994). In Group IV (Catechin control group) the animals were intraperitoneally (i.p) injected with 100mg/kg of catechins daily from the seventh day to day 16 (Ahmed et al., 2008). In Group V (Methotrexate treated group) the animals were treated with CFA as illustrated in Group II for arthritis induction, then treated with methotrexate from the seventh day as illustrated in Group III. In Group VI (Catechin-treated group) arthritis was induced in experimental animals as shown in Group II, and then they were treated with catechin by the method described in Group IV. Group V (Catechin-methotrexate-treated group) CFA was used for arthritis induction into the experimental animals as described in group II, then after the seventh day of immunization, both methotrexate and catechin were used for RA animals’ treatment as described in groups III, IV respectively.

After the arthritic score reached its peak on the seventh day of immunization and the treatment strategy gave the best result in relieving these arthritic features, the experiment ended on day 28 then all animals were sacrificed and their blood samples were collected into two types of
tubes, EDTA tubes for hematological blood tests analysis and the other on dry ones for serum separation. All tubes were stored at -20 °C until used.

**Hematological examination**

Total white blood count (WBC), red blood count (RBCs), hemoglobin (Hg) level, hematocrit percentage (Hct %), and platelet count were measured for each group. The Neubauer counting chamber was used for manually counting the number of nucleated cells as an indication of total WBCs (Chabot-Richards and George, 2015), RBCs in a cubic millimeter of the blood sample (Priya et al., 2011), and the platelets count per liter of a diluted blood sample using ammonium oxalate as a diluting solution. Hemoglobin level and Hct% were determined using a complete blood picture test.

**Liver Function Analysis**

The serum levels of both alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined using diamond diagnostic colorimetric kits as illustrated in the study of (Young and Friedman, 2001). While alkaline phosphatase level was measured by reading the absorbance of a colored complex formed as a result of the reaction of a phenolic compound in the presence of potassium ferricyanide and 4-aminoantipyrine at pH10 (Kay, 1930; Kind and King, 1954).

**Kidney Function Analysis**

Both urea and creatinine levels were determined in rat serum using bio-diagnostic kits and the colorimetric method described by (Young, 2001), (Larsen, 1972) respectively.

**Statistical analysis**

GraphPad Prism software 6 (San Diego, CA) was used for analyzing the significant degree of both treated groups compared with controls through one-way ANOVA and Tukey’s test as p-values < 0.05 indicate a statistically significant value. All data used are expressed as mean ± SD.

**Results**

**Catechin extraction and purification from green tea**

The hot water extraction method for catechin isolation gave a high yield equaled 57mg catechin /g crude extract, this indicates green tea is a good source of catechins. The unknown catechin concentration in the test compound was measured from the catechin standard curve performed through the reaction of catechins with ethanolic vanillin solution that equaled 0.4 mg. The test compound was undergone into several purification steps as applied into a silica gel column chromatography using a mixture of specific solvents as a mobile phase, the elution profile of catechin separation by silica gel column was shown in (Fig. 1). Other spectrophotometric tests were performed for the quantitative
Therapeutic efficacy of catechins on systemic complications of rheumatoid arthritis in animal model determination of catechin concentration in green tea crude extract including DMACA which gave high absorbance indicating high catechin content in the test sample. Also reading absorbance at different wavelengths by UV spectrophotometry represented that the test compound showed a peak absorbance very close to that of the standard catechin. FTIR spectrum of the separated catechin showed the presence of the O-H group at 3422 cm\(^{-1}\), C=C group at 1618 cm\(^{-1}\), and C-O group at 1049 cm\(^{-1}\) compared with the standard catechin at 3400, 1627, and 1037 respectively as expressed in Fig. (2). All these results proved a good catechin separation from green tea leaves.

![Absorbance at 500nm](image1.png)

**Fig. (1):** Green tea catechin crude extract elution profile using silica gel column chromatography

![FTIR spectrum](image2.png)

**Fig. (2):** FTIR spectrum of both the standard and isolated catechin, A: Standard catechin spectrum, B: Isolated sample spectrum

**Hematological profile**

Complete blood picture assays showed alterations and hematological complications in CFA injected groups compared with the normal control ones as there was a significant increase in total WBC count, with a reduction in RBC count and hemoglobin level (Table 1). After treatment with catechin, rats' plasma levels of both RBCs and Hb% increased while the total WBC count decreased. Methotrexate treatment had a bad effect causing severe anemia due to decreasing in RBC count. The combinational treatment helped in
relieving these bad effects caused by methotrexate alone. These results indicate that green tea catechin was a good choice for RA treatment with no side effects on blood composition caused by other traditional treatments.

Table (1): Hematological profile

<table>
<thead>
<tr>
<th>Groups</th>
<th>WBC (x10^3/uL)</th>
<th>RBCs (x10^6/uL)</th>
<th>Hb (g/dL)</th>
<th>Platelet (x10^3/uL)</th>
<th>Hot (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>Mean ± SE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8.73 ± 0.46</td>
<td>4.82 ± 0.27</td>
<td>11.56 ± 0.6</td>
<td>301 ± 18.5</td>
<td>44.1 ± 0.09</td>
</tr>
<tr>
<td>Arthritis control</td>
<td>Mean ± SE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>12.65 ± 0.7</td>
<td>2.15 ± 0.24</td>
<td>10.8 ± 0.5</td>
<td>423 ± 19.5</td>
<td>28.3 ± 0.04</td>
</tr>
<tr>
<td>Methotrexate control</td>
<td>Mean ± SE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8.65 ± 0.48</td>
<td>2.15 ± 0.24</td>
<td>10.8 ± 0.5</td>
<td>423 ± 19.5</td>
<td>28.3 ± 0.04</td>
</tr>
<tr>
<td>Catechin control</td>
<td>Mean ± SE</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>9.01 ± 0.5</td>
<td>4.1 ± 0.386</td>
<td>10.8 ± 0.21</td>
<td>363 ± 18.5</td>
<td>38.5 ± 0.09</td>
</tr>
<tr>
<td>Methotrexate treated</td>
<td>Mean ± SE</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>3.79 ± 0.18</td>
<td>3.84 ± 0.07</td>
<td>6.78 ± 0.21</td>
<td>423 ± 29.1</td>
<td>28.3 ± 0.04</td>
</tr>
<tr>
<td>Catechin treated</td>
<td>Mean ± SE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.95 ± 0.12</td>
<td>5.01 ± 0.18</td>
<td>10.96 ± 0.19</td>
<td>388 ± 19.5</td>
<td>42.0 ± 0.08</td>
</tr>
<tr>
<td>Catechin-methotrexate</td>
<td>Mean ± SE</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>2.41 ± 0.17</td>
<td>2.75 ± 0.19</td>
<td>8.15 ± 0.318</td>
<td>402 ± 15.5</td>
<td>33.8 ± 0.1</td>
</tr>
</tbody>
</table>

Values are expressed as the mean ± SE (n= 7) of the experimental group. ns: non-significant, a: p < 0.05 showed significance vs. normal control, b: p < 0.05 showed significance vs the RA control group, c: p < 0.05 showed significance vs. the MTX control group. (+, *, ) referring to the degree of significance to the normal control, arthritis control, and methotrexate control respectively.

Liver Function Analysis

Liver enzymatic biomarkers alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) levels were measured in rat's sera as an indicator of liver function. Induction of arthritis in rats causes systemic effects affecting the liver, causing liver damage and an elevation of these enzymatic biomarkers leading finally to liver function impairments. Methotrexate treatment reduced the serum level of these enzymes in a dose-dependent manner. Treatment with catechin returned these enzymes to a normal value and gave a good disease outcome as shown in Fig. (3).
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**Fig. (3):** Liver function enzymatic biomarkers of all groups. A: Serum ALT, B: Serum AST, and C: Serum ALP activities. Data are expressed as the means ± S.E (n=7). a*: showed significance ($p < 0.05$) vs. the normal control group. b*: showed significance ($p < 0.05$) vs. the arthritis control group. c*: showed significance ($p < 0.05$) vs. methotrexate control group. (+,*), referring to the degree of significance to the normal control, arthritis control, and methotrexate control respectively.

**Kidney function analysis**

Kidney function tests include measuring serum levels of both urea and creatinine, as a complication of RA, the CFA-injected rats showing increasing serum urea and creatinine levels that significantly decreased after treatment with catechins. Methotrexate treatment affects blood urea and creatinine in a dose-dependent matter shown in **Fig. (4).**

**Fig. (4):** Kidney function tests of all experimental groups. A: Serum level of urea and B: Serum level of creatinine. Data are expressed as the means ± S.E (n=7). a*: showed significance ($p < 0.05$) vs. the normal control group. b*: showed significance ($p < 0.05$) vs. the arthritis control group. c*: showed significance ($p < 0.05$) vs. the methotrexate control group. (+,*), referring to the degree of significance to the normal control, arthritis control, and methotrexate control respectively.
Discussion
Rheumatoid arthritis is a chronic disease affecting synovial membrane lining joints causing synovitis, joint destruction, and cartilage damage. The late stage of untreated RA was associated with some extra-articular manifestations and systemic complications affecting many other organs so early diagnosis and effective treatment are important for the reduction of these harmful manifestations and achieving a good disease outcome (Smolen et al., 2014).

In this study, we focused on studying the effect of green tea catechin, especially EGCG, the most abundant polyphenolic compound found in green tea that exhibited strong anti-inflammatory, antioxidant, and anti-arthritic activities on the RA systemic effects including hematologic manifestations, liver, and kidney functions impairments. Previous studies studied the role of EGCG as an anti-carcinogenic, antibacterial, and cardio-protective agent. In our study, we used a hot water extraction method for catechin isolation from green tea leaves as illustrated in the study by (Price and Spitzer, 1993). Many other studies (Row and Jin, 2006, Bazinet et al., 2007; Liang et al., 2007; Hu et al., 2009; Dong et al., 2011) depend on liquid-liquid extraction, while the study of (Hu et al., 2009) illustrated other extraction methods either by ethanolic or citric acid extraction. The isolated catechin was then introduced onto a silica gel column for further purification as described by (Tanizawa et al., 1983 and Amarowicz et al., 2003). Previous studies used Sephadex LH-20 chromatography (Hoefler & Coggon, 1976; Amarowicz & Shahidi, 1995; Amarowicz & Shahidi, 1996; Chen & Ho, 1995). Several spectrophotometric assays were performed on the isolated fractions for quantitative estimation of catechin including DMACA (Ivanova et al., 2010) and vanillin test (Sun et al., 1998), that reading high absorbance as an indication of high catechin content also UV light spectrophotometry at a wavelength range of 260-400 nm (Arnow et al., 2001; Poudel et al., 2008) showed peak absorbance of the sample approximately close to the peak of standard catechin, also comparing with the FTIR spectrum of standard catechin, the isolated catechin represented the same function groups. These observations indicate good isolation and high catechin content in the test compound.

Our results illustrated that microcytic hypochromic anemia, leukocytosis with
neutropenia, and thrombocytosis were common Hematological alternations of rheumatoid arthritis as the CFA treated groups showed a significant increase in total WBCs, decreased RBCs with low hemoglobin level than non-treated ones. After treatment with catechin, these abnormalities were recovered up to normal values, while methotrexate treatment groups showed worse hematologic features as a side effect of methotrexate injection. The complete blood picture of this group showed severe iron deficiency anemia while in the combinational treatment, catechin helped in raising hemoglobin levels. These results agree with the study of (Dkhil and Mezher, 2014) that illustrated the role of IL-6 the major inflammatory cytokine in rheumatoid arthritis pathophysiology on WBCs, RBCs, hemoglobin and HCT% as the higher serum level of IL-6, the lower RBCs, hemoglobin and HCT% with increasing in WBCs as a result of activation of growth factor erythropoietin that responsible for erythropoiesis (Kopf et al., 1994). Additionally, regarding the impact of RA on the liver, there was an elevation in liver enzyme biomarkers ALT, AST, and ALP due to liver impairment. Our results show that CFA arthritic-induced rats exhibited high serum levels of these biomarkers compared with normal control rats. Methotrexate-treated rats also showed serum evaluation of this enzyme as a severe side effect of methotrexate, while treatment with catechin alone or in combination with methotrexate helped in reducing the serum level of these enzymes near to the normal values of the control ones, these results came on the same line of the study of (Radovanović-Dinić et al., 2018) that reported that both the hepatic manifestations causing liver damage associated with RA infection and the hepatotoxic medications of RA causing liver damaging. It also reported that people with RA were more susceptible to autoimmune liver disease. Another study by (Podgórska et al., 2020) assured these results as explicated that the liver damage associated with RA occurred either as a primary rheumatic disease with hepatic manifestations or anti-rheumatic drug-induced liver disease. Furthermore, RA also affects kidneys causing kidney function impairment. This study illustrated that induction of arthritis into rats and methotrexate-treated rats showed high serum levels of urea and creatinine but in catechin-treated groups, there was a reduction in rats serum urea and creatinine levels. These results confirmed that kidney damage was associated with RA as an extra-articular manifestation or because of RA.
medication side effects. These were the same results of another study (Icardi et al., 2003) that illustrated that Kidney injury of RA patients because of rheumatoid nephropathy is an extra-articular manifestation or nephrotoxic effects of anti-rheumatic drugs. Another study by (Chiu et al., 2015) reported that RA patients had a higher risk of developing chronic kidney diseases and glomerulonephritis.

**Conclusion**

Green tea leaves contain high catechins content. The hot water extraction method gave a high yield of these isolated catechins. RA is a complex systemic disorder. The impact of green tea catechins on all the hematological alterations, hepatic manifestations, and kidney function impairment as an RA complication was studied, and it was concluded that treatment with catechins exhibited approximately normal hematological profile, and an improvement in both the liver and kidney functions. Catechins also reduced methotrexate-induced toxicity, which indicates that catechins are good natural anti-arthritic agents with no toxicity.

**Declarations**

**Acknowledgments**
The authors of this study thank all members of the Faculty of Science.

**Ethics approval and consent to participate**
The experiments will be monitored by the Egyptian Ethical Committee of Tanta University’s Faculty of Science (#IACUC-SCI-TU-0174).

**Consent for publication**
Not applicable

**Availability of data and materials**
All data generated or analyzed during this study are included in this published article.

**Competing interests**
The authors declare that they have no competing interests.

**Funding**
Not applicable

**Authors’ contributions**
RE: methodology, writing, and interpreting the data of the original draft. ED: conceptualization and formal analysis. MT: supervision and investigation, reviewing and editing. KA: analyzed and interpreted the data and edited. The authors read and approved the final manuscript.

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YOUNG, D. S. & FRIEDMAN, R. B. 2001. Effects of disease on clinical laboratory tests. *(No Title).*
الفعالية العلاجية لمركبات الكاتيكن على مضاعفات الاالتهاب الروماتيدي في الفئران المصابة بالروماتويد

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تعتبر مركبات الكاتيكن المستخلصة من الشاي الأخضر مركبات عديدة الفينولات ذات خصائص مضادة للأالتهاب ومضادة للأكسدة وذات فعالية على مرض الاالتهاب الروماتيدي خاصة مركب الأبي جالو كاتيكن جاليت والذي يعد أحد مركبات الكاتيكن الموجودة في الشاي الأخضر والأكثر أهمية وانتشارا.

يعد الهدف الرئيسي من هذه الدراسة هو دراسة تأثير مركبات الكاتيكن المستخلصة من الشاي الأخضر على التغييرات الطارئة على مكونات الدم وقشور عمل وظائف كل من الكبد والكلى كمضاعفات مصاحبة لمرض الاالتهاب الروماتيدي.

وت最常见的 النتيجة أن مركبات الكاتيكن المستخلصة من الشاي الأخضر تعمل على تحسين حالة الألتهاب المصاحبة للأمراض المزمنة مثل الاالتهاب الروماتيدي حيث تعمل على ارتفاع عدد كرات الدم الحمراء كما تعمل على انخفاض الزيادة في كرات الدم البيضاء الناتجة عن الألتهاب بالروماتيدي. كما تعمل مركبات الكاتيكن أيضاً على تحسين وظائف الكبد في الفئران المصابة بالروماتيدي حيث تساعد على انخفاض معدلات أنزيمات الكبد في الدم كما أن لها تأثير فعال على خفض الزيادة في معدل كل من مستوى البوليا و الكرياتينين في فئران الفئران المصابة مما يساهم في تحسين وظائف الكبد لديهم.

ينتضح من هذه النتائج مدى الفعالية العلاجية لمركبات الكاتيكن المستخلصة من الشاي الأخضر على المضاعفات المصاحبة للإالمراض الروماتيدي في الفئران المصابة بالروماتويد.