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# Protective Effect of Clinacanthus nutans on Carbon Tetrachloride Induced Hepatotoxicity in Wistar Rats

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**ABSTRACT**

**Background:** The liver is crucial for drug metabolism and is often impacted by toxins from natural, domestic, and industrial sources. The cause of hepatotoxicity is unclear, but studies suggest carbon tetrachloride is a causative factor. A major medical concern, drug-induced liver damage (DILI) has emerged due to the rising use of herbal remedies. Among the many possible traditional medical applications of the Clinacanthus nutans leaf are the alleviation of skin rashes, bites from insects, herpes simplex virus lesions, diabetes, and gout. Clinacanthus nutans leaf extract may have a protective impact on DILI in rats, although this has not been established in any research.

**Objectives:** The purpose of the research was to evaluate Clinacanthus nutans' ability to shield Wistar rats from the hepatotoxic effects of carbon tetrachloride.

Methodology: Male Wistar rats were used, and they were divided into five groups at random: control, disease, low dosage, standard, and high dosage. They received oral dosages of 200 mg/kg of saline, carbon tetrachloride, CCl4, Silymarin, and leaf extract from Clinacanthus nutans over a period of seven days. The 28th day saw the evaluation of biochemical parameters.

**Results:** The findings demonstrated that the injection of CCl4 caused harm to the liver. Biochemical indicators such as total blood bilirubin, ALT, AST, and ALP levels were enhanced, and total protein levels were significantly reduced, confirming that CCl4 caused hepatotoxicity in this research. This research found that extracts from the leaves of the Clinacanthus nutans plant were able to reverse liver damage in rats that had been induced by CCl4 exposure.

**Conclusion**: In the present study treatment with Clinacanthus nutans has shown protective effect on Carbon tetrachloride induced hepatotoxicity in wistar rats.

**Key words:** Hepatotoxicity, Carbon tetrachloride, Clinacanthus nutans, Silymarin, Hepatotoxicity, liver.

## I. INTRODUCTION:

The largest glandular organ in the body, the liver carries out several essential functions. It is in charge of breaking down proteins, lipids, and carbs. Bile production is centered here, and bile is vital for waste elimination and digestion. The liver excretes 800–1000 mL of bile daily from its hepatocytes. This mixture is mainly made up of water, bile salts, cholesterol, and bilirubin, which are essential for the breakdown of old red blood cells. The components like stercobilin that give our stools their characteristic color are produced by this breakdown. In addition to producing bile, the liver is an incredible organ that can perform a multitude of other functions, such as hormone regulation, bloodstream detoxification, nutrient storage, clotting factor manufacturing, and even growth hormone activity. Due to the liver's relatively large reserve in converting T4 to T3, hypothyroidism is uncommon in patients with liver disease. The liver modifies the function of growth hormone (GH) secreted by the pituitary gland. It plays a significant role in drug metabolism as well because of enzymes like cytochrome P450, which slowly break down medications and can render them inactive or occasionally cause pharmacological activation. A liver disease's ability to function can be significantly impacted by a variety of causes, including infections, hereditary factors, toxins, or drug reactions. These conditions often require liver transplants in order to survive. This complicated organ impacts nearly every other system in our body and has a significant impact on overall wellness.

## II. MATERIALS AND METHODS:

### 2.1 Drug and Reagents:

The aspartate transaminase, alanine transaminase, total bilirubin, and cholesterol kits

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and reagents were bought from Erba Diagnostics in India. Carbon tetrachloride (S. D. Fine Chemicals, India), Silymarin (Sigma-Aldrich Co, USA). Total Protein Kit (Span Diagnostics Ltd, Surat) Thiopental Sodium injections (Neon Laboratories Limited, Mumbai).

**2.2 Acute Toxicity**

The decision to accept a novel medicine for clinical usage is aided by toxicological research. According to OECD 401, 423, and 425, a medicine cannot be used in a clinical setting without first undergoing a clinical trial and toxicity tests. The term "acute toxicity" describes the negative reactions that might happen with just one dosage of a chemical. For the detection of skin and eye effects (such as corrosion, irritation, and sensitization; topical or local toxicity), separate tests are required. The primary objective of the research was to determine whether or not a methanolic extract of Clinacanthus nutans had any harmful effects, so that it might be used to assess various biological procedures. Research on the extract's acute toxicity profile was therefore conducted in Swiss Albino mice in relation to their behavioural traits. Doses of 20, 200, and 2000 milligrammes per kilogramme of body weight were the highest permissible for testing, in line with OECD guidelines.

**2.3 Experimental Animals**

The protocol for the acute oral toxicity test followed OECD (Organization for Economic Co-operation and Development) 423. The study's animals came from Uppal, Hyderabad, India's Sainath Agencies. There was an abundance of food and drink available at all times, and each animal had its own secure enclosure with a temperature control system and a 12-hour light/dark cycle. Because they were more sensitive to treatment, we selected healthy, young adult, non-pregnant Swiss albino female mice weighing 25-32 g. All experimental techniques were conducted in strict accordance with the guidelines established by the Committee for the Purpose of Control and Supervision of Experiments on Animals (320/CPCSCEA dated 03-01-2001). In Hyderabad, India, the G. Pulla Reddy College of Pharmacy's Institutional Animal Ethics Committee reviewed and authorised the study (GPRCP/IAEC/ 09/19/11/PCL/AE-7-Mice-M/F-15).

**2.4 Housing and Diet**

In a controlled environment maintained at 23 ± 2ºC, the animals were housed in 55 × 32.7 x 19 cm polypropylene cages that were filled with sawdust. A 12-hour light-and-12-hour dark cycle was established, with the humidity maintained at 45%. A card with the test drug code, animal weight, dosage, delivery route, and cage number identified each cage. They were fed conventional lab animal meal pellets and given access to water. One dosage of the test drug was given by gavage using an oral needle that was specially designed for the purpose. Animals were starved 3 h before treatment. A specifically developed oral needle was used to give the test material in a single dosage by gavage. The animals were given their doses after a three-hour fast. The dosage was 1 millilitre per kilogramme of the animal's weight. The amount of the experimental chemical was determined by calculating the animal's body weight on the day of treatment. The animals were watched one by one for half an hour at regular intervals during the first twenty-four hours, with extra care provided for the first forty-eight hours, and then every day for the next seven days. At least twice a day, we checked up on the mice to make note of any changes in their behavior or signs of illness. Among the wellness measures that were recorded were skin, fur, eyes, mucous membranes, behavioral pattern, salivation, sleep length, tremors, lethargy, diarrhea, and coma. Also, the LD50 and mortality rates are determined according to OECD standards. After the test drugs were given, the subjects were instructed to fast for another one to two hours. After 24 hours, the number of survivors was recorded. Following this, they were kept under constant monitoring for another seven days.

**2.5 Experimental Animals**

Thirty albino Wistar rats, two male and two female, weighing 200–250 grams, were purchased from Sainath Agencies in Uppal, Hyderabad, India. In a controlled environment with a 12-hour light/dark cycle, the rats were housed individually and provided with free access to food and drink. After the rats were allowed to acclimate for seven days, they were divided into different experimental groups at random.

**2.6 Experimental Protocol:**

Albino Wistar rats weighing 200–250 grams, of any gender, were utilized in this 28-day research. The following is a description of the five

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groups (each including six rats) to which the experimental animals were randomly assigned:
Group 1: Normal Control (orally administered 1 ml/kg of normal saline)
Group 2: Disease Control (subcutaneous injection of 1 milligram of carbon tetrachloride per kilogram of body weight)
Group 3: Standard (oral administration of silymarin at 100 mg/kg body weight)
Group 4: Low Dose Treatment: Oral administration of CCl4 and Clinacanthus nutans leaf extract at a dose of 200 mg/kg body weight was performed.
Group 5: High Dose Treatment: 400 mg/kg body weight of oral Clinacanthus nutans leaf extract was administered along with CCl4.

The subcutaneous delivery of carbon tetrachloride (CCl4) started on day 5. The standard drug Silymarin and the Clinacanthus nutans leaf extract were orally administered one day after the CCl4 administration commenced.

**2.7 Evaluation of Biochemical Parameters**

The rats were all brutally slain by cervical dislocation within one day after the last treatment. Samples of blood were taken from the retro-orbital sinus plexus prior to the patient's death, while they were under light ether anaesthesia. After clotting, the drawn blood was separated into serum and centrifuged for 15 minutes at 3500 rpm. This process preserved the blood for subsequent investigation of multiple biochemical markers, including cholesterol, SGPT, SGOT, total protein level, SOD, and bilirubin. These studies were carried out with chemicals that were specially designed for an Automatic Analyzer. In order to conduct biochemical and histological analyses, a section of the liver was removed.

**2.7.1 Estimation of Alkaline Phosphatase (ALP):**
The method described by King (1965) was used to estimate the serum alkaline phosphatase.

**2.7.2 Estimation of Alanine Transaminase (ALT):**
Alanine Transaminase levels were measured according to the Modified Kinetic Method.

**2.7.3 Estimation of Aspartate Transaminase (AST)**
Aspartate Transaminase levels were measured according to the Modified Kinetic Method.

**2.7.4 Estimation of Total Bilirubin**
Bilirubin levels were measured according to the method Diacetyl Monoxime Method (DAM).

**2.7.5 Estimation of Total Cholesterol**
Cholesterol levels were measured according to the method Enzymatic-colorimetric method (Liebermann Burchard's Method).

**2.7.6 Estimation of Total Protein**

A coloured chelate is formed when cupric ions react with protein peptide links in an alkaline solution; this chelate's absorbance is measured at 578 nm. This combination remains soluble at alkaline $p^H$ thanks to the sodium-potassium tartrate in the Biuret reagent. In a sample, the amount of total protein has a direct correlation to the absorbance of the final colour.

**2.7.7 Estimation of Superoxide Dismutase**

The levels of superoxide dismutase were determined using the techniques described by Tilly et al., Keller et al., and Beckman et al.

**2.7.8 Estimation of Catalase**
The method developed by Hadwan MH et al. was used to test the levels of catalase.

**2.8 Histopathology of Liver**

Using a precise microtone (Buchi type) dissectometer, we extracted liver lobule tissue samples. A 10% phosphate-buffered neutral formalin fixative was used to cut 50-micron sections, which were then dehydrated in a graded alcohol solution ranging from 50% to 100% by weight, and then embedded in paraffin. Hemoxylin and Eosin were used to stain the sections. At 40X and 100X magnification, stained sections were examined. In order to determine histopathological scratch in hepatic tissues, a qualitative preliminary evaluation was conducted. When observing, we took into account the presence or absence of inflammation and necrosis in addition to the regular component constitution.

## III. STATISTICAL ANALYSIS

The mean ± SEM is used to represent the results. A graphpad A one-way analysis of variance (ANOVA) with Tukey's post hoc test was performed in Prism for statistical analysis. Catalase activity was measured in units per milligramme of protein. 9 when comparing more than two groups. Statistical significance was deemed to have been achieved when $p<0.05$.

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## IV. RESULTS AND DISCUSSION

### 4.1 Acute Toxicity Studies

According to the current research that followed the OECD standards 423, the chosen plant extracts did not cause any deaths throughout the seven-day trial. Table 3 shows the average weight of the animal after treatment with 2000 mg/kg of plant extract. In Table 4, we can see the treated animals' clinical signs. We also note that the wellness measures used to assess toxicity did not show any significant alterations. The skin, hair, eyes, mucous membranes, behavioural pattern, salivation, and sleep of both the treated and control animals were found to be normal. Tremors, lethargy, diarrhoea, and unconsciousness were not experienced by any of the animals. According to Table 2, there was no indication of such death. It did not appear to be hazardous at a dosage of 2000 mg/kg of the Clinacanthus nutans plant extract, according to the acute oral toxicity study. According to the calculations from Acute Oral Toxicity (Guideline 423), the LD50 of Clinacanthus nutans for acute oral toxicity was over 2000 mg/kg body weight.

**Table No 1: Effect of Clinacanthus nutans leaf extract on Body weight**

| Dose | Animal No. | Body Weight (gm) |
|---|---|---|
| C. Nutans (2000 mg/kg) | 1 | 28 |
| | 2 | 29 |
| | 3 | 28 |
| | 4 | 28 |
| | 5 | 27 |

**Table No 2: Effect of Clinacanthus nutans leaf extract on Mortality** Nil: No death reported

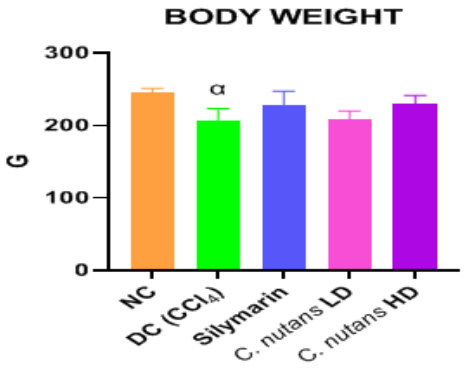
| Dose | Animal No. | Mortality |
|---|---|---|
| C. Nutans (2000 mg/kg) | 1 | Nil |
| | 2 | Nil |
| | 3 | Nil |
| | 4 | Nil |
| | 5 | Nil |

### 4.2 Hepatoprotective Activity

#### 4.2.1 Measurement of Body Weight, Change in Body Weight, Liver Weight and Relative Liver Weights

After receiving CCl4, neither the liver weight nor the body weight of the normal control group increased statistically. The increased liver weight was considerably decreased in the pre-treatment C. nutans group (200mg/kg, 400mg/kg) compared to the disease control group. When given at a dosage of 100 mg/kg, the conventional hepatoprotective medication silymarin significantly reduced liver weight and relative liver index to normal range.

**Figure No 1: Effect of *Clinacanthus nutans* leaf extract on Body Weight (g) in carbon tetrachloride in induced hepatotoxicity in wistar rats.**



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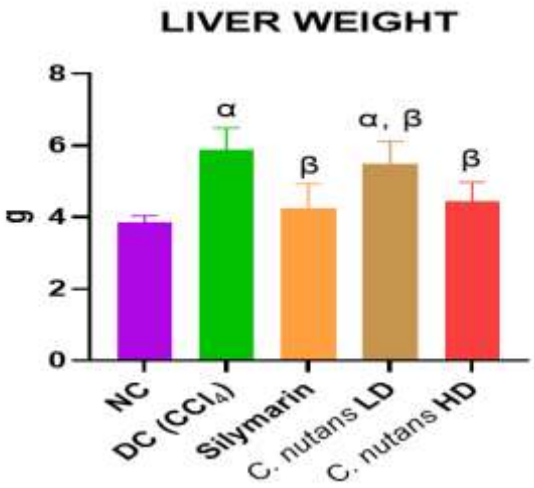
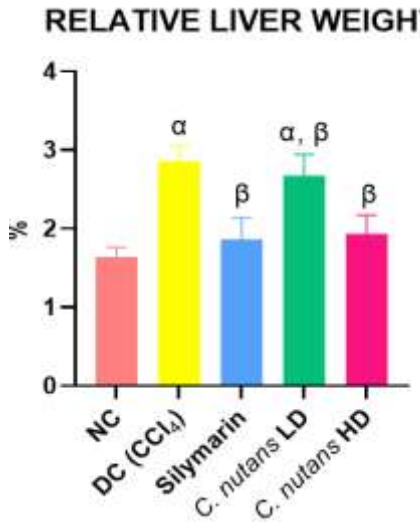
An analysis of variance with one way of separation is used to examine the data (ANOVA). The mean ± SEM (n = 6) is used to display the results for each group. Tukey's test is then used to compare means. By αp < 0.05, we can see that the normal control group and the illness control group vary significantly from each other.

**Figure No 2: Effect of *Clinacanthus nutans* leaf extract on Liver Weight (g) in carbon tetrachloride in induced hepatotoxicity in wistar rats.**




A one-way analysis of variance is used to analyse the data (ANOVA). The mean ± SEM of each group's results (n = 6) are shown. Tukey's test is then used to compare means. Differences between the normal control group and the illness control group are indicated by αp < 0.05, respectively.

**Figure No 3: Effect of *Clinacanthus nutans* leaf extract on Relative *Liver Weight* (%) in carbon tetrachloride in induced hepatotoxicity in wistar rats.**




An analysis of variance with one way of separation is used to examine the data (ANOVA). The mean ± SEM (n = 6) is used to display the results for each group. Tukey's test is then used to compare means. Both the normal control group and the sickness

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control group show significant differences with αp < 0.05.

Multiple animal and human investigations have shown that CCl4 may cause Cen trizonal hemorrhagic liver necrosis. This research found that CCl4 increased liver weight in mice by causing inflammation, vacuolization, and infiltration in the livers of the rats. As a result, the rats' body weights dropped but their liver weights rose. Rats treated with silymarin (100 mg/kg), methanol leaf extract of C. nutans (200 mg/kg, 400 mg/kg), or both failed to differ significantly in body weight from the disease control group, but they did differ significantly in liver weight and relative liver weight.

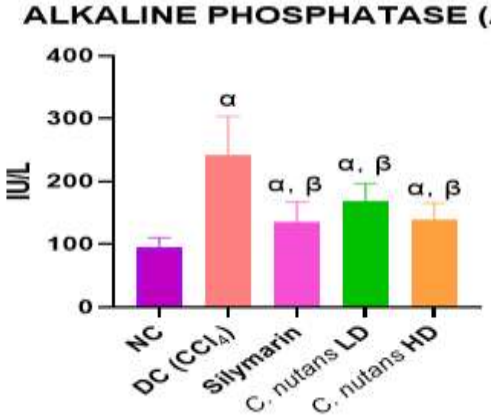
**4.3 Determination of Biochemical Parameters**

Using the corresponding assay kits and following the techniques provided, the serum was analyzed for biochemical markers such as SGOT, SGPT, ALP, total bilirubin, and cholesterol.

**4.3.1 Estimation of Alkaline phosphatase (ALP)**

Effect of Clinacanthus nutans and $CCl_4$ on ALP level of rats is shown in table 9. A considerable dose-dependent decrease in blood ALP was detected after treatment with methanolic extract of C. nutans leaf extract, in contrast to the normal group, which showed a marked increase in serum ALP levels after administration of CCl4 (1ml/kg).

**Figure No 4: Effect of *Clinacanthus nutans* leaf extract on *Alkaline phosphatase* (ALP) levels (IU/L) in carbon tetrachloride in induced hepatotoxicity in wistar rats.**




An analysis of variance with one way of separation is used to examine the data (ANOVA). The mean ± SEM (n = 6) is used to display the results for each group. Tukey's test is then used to compare means.

By αp < 0.05, we can see that the normal control group and the illness control group vary significantly from each other.

The role of hepatic cells is associated with serum ALP. An elevation in serum ALP levels is caused by an increase in synthesis, which occurs in the context of an increase in biliary pressure. The substantial liver damage caused by the toxin was corroborated by the higher levels of all of these marker enzymes in rats treated with CCl4. Polyphenolics may have had a role in the current study's reported decrease in ALP concentration after administration of plant extracts.

**4.3.2 Estimation of Alanine aminotransferase (ALT) levels**

Fig. 5 shows the effects of CCl4 and Clinacanthus nutans leaf extract on the ALT (SGPT) level of rats. The normal group's serum ALT (SGPT) level was significantly lower after treatment with methanolic extract of Clinacanthus nutans leaf extract, but the experimental group's level was significantly higher after administration of CCl4 (1 ml/kg). In comparison to the CCl4-treated group, enzyme levels were lower in the extract-and silymarin-treated groups.

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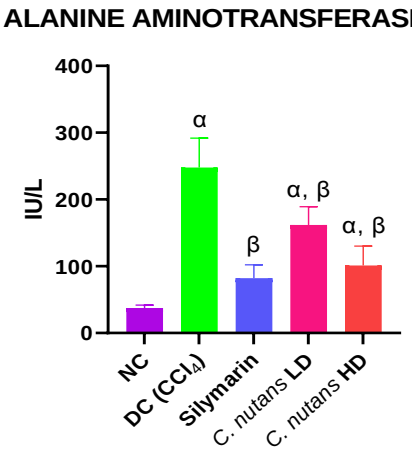
**Figure No 5: Effect of *Clinacanthus nutans* leaf extract on Alanine aminotransferase (ALT) levels (IU/L) in carbon tetrachloride in induced hepatotoxicity in wistar rats.**




An analysis of variance with one way of separation is used to examine the data (ANOVA). The mean ± SEM (n = 6) is used to display the results for each group. Tukey's test is then used to compare means.

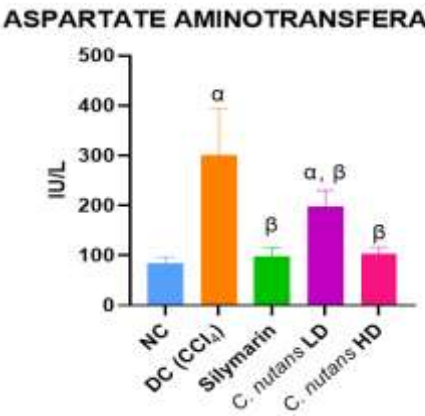
Both the normal control group and the sickness control group show significant differences with αp < 0.05.

An abnormally high serum ALT (SGPT) level after CCl4 treatment suggests cellular leakage and a decline in the functional integrity of the liver's cell membranes, which disrupts its usual function. The damage induced by the CCl4 was dramatically reversed (p<0.001) by the methanolic extract of C. nutans. The plant extract in question may have reduced the leakage of intracellular enzymes and reversed the increased serum enzymes in CCl4-induced liver damage by stabilising the cell membrane.

**4.3.3 Estimation of Aspartate aminotransferase (AST) levels**

Fig. 6 shows the effects of CCl4 and Clinacanthus nutans leaf extract on the AST (SGOT) level in rats. When compared to the normal group, the serum AST (SGOT) level was significantly elevated after CCl4 (1 ml/kg) administration; however, a dose-dependent reduction was detected after treatment with the methanolic extract of Clinacanthus nutans leaf extract. In comparison to the CCl4-treated group, enzyme levels were lower in the extract-and silymarin-treated groups.

**Figure No 6: Effect of *Clinacanthus nutans* leaf extract on Aspartate aminotransferase (AST) levels (IU/L) in carbon tetrachloride in induced hepatotoxicity in wistar rats.**



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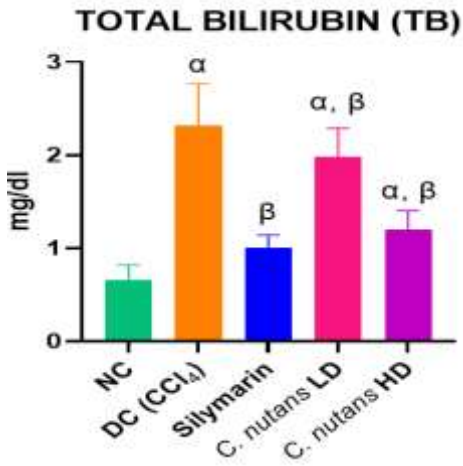
An analysis of variance with one way of separation is used to examine the data (ANOVA). The mean ± SEM (n = 6) is used to display the results for each group. Tukey's test is then used to compare means.
By αp < 0.05, we can see that the normal control group and the illness control group vary significantly from each other.

There was a significant increase in the serum AST (SGOT) level after CCl4 treatment, which means that the liver's normal functioning has been disrupted due to cellular leakage and lack of effective membrane integrity. The damage induced by the CCl4 was dramatically reversed (p<0.001) by the methanolic extract of C. nutans. The potential membrane stabilising ability of the plant extract under investigation might have prevented intracellular enzyme leakage and reversed the elevated serum enzymes seen in CCl4-induced liver injury.

**4.3.4 Estimation of Total Bilirubin levels**

Figure No. 7 shows the blood bilirubin level as a function of CCl4 and Clinacanthus nutans leaf extract. There was a considerable increase in serum bilirubin level after $CCl_4$ administration which was then reversed by C. nutans leaf extract.

**Figure No 7: Effect of *Clinacanthus nutans* leaf extract on Total Bilirubin levels (mg/dl) in carbon tetrachloride in induced hepatotoxicity in wistar rats.**




A one-way analysis of variance is used to analyse the data (ANOVA). The mean ± SEM of each group's results (n = 6) are displayed. Tukey's test is then used to compare means.
By αp < 0.05, we can see that the normal control group and the illness control group vary significantly from each other.

The buildup of bilirubin is a measure of the binding, conjugation, and excretory ability of hepatocytes, and it is one of the most helpful clinical cues to the degree of necrosis. Serum bilirubin levels were shown to decrease after extract administration in CCl4-induced liver injury, suggesting that the extracts were successful in restoring normal liver function.

**4.3.5 Estimation of Total Cholesterol levels**

When contrasted with the control group that received normal saline (P.O.), the CCL4-treated group had significantly higher cholesterol levels. Groups treated with silymarin or C. nutans leaf extract had lower cholesterol levels than the CCL4 group. GROUP-V (400 mg/kg + 1 ml/kg $CCl_4$ (S.C)) was showed more effect on the Cholesterol levels in $CCL_4$ induced Hepatotoxicity when compared to the GROUP-IV (200mg/kg +1 ml/kg $CCl_4$ (S.C)).

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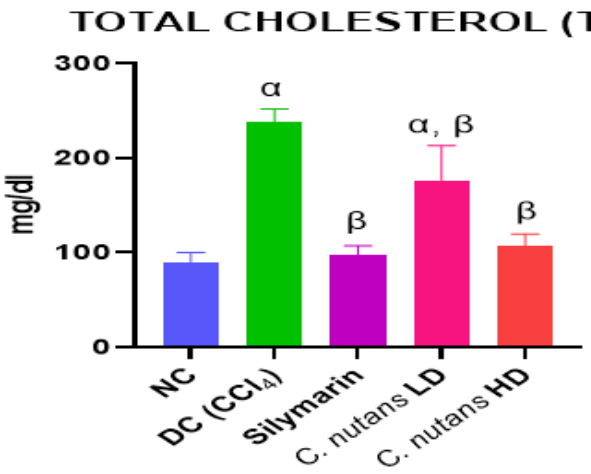
**Figure No 8: Effect of *Clinacanthus nutans* leaf extract on Total Cholesterol levels (mg/dl) in carbon tetrachloride in induced hepatotoxicity in wistar rats.**

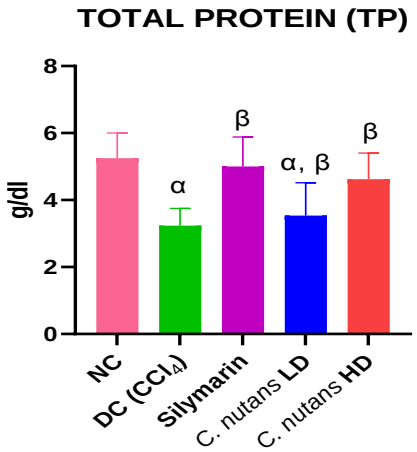



In this data study, a one-way analysis of variance is used (ANOVA). The means ± SEM (n = 6) of each group are shown. Tukey's test is then used to compare means. By αp < 0.05, we can see that the normal control group and the illness control group vary significantly from each other.

**4.3.6 Estimation of Total Protein levels**

Compared to the control group that received 0.9 percent W/V normal saline, the CCl4 group showed lower total protein levels (P.O.). In contrast to the animals in the CCl4 (1 ml/kg; S.C.)-induced group, those given C. nutans leaf extract (200 mg/kg, 400 mg/kg) or silymarin (100 mg/kg) had higher total protein levels. The high dose of C. nutans was showed more effect in CCl4 induced hepatotoxicity.

**Figure No 9: Effect of *Clinacanthus nutans* leaf extract on Total Protein levels (g/dl) in carbon tetrachloride in induced hepatotoxicity in wistar rats.**



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An analysis of variance with one way of separation is used to examine the data (ANOVA). The mean ± SEM (n = 6) is used to display the results for each group. The next step is to compare means using Tukey's test. Comparing the normal control group with the illness control group, we find that there are significant differences ($\alpha p < 0.05$).

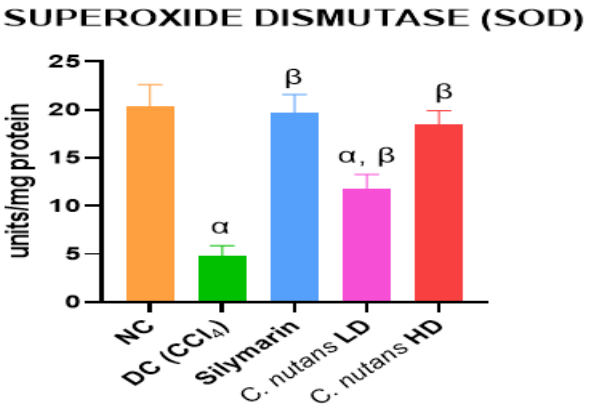
**4.3.7 Estimation of serum antioxidant enzymes**

The marker enzymes for oxidative damage like superoxide dismutase (SOD) and catalase were estimated for the measurement of protective activity exhibited by C. nutans leaf extract. Toxic dose of $CCl_4$ caused alteration in the antioxidant enzymes level which is an indicative of hepatocellular damage. Treatment of animals with the C. nutans leaf extract caused significant setback of the enzyme levels to near normal values.

**4.3.7.1 Estimation of Superoxide Dismutase (SOD)**

Compared to the GROUP-I (0.9 percent W/V Normal Saline (P.O.)) group, the CCL4 group had lower levels of SOD (4.32±0.17), while the control group had 8.512±0.17. and enzyme levels were greater in the silymarin and extract groups than in the control group. All the treated groups were showed more effect on the SOD levels in CCL4 induced Hepatotoxicity.

**Figure No 10: Effect of *Clinacanthus nutans* leaf extract on SOD levels (units/mg protein) in carbon tetrachloride in induced hepatotoxicity in wistar rats.**




An ANOVA with one way of analysis is used to examine the data. The results for each group are displayed as the average plus or minus the standard error of the mean (n = 6). Tukey's test is then used to compare means.

Both the normal control group and the sickness control group show significant differences with $\alpha p < 0.05$.

As one of the most important enzymes in the enzymatic antioxidant defence system, superoxide dismutase (SOD) activity declines, which is the most sensitive enzymatic marker in liver damage. Hepatocellular injury is sensitively indicated by a decline in superoxide dismutase activity. Hydrogen peroxide is formed when the superoxide anion is scavenged, reducing the harmful effects of this radical. Hepatic SOD activity was significantly increased by the C. nutans leaf extract, indicating that reactive free radical produced oxidative damage to the liver is reduced.

**4.3.7.2 Estimation of Catalase (CAT)**

Catalase levels were lower in the group treated with CCl4 (23.41±1.27) compared to the control group (8.512±0.17) that was treated with GROUP-I (0.9%W/V Normal Saline (P.O.). and Catalase were both reduced in the groups treated with extract or silymarin as compared to the control group. All the treated groups were showed more effect on the SOD levels in CCl4 induced Hepatotoxicity.

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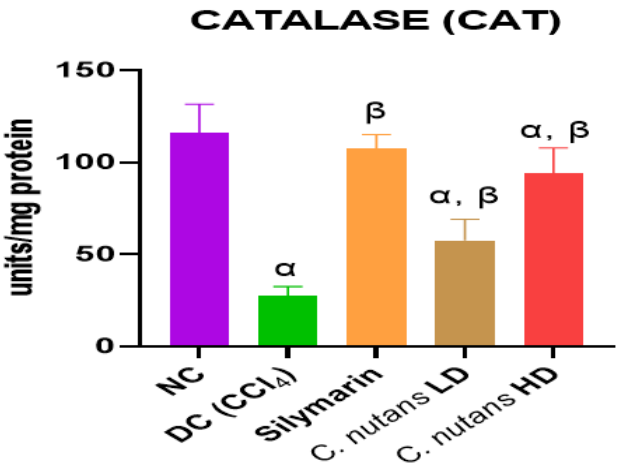
**Figure No 11: Effect of *Clinacanthus nutans* leaf extract on Catalase levels (units/mgprotein) in carbon tetrachloride in induced hepatotoxicity in wistar rats.**




An analysis of variance with one way of separation is used to examine the data (ANOVA). The mean ± SEM (n = 6) is used to display the results for each group. The next step is to compare means using Tukey's test.

Both the normal control group and the sickness control group show significant differences with αp < 0.05.

Red blood cells and the liver contain the most active forms of the enzyme antioxidant catalase (CAT), but it is present in every tissue of an animal. Catalase inhibits the formation of tissue-damaging hydroxyl radicals and breaks down hydrogen peroxide. As a consequence, the absorption of hydrogen peroxide and superoxide radical may cause a variety of harmful effects if CAT activity is reduced. The C. nutans leaf extract significantly elevated the reduced level of catalase caused by toxic dose of $CCl_4$.

**4.4 Histopathological Studies**

Figure 13 (a) shows the findings of the histological tests on the control animals' livers. The usual architecture of the hepatic cells is present, with well-defined cytoplasm, sinusoids, a conspicuous nucleus, and a central vein. In Figure 13 (b), inflammatory cell infiltration, severe coagulative necrosis, and hemorrhage were seen in the liver tissue sections of the rats in the disease control group that were administered 1ml/kg CCl4. Figure 13 (c) shows that rats treated with silymarin (100 mg/kg body weight) and then incubated with CCl4 maintained normal architectural hepatocytes. Fig.13 (d) shows that treatment with C. nutans leaf extract (200 mg/kg body weight) reduced coagulative necrosis, vacuole formation, and damage in rats. Figure 13 c shows that rats given with 100 mg/kg of silymarin had normal livers with moderate inflammation, texture, and cell arrangement; similarly, rats treated with 400 mg/kg of C. nutans leaf extract had normal livers with comparable inflammation, texture, and cell arrangement. Therefore, at a high dosage level of 400 mg/kg, the C. nutans leaf extract antagonistically inhibited the CCl4-induced hepatotoxicity in rats. The C. nutans leaf extract showed enhanced hepatoprotective potential by preventing histological changes. All of the observed histological alterations corroborated the liver's biochemical and functional measures (Figure 13).

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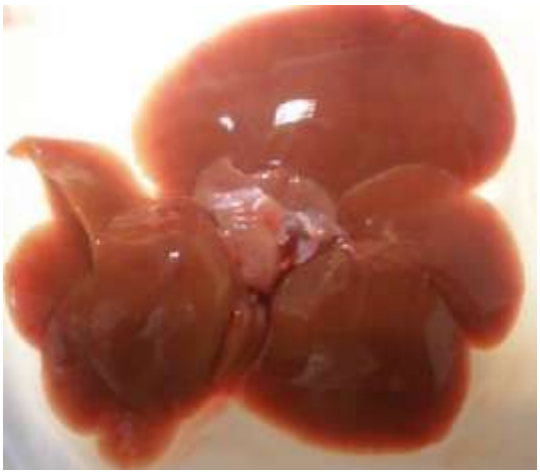
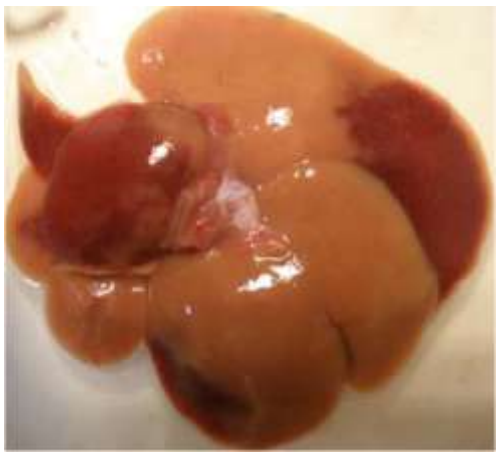
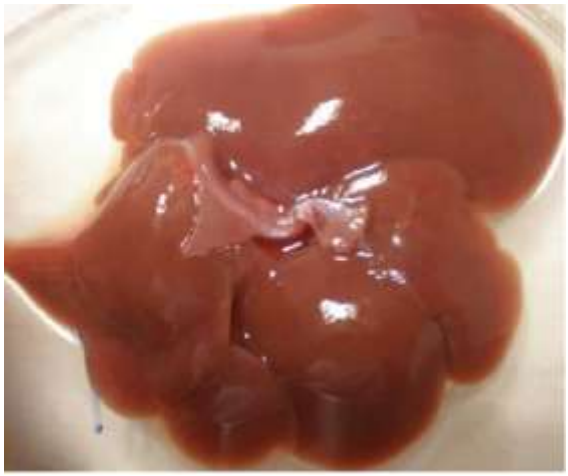
**Figure No 12: Photographs of liver**



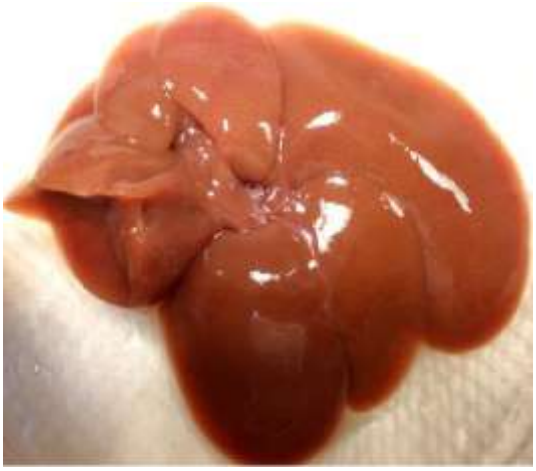
**a. Normal Control (Saline; P.O)**



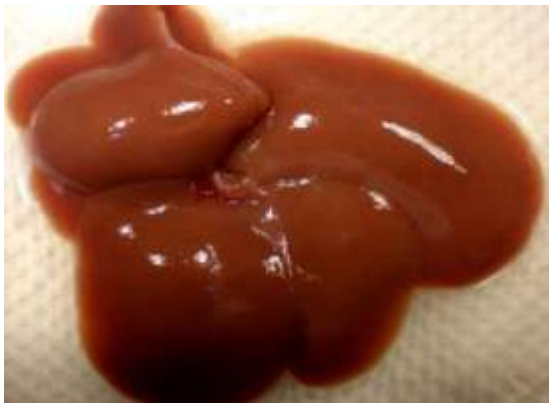
**b. Disease Control ($CCl_4$ 1ml/kg; S.C)**



**c. Silymarin (100 mg/kg; P.O)**



**d. *C. nutans* (200mg/kg; P.O)**



**e. *C. nutans* (400mg/kg; P.O)**

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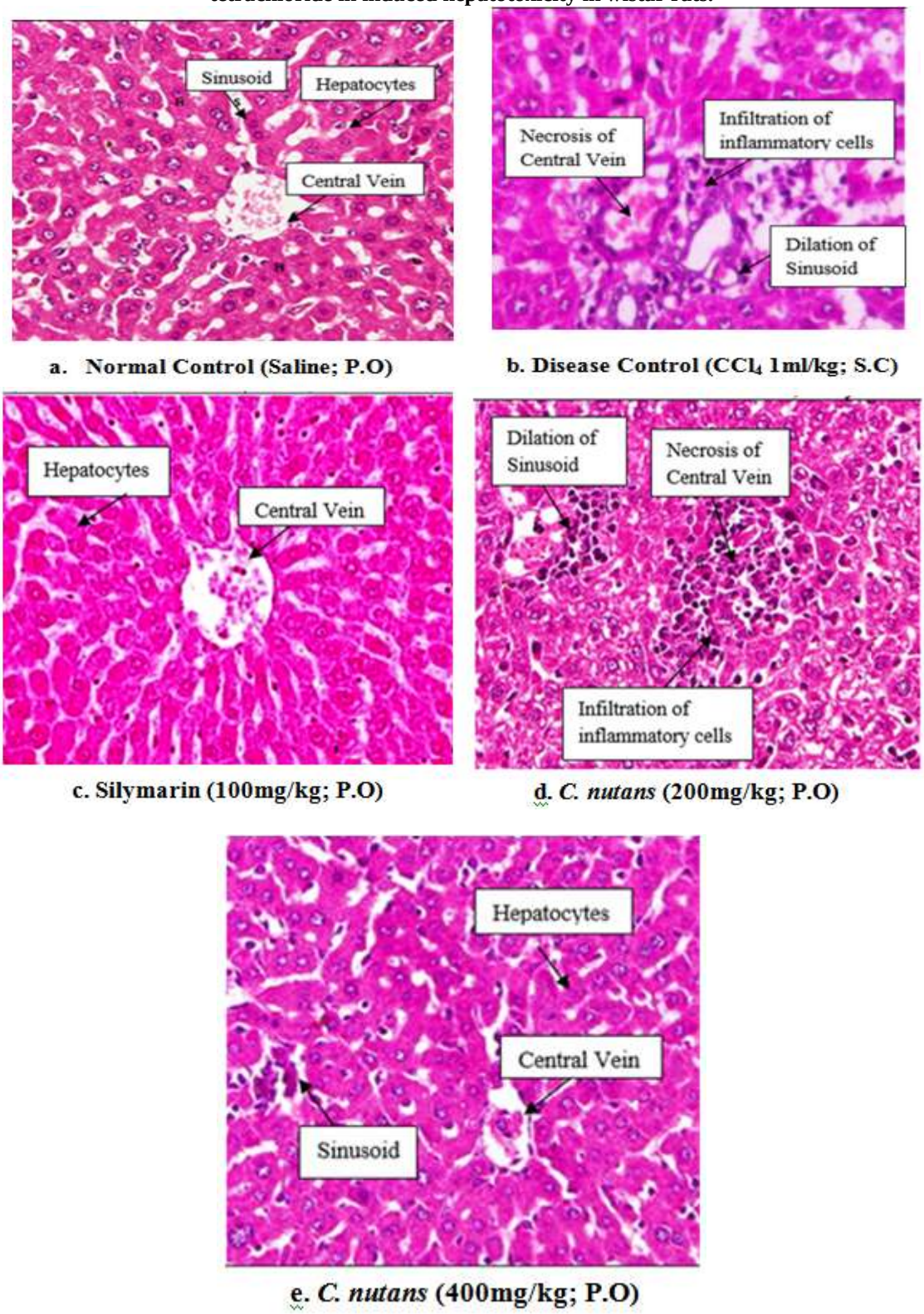
**Figure No 13: Effect of Clinacanthus nutans leaf extract on Histopathology of liver in carbon tetrachloride in induced hepatotoxicity in wistar rats.**




a. Normal Control (Saline; P.O)

b. Disease Control ($CCl_4$ 1ml/kg; S.C)

c. Silymarin (100mg/kg; P.O)

d. *C. nutans* (200mg/kg; P.O)

e. *C. nutans* (400mg/kg; P.O)

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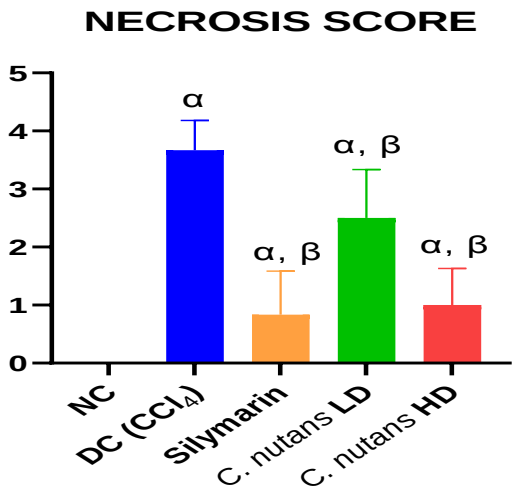
Furthermore, the liver pathology was scored as described previously (French et al., 2000), as follows:
Score 0 = No visible cell damage.
Score 1 = Focal hepatocyte damage in less than 25% of the tissue.
Score 2 = Focal hepatocyte damage in 25–50% of the tissue.
Score 3 = Extensive, but focal, hepatocyte lesions.
Score 4 = Global hepatocyte necrosis.

**Figure No 14: Effect of *Clinacanthus nutans* leaf extract on necrosis score in carbon tetrachloride in induced hepatotoxicity in wistar rats.**




An analysis of variance with one way of separation is used to examine the data (ANOVA). The mean ± SEM (n = 6) is used to display the results for each group. Tukey's test is then used to compare means. By $\alpha p < 0.05$, we can see that the normal control group and the illness control group vary significantly from each other.

## V. DISCUSSION

The acute toxicity test is crucial for assessing the safety and effectiveness of traditional herbal products as it helps identify potential injuries caused by high doses. It is recommended to use a limit test when existing data indicates low mortality risk at the highest starting dose level. This study assessed hepatoprotective activity through blood biochemical parameters and histopathological studies, focusing on the hematopoietic system, a sensitive target for toxic compounds in humans and animals. The liver's structural integrity is assessed by the elevated levels of hepato-specific enzymes like ALP, ALT, and AST in serum, which are released after cellular damage. Doses of C. nutans methanolic extract up to 2000 mg/kg body weight had no effect on the mice's weight, behaviour, toxicity markers, or death, according to the researchers. Another study looked at whether or not C. nutans ethanolic extract may prevent carbon tetrachloride (CCl4)-induced liver damage in rats. In contrast to rats treated with CCl4, those given the methanolic extract showed considerable improvements in total protein and catalase levels and marked decreases in high levels of many biochemical markers. Histopathological investigations confirmed these results, revealing that rats treated only with CCl4 suffered considerable liver damage. Rats pre-treated with silymarin or C. nutans extracts, on the other hand, showed less damage and kept their livers structurally intact. Particularly, the extract

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significantly protected rats' livers from CCl4-induced toxicity by promoting cell regeneration, maintaining membrane integrity, and reducing enzyme release.

## VI. CONCLUSIONS

The potential of medicinal plants as a tool in the fight against or treatment of liver disease is the focus of this investigation. The medicinal properties of Clinacanthus nutans leaves have been the subject of numerous studies, and this article concentrates on those leaves. Finding toxic-fighting defensive components inside these plants is the primary goal of the research. Plants have long been used for various ailments, and their use in medicine offers potential advantages due to lower toxicity compared to synthetic compounds. The study aims to find simple, effective herbal remedies for liver disorders in India.

**Ethical Issues:**
All the experimental procedures were carried out in accordance with Committee for the Purpose of Control and Supervision of Experiments on Animal (320/CPCSCEA dated 03-01-2001) guidelines. The study was reviewed and approved by the Institutional Animal Ethics Committee **(GPRCP/IAEC/09/19/11/PCL/AE-7-Rats-M/F-30)**, G. Pulla Reddy College of Pharmacy, Hyderabad, India.

**Conflict of interest:**
The authors have no conflicts of interest regarding this investigation.

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