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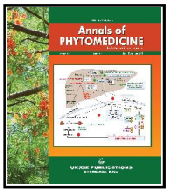

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# Exploration and treatment of salt-induced hyperlipidemia, adiposity and renal dysfunction by utilizing of *Matricaria recutita* L. whole plant concentrate in wistar rat model

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**Abstract**

A high-sodium diet (HSD) can lead to hypertensive rubor associated with hyperlipidemia, renal atrophy and adipocyte proliferation. This study aimed to investigate the *in vivo* function of *Matricaria recutita* L. whole plant phenol-rich extract (PRE) in ameliorating hyperlipidemia, obesity and renal recovering capacity in a high salt meal intake animal model. The study involved 35 male wistar rats each weighing between 170-180 g, divided into five groups and given different treatments. Group 1 was given rat food, Group 2 was given HSD, Group 3 was given HSD + 75 mg/kg body weight of PRE, Group 4 was given 100 mg/kg body weight of PRE and Group 5 was given 150 mg/kg body weight of PRE. The experimental rats' weights were noted everyday and at the conclusion of eight weeks, the animals were put to sleep after fasting for a whole night. Their kidneys were then isolated and blood was withdrawn through belly incisions for a subsequent histological and biochemical study. The study findings demonstrated that there was substantial ($p \leq 0.05$) histopathological alteration in the renal techtonics of Group 2 as well as increase in the lipid profile, body fat deposit (54.5%) and amount of renal bioproducts present, including urea, uric acid, creatinine and albumin. When compared with rats who were fed their normal diet, it was also found to be similarly associated with a dose-dependent recovery potential in the groups that were co-treated with PRE. The study concluded that PRE has the potential to be utilized as a therapeutic agent in the near future for the treatment of pathological derangements caused by high-sodium diet in terms of hyperlipidemia, adiposity and renal dysfunctionality. The study findings suggests that *M. recutita* whole plant phenol-rich extract (PRE) could be a promising natural health product for the treatment of hyperlipidemia, obesity and renal dysfunctionality.

## 1. Introduction

Despite all of the health issues that are afflicting the world, there have been a huge number of customer testimonials and success stories demonstrating the unquestionable effectiveness of natural therapies. This is another evidence that natural therapies should, without a shadow of a doubt, be incorporated into the administration of health care delivery. Since the birth of humanity, humans have used herbs as food and medicine. Medicinal plants make up a sizable amount of humanity's natural resources. Herbs have long been emphasized in Indian rights and culture (Vaidya *et al.*, 2022). Our ancestors have employed herbal plants for different antibacterial activities from the beginning of time, and this practice has been passed down from generation to generation (Deshmukh *et al.*, 2022). In particular, in our age that has become accustomed to consuming junk food, and a healthy momentum cannot be overlooked. A high-salt diet (HSD) is associated with junk food and prepared meals, which has been documented to cause hypernatremia, which has been linked to obesity, hyperlipidemia, and renal dysfunction in both people and animal models (Elagib *et al.*, 2012). More specifically, intake of junk food and prepared meals connected to high-salt diets (HSD) has also been documented globally (Akomolafe *et al.*, 2014). People have recently started switching from a homemade diet to ready-made meals that are readily available and most of which are preserved using high salt condiments. This change just took place recently. People who rely to consume more salt, which is harmfully undermining metabolic inertia and may be utilized as a preservative, flavoring, or seasoning in the latter (Disi *et al.*, 2016). Numerous international investigations that were undertaken have demonstrated this (WHO, 2012). Over the past few years, there has also been a lot of interest in the recommendation of dietary salt (sodium chloride) intake, which is particularly important in the regulation of associated pathologies like cardiovascular diseases (Elagib, 2012), obesity, and tunica intima stiffness (Amarini *et al.*, 2016). It has also been demonstrated that a high salt diet intake is the main contributor to oxidative damage,

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hepatotoxicity, obesity, stroke, and nephrotoxicity (Rodriguez-Iturbe and Johnson, 2010), as well as hyperlipidemia (Andonova *et al.,* 2022; Ara *et al.*, 2009).

Natural products are the most potent source of medication, according to our research. Since the dawn of time, almost all communities have turned to medicinal plants as a source of healing (Telrandhe and Gunde, 2022). According to theories put up by researchers (Ara *et al.*, 2009; Assink *et al.*, 1984), it has a substantial impact on the fibrosis, atherosclerosis, and myocardial infarction processes. Additionally, hypernatremia-related oxidative damage to lipidemic layers that simultaneously produced hyperlipidemia, organ toxicity, and glomerulus degradation has been shown in animal models. This implies that hypernatremia may contribute to the development of these diseases (Bhat, 2022). It is crucial to assess the toxicity of all medications, whether they are synthetic or natural. The toxicity analysis aids in choosing a safe dose in addition to foretelling any hazardous consequences (Potbhare *et al.*, 2022). Only a few numbers of research have shown that high salt-sensitive mice had decreased leptin responsiveness, which resulted in obesity, hyperlipidemia (Boegehold, 2013), and eventually hypertension (Konate *et al.*, 2012). Another study (Bruce, 1985) confirmed the effects of hypernatremia on the functioning and structural integrity of the kidneys (Busher, 1990), affecting biomarkers for urea, uric acid, creatinine, and albumin. According to reports, these impacts are mediated by the kidney's subvention of renin-modules angiotensin of operandi (Carre *et al.*, 2015).

It has been reported that plant origin can protect against hypernatremia-driven renal injury and the associated health problems (Chenevard *et al.*, 2006). This protection may be achieved by inhibiting the actions of angiotensinogen, which essentially peptides angiotensin I (Cheon *et al.*, 2017). This study was carried out as a part of an inquiry to address the metabolic infringement that existed (Chinedu *et al.*, 2013). Furthermore, phytoconstituents, most notably phenols, terpenes, and alkaloids, are also well classified and documented agents that are beneficial as protective therapeutics against the endemic development of some diseases (Fasan, 2021) and depletion on the levels of some renal and lipid related biomarkers such as globulin (Tojo and Kinugasa, 2012) and high-density lipoprotein (HDL) respectively (Olaleye *et al.*, 2013). The human body naturally produces the leptin hormone, which controls the kidney's ability to influence renin-angiotensin functions. This hormone may restore and safeguard the body's balance against a certain level of health instability brought on by excessive salt infringement if it is not overworked. Despite this, long-term exposure to a high-salt diet causes the body's inertia to eventually develop hypernatremia-related pathology (Roson *et al.*, 2011), along with hyperlipidemia and obesity, both of which are linked to a high mortality rate (Dobrian *et al.*, 2003).

With this knowledge in mind, the study's goal was to give a general review of the application of the M. recutita medicinal herb. In further detail, the study examined the plant's phenolic-rich bioactive components as well as its fatal dosage, antiobesity, kidney recuperation, and antihyperlipidemic potential in an animal model that had consumed a high-sodium diet. *M. recutita*, is a medicinal plant that belongs to the Asteraceae family (Michel *et al.,* 2013). It is most frequently used as a "bedtime tea," and it has been postulated that the sedative properties of herb could aid to alleviate feelings of alertness or anxiety during the evening, as well as encourage falling asleep.

## 2. Materials and Methods

### 2.1 Plant materials

Local sources of complementary and alternative medicine provided the whole *M. recutita* plant. The plant specimen that was tagged to Voucher No. 0258, identified and authenticated by "Post graduate" Teaching Department of Botany, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur.

### 2.2 Plant preparation

The whole *M. recutita* plant was subjected to a thorough washing with sterile water, followed by air-drying at room temperature for five weeks, as described by Weidner *et al.* (2013). Subsequently, the plant material was crushed using an industrial fine grinding machine, as reported by Sulaiman *et al.* (2011). The powdered sample was ultimately transferred into an amber-colored, sealed vial and thereafter kept in a refrigerator to facilitate future extraction.

### 2.3 Extraction of plant material and preparation of phenolic rich extract (PRE)

Combining the procedures described in (Konate *et al.*, 2012; Zlotek, 2016) with just minor modifications allowed for the production of a phenolic-rich extract. The powdered preserved sample of the dried whole plant *M. recutita* (300 g) was extracted by using a Soxhlet extractor apparatus with 1000 ml of acetic acid, water, and ethanol (2:28:70 v), and the mixture was extracted over a course of 72 h. Subsequently, the menstruum underwent filtration utilizing paper with a thickness measuring 150 mm.

Following this, it was subjected to rotary evaporation at a temperature of 40$^{o}$C, while being maintained under vacuum pressure. Finally, freeze drying was employed to get the dried extract, which was rich in phenolic compounds. Following that, the rich phenolic yield of 10.20 per cent was placed in amber bottles, which were then stored in the refrigerator so that further research could be conducted.

### 2.4 Qualitative test

The qualitative test going to be confirmatory test for phenolic content. A 5 g portion of the stock, containing a high concentration of phenolic compounds, was added to 2 ml of a 5% aqueous solution of ferric chloride in a test tube. After giving it a moderate shake, the test color tubes changed to a glittering blue, which showed the presence of a highly rich phenolic phytocomponent.

## 3. Animals used in research

In accordance with CPCSEA, the Institutional Animal Ethical Committee (IAEC) on animal usage of the post graduate school gave their stamp of approval to the study's protocol and assigned it an ethical approval number (DMIHER/IAEC/2022-23/012). The study employed healthy adult male Wistar rats with a weight range of 170-180 g.

The rats utilized in this study were acquired from the animal unit within the Department of pharmacology and afterwards housed within the department's animal facility. There were total of 35 rats used in the experiment. A commercial 8% high salt and normal rat chow was given to them (Vivo Bio Tech Ltd. Telangana, India), and they were given an abundant supply of water. Additionally, they were allowed to acclimate for two weeks prior to the beginning of the co-administration.

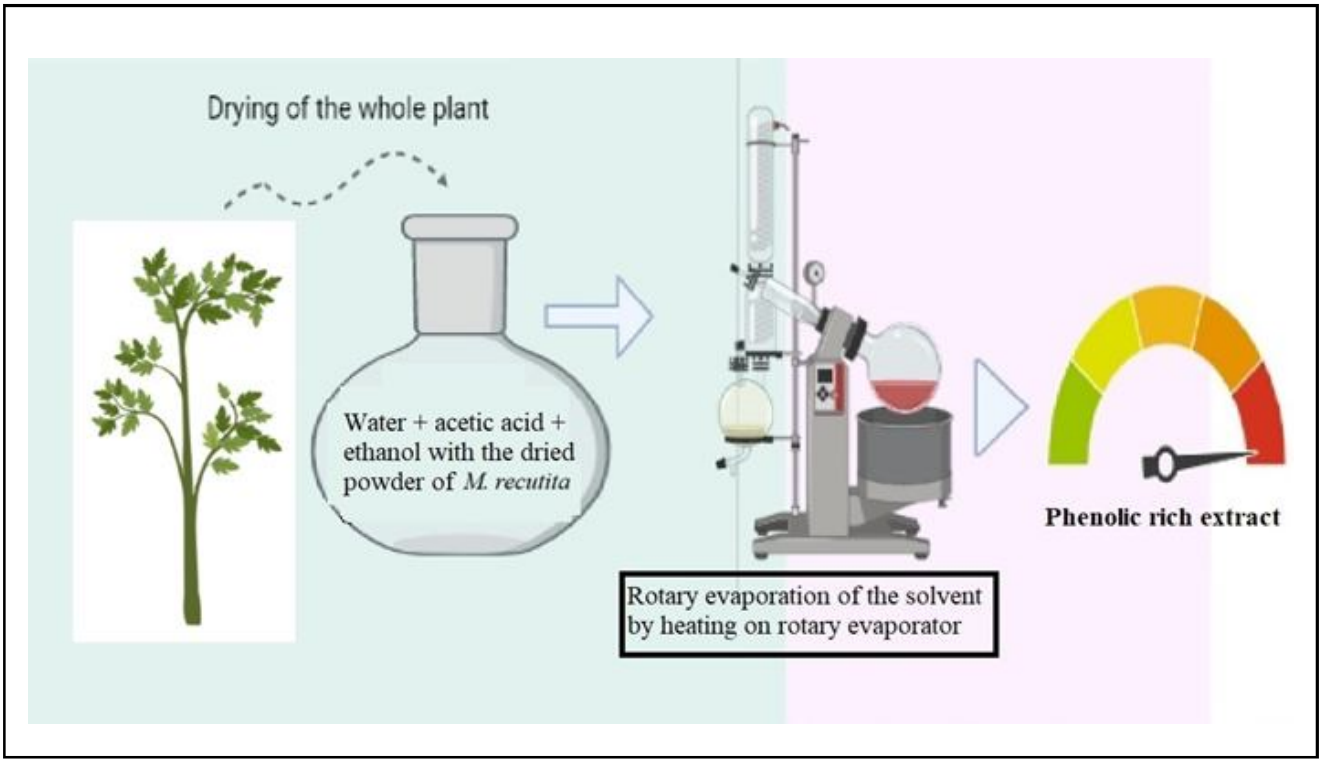


**Figure 1: Extraction of phenolic rich compounds in *M. recutita*.**

### 3.1 Selection of animals

A total of 35 wistar rats were divided into five groups, with each group consisting of seven rats. The distribution of rats in the groups is as follows:

**Group 1** rats on a standard fare (Chow diet).

**Group 2** rats were given an oral diet high in salt.

**Group 3** received HSD in addition to 75 mg/kg/bodyweight of PRE.

**Group 4** received HSD in addition to 100 mg/kg/bodyweight of PRE.

**Group 5** received HSD in addition to 150 mg/kg/bodyweight of PRE.

### 3.2 Acute lethal toxicity

Extracts high in phenol from the entire plant of *M. recutita* were tested for their acute and fatal toxicity. The acute lethal toxicity ($LD_{50}$) of phenol rich extracts (PRE) was calculated using up and down techniques in this study (Israel *et al.*, 2021). This is supported by the findings of the aforementioned studies (Bruce, 1985; Chinedu *et al.*, 2013).

### 3.3 Animal sacrificing

Following an overnight period of fasting and subsequent to the most recent administration, the animals were subjected to anesthesia using chloroform vapors for duration of approximately two minutes. Subsequently, the animals were sacrificed through an incision in the abdominal region, and the renal organs were extracted. Blood samples were collected using 5 ml syringes and divided into separate containers, one containing ethylene diamine tetraacetic acid (EDTA) and the other containing serum. The serum that was produced after the latter was allowed to stay for 45 min and then centrifuged at 3000 rpm for 15 min was used for the biochemical analysis. This study aims to assess the biochemistry and estimate the serum cholesterol profile. According to Olaleye *et al.* (2013) analytical protocol, test kits purchased from Dange Traders Ltd. were used to determine the concentrations of the following:

#### 3.3.1 Fat cholesterols in the serum

- Total cholesterol (TC)
- Triglycerides concentrations (mg/dl)
- Low density lipoprotein (LDL)
- Cholesterols made up of high-density lipoproteins (HDLc)

The following is the calculation that was used to determine the total cholesterol concentration:

$$\frac{\text{Absorbance of sample} - \text{Concentration of standard}}{\text{Absorbance of standard}}$$

Conversion factor, mg/dl × 0.0258 = mmol/l

#### 3.3.1.1 Estimation of triglycerides (TG)

Triglyceride concentration was determined by the following formula:

$$\text{Triglycerides (TG) (mg/dl)} = \frac{\text{Absorbance of sample} \times 200}{\text{Absorbance of standard}}$$

Conversion factor, mg/dl x 0.0113 = mmol/l

#### 3.3.1.2 Calculation of high density and low-density lipoproteins

The HDL and LDL/VLDL cholesterol measurement kit offers a straight forward approach for the efficient separation of HDL and VLDL

(very low-density lipoprotein) in blood samples. This kit allows for the separate quantification of HDL and VLDL by the utilization of cholesterol oxidase mediated reactions, hence providing a handy and readily measurable technique. The feasibility of this process is enabled by the incorporation of a kit that presents research suggesting a direct method to enhance the effective separation of high-density lipoprotein (HDL) and very low-density lipoprotein (VLDL) particles. After heating the reaction mixture to 37°C for an hour, an absorbance reading was taken at 570 nm using a microtiter plate reader having 96 wells and 4303 model.

#### 3.3.2 The formula for calculating HDLc concentration

$$\frac{\text{Absorbance of sample} \times \text{Concentration of standard} \times \text{Dilution factor (200)}}{\text{Absorbance of standard}}$$

where, LDL-cholesterol was quantified as:

LDL cholesterol = Total cholesterol – HDLc – (TG/5)

#### 3.3.3 Calculation of kidney function biomarkers

##### 3.3.3.1 Serum creatinine determination

The method that was described by Zuo *et al.* (2008) was used to quantify the serum creatinine levels.

##### 3.3.3.2 Serum urea determination

In the presence of urease, urea is hydrolyzed enzymatically into ammonia, and the ammonia subsequently experiences a change in color to blue when phenol and hypochlorite solution are present. The content of urea was quantified by the utilization of photometric measurements, specifically by assessing the absorbance of the blue solution at a wavelength of 546 nm, which in turn gave the concentration of the urea. For the assay, a commercial kit that was purchased from LABKIT was utilized.

The following formula was used to determine the concentration of urea in milligrams (mg/dl):

$$\frac{\text{Absorbance for sample} - \text{Absorbance for standard}}{\text{Standard concentration}}$$

##### 3.3.3.3 Serum uric acid determination

The approach that was presented by Zuo *et al.* (2008) was utilized in order to perform the calculation necessary to determine the concentration of uric acid.

##### 3.3.3.4 Measurement of total protein in serum

The total amount of protein was computed using the method outlined by Sedlak and Lindsay (1968).

##### 3.3.3.5 Serum albumin determination

According to the findings of Assink *et al.* (1984), we were able to determine the albumin concentration in the serum.

##### 3.3.3.6 Serum globulins determination

As a consequence of this, the total globulin fraction was determined by reducing the total protein content of the serum by the albumin concentration (Busher, 1990).

Globulin level (g/dl) = Total protein concentration (g/dl) - albumin concentration (g/dl)

#### 3.3.4 Calculation of the weight of both the animals and the kidneys

We also determined the body weights of the rats that had PRE and HSD delivered concurrently, as well as the weight of the kidney that was removed for analysis.

##### 3.3.4.1 Histological analysis of the kidneys

After being fixed in a phosphate-buffered solution with a pH of 7.4, the renal architecture was examined after being stained with eosin and then preserved with formaldehyde at a concentration of 4%. After being premounted on slides, tissues were inspected at X100 (H&E) magnification and recorded (Olorunnisola *et al.,* 2021).

#### Statistical analysis of data using Newman-Keuls Multiple Comparison test

An examination of the Statistics the data were analyzed using a Newman-Keuls Multiple Comparison test, which is a streamlined version of a one-way analysis of variance (ANOVA). Graph Pad Prism was utilized in the process of carrying out the statistical analysis (ver.5.0a). In addition to this, all of mean and standard deviation were used to summarize the data (n=6), and statistical significance was determined using the $p$ value less than 0.05. The data with various superscripts along the same column were put next to the data from the control group, and the results showed that they are statistically distinct.

## 4. Results

After 8 weeks, a significant ($p$>0.05) percentage increase in the body weight of the positive group (HSD diet) (54.5%). After comparing the negative rats fed a standard chow diet (51.2%), was intimated in this study. This finding is depicted in Figure 2, and the study revealed a statistically significant difference observed in body weight growth across the groups having positive group and negative treated groups. Furthermore, the observed prevalence of obesity is consistent with the conclusions drawn by Lanaspa *et al.* (2018). Their study identified a correlation between the lack of response to leptin, a diet rich in salt, and obesity in rats. This was accompanied by the activation of endogenous fructose metabolism, which further contributed to the development of fatty liver. In contrast to the control group, the rats that received a phenolic-rich extract (yielding 10.20 per cent) from the entire plant exhibited a notable and statistically significant ($p$<0.05) reduction in body weight. This reduction was observed to be dose-dependent, with percentages of 34 per cent, 29.3 per cent and 22.9 per cent for different doses of the extract. This was observed in all three groups. The antiobesity findings of, on normoglycemic wistar rats, to which they fed aqueous extract of the same plant, is consistent with the established dose-dependent and co-relational decreases ($p$<0.05). Because of this, the effect that *M. recutita* extract has on body weight can be linked to the abundant phenolic phytoconstituents that are contained in this concentrate.

In addition to this, it can adequately explain, as seen in Table 1, a significant ($p$>0.05) rise in the total amount of lipid profile, total cholesterol, total fat, very low-density lipoprotein, and low-density lipoprotein, with a concomitant ($p$<0.05) drop in the level of HDL in the group fed high salt diet exclusively (Group 2) when collate with

rodents on standard rat meal after 8 weeks of treatment (Lanaspa *et al.,* 2018). The results showed that PRE has the ability to dramatically and dose-dependently bring the co-administered group's impaired lipid profile back to a level that was almost normal (Ara *et al.,* 2009). This was linked to an HDL level improvement that was dose-dependent as well as metabolic. It is crucial to emphasize the detrimental increase in low-density lipoprotein (LDL) levels compared to other lipid parameters in the group exposed to excessive salt. This observation aligns with the findings reported by Olaleye *et al.* (2013) in their study.

**Table 1: Results of feeding rats a meal consisting of 8% more salt than normal a phenol rich extract (PRE) of the *M. recutita* entire plant and measuring their lipid profiles in mg/dl**

| Group | TC | HDL | TG | LDL | VLDL |
|---|---|---|---|---|---|
| **Group 1** (Rat food) | 91.21 ± 1.21 | 80.97 ± 1.14 | 70.11 ± 1.15 | 9.31 ± 1.00 | 14.62 ± 0.33 |
| **Group 2** (HSD) | 158.50 ± 1.26* | 48.38 ± 0.55* | 180.30 ± 1.30* | 58.05 ± 0.15* | 43.32 ± 0.25* |
| **Group 3** (HSD + 75 mg/kg PRE) | 130.30 ± 0.03* | 54.31 ± 0.33* | 161.20 ± 1.20* | 38.41 ± 0.08* | 29.21 ± 0.52* |
| **Group 4** (HSD + 100 mg/kg PRE) | 115.30 ± 1.35* | 72.51 ± 1.29* | 140.50 ± 0.15* | 21.52 ± 0.01* | 20.55 ± 0.16* |
| **Group 5** (HSD + 150 mg/kg PRE) | 99.21 ± 0.15* | 75.32 ± 1.19 | 100.30 ± 0.13* | 13.51 ± 0.03* | 15.59 ± 0.25 |

**Note:** Values are expressed as mean ± standard deviation (SD). TC: Total Cholesterol, HDL: High-Density Lipoprotein, TG: Triglycerides, LDL: Low-Density Lipoprotein, VLDL: Very Low-Density Lipoprotein. *$p$<0.05 compared to Group 1. The statistical significance was determined using the Newman-Keuls Multiple Comparison test.

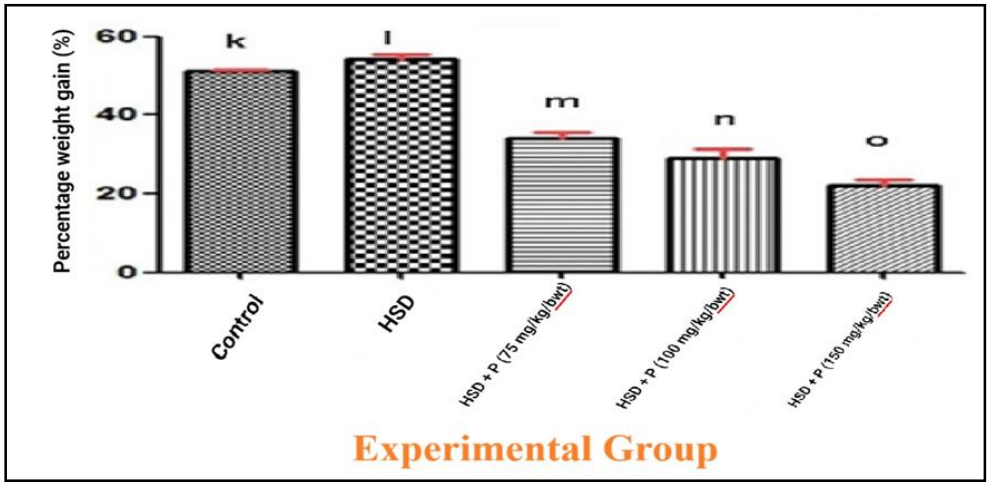


**Figure 2: The effects that a phenolic rich extract of the whole *M. recutita* plant had on the body weight of rats that had been fed a diet consisting of 8% high salt.**

In comparison to animals that were provided with a standard diet, it was observed that rats who were fed with high-salt diet (HSD) exhibited a dose-dependent rise in blood levels of urea, uric acid, creatinine, and albumin. This increase was shown to be statistically significant ($p$>0.05).

The aforementioned observation was made in Table 2. Nevertheless, when Groups 3, 4 and 5 were simultaneously administered with PRE at doses of 75, 100 and 150 mg/kg/body weight, respectively, a reduction in the levels of kidney biomarkers (urea, uric acid, creatinine, and albumin) was observed in a dose-dependent manner, approaching normal levels. Concurrently, there was an elevation in the levels of the kidney marker, globulin. Most importantly, when compared to the group that was fed regular chow, PRE was able to restore all renal parameters that had been compromised to levels that were nearly normal after being treated with the co-treatment.

**Table 2: Results of the phenol rich extract (PRE) of the *M. recutita* on some markers of HSD-induced kidney toxicity.**

| Group | Urea (mg/dl) | Uric acid (mg/dl) | Creatinine (mg/dl) | Albumin (g/dl) | Total protein (g/dl) | Globulin (g/dl) |
|---|---|---|---|---|---|---|
| Group1 | 31.80 ± 0.53 | 2.21 ± 0.02 | 0.62 ± 0.05 | 3.20 ± 0.01 | 6.05 ± 0.03 | 2.85 ± 0.02 |
| Group2 | 58.21 ± 0.05* | 6.05 ± 0.05* | 1.41 ± 0.07* | 5.62 ± 0.02* | 7.05 ± 0.12 | 1.43 ± 0.10* |
| Group3 | 50.15 ± 0.02* | 4.32 ± 0.02* | 1.10 ± 0.01* | 4.74 ± 0.10* | 6.05 ± 0.12 | 1.31 ± 0.02* |
| Group4 | 43.21 ± 0.02* | 3.96 ± 0.05* | 1.05 ± 0.03* | 3.83 ± 0.15* | 6.05 ± 0.55 | 2.22 ± 0.40 |
| Group5 | 39.30 ± 0.08* | 3.01 ± 0.02* | 0.92 ± 0.04* | 2.98 ± 0.11 | 6.05 ± 0.12 | 3.07 ± 0.01* |

**Note:** Values are expressed as mean ± standard deviation (SD). Urea, uric acid, creatinine, albumin, total protein, and globulin were measured to evaluate renal function. *$p$<0.05 compared to Group 1. The statistical significance was determined using the Newman-Keuls Multiple Comparison test. Tests of renal function have utility in identifying the presence of renal disease, monitoring the response of kidneys to treatment, and determining the progression of renal disease. Regular testing helps healthcare providers track the health of patients and any underlying conditions. The results of this study suggest that Matricaria recutita L. whole plant phenol-rich extract (PRE) has the potential to be utilized as a therapeutic agent for the treatment of pathological derangements caused by a high-sodium diet in terms of hyperlipidemia, adiposity, and renal dysfunctionality.

**Citations:**

[1] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9689510/

[2] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6615299/

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[4] https://www.ahajournals.org/doi/10.1161/CIRCULATIONAHA.122.059038

[5] https://www.scielo.br/j/bjmbr/a/5Q975nnbbKfPyLxcNwNff6M/

[6] https://ordspub.epa.gov/ords/eims/eimscomm.getfile?p_download_id=458780

### 4.1 Histological analysis

As shown in Figure 3, the initial set of rats, which were provided with standard rat chow, had typical anatomical representations of the kidney. When compared to Group 2, which was fed only HSD, these were demonstrated by normal kidney total weight, glomerulus size, and afferent arteriole size (blue arrow). The kidney weight, renal topology, and the cortical histo-architecture were all eventually distorted as a result of the gradual consumption of high-salt diets, which ultimately led to nephrosclerosis. However, in a study conducted by Bhat (2022), it was observed that Groups 3, 4, and 5, which received concurrent administration of PRE at doses of 75, 100, and 150 mg/kg/body weight, respectively, exhibited a notable and progressive improvement in kidney integrity. Notably, Group 5 demonstrated a restoration of kidney parenchyma, mesangial cells, and renal weight, approaching levels comparable to those observed in healthy individuals.

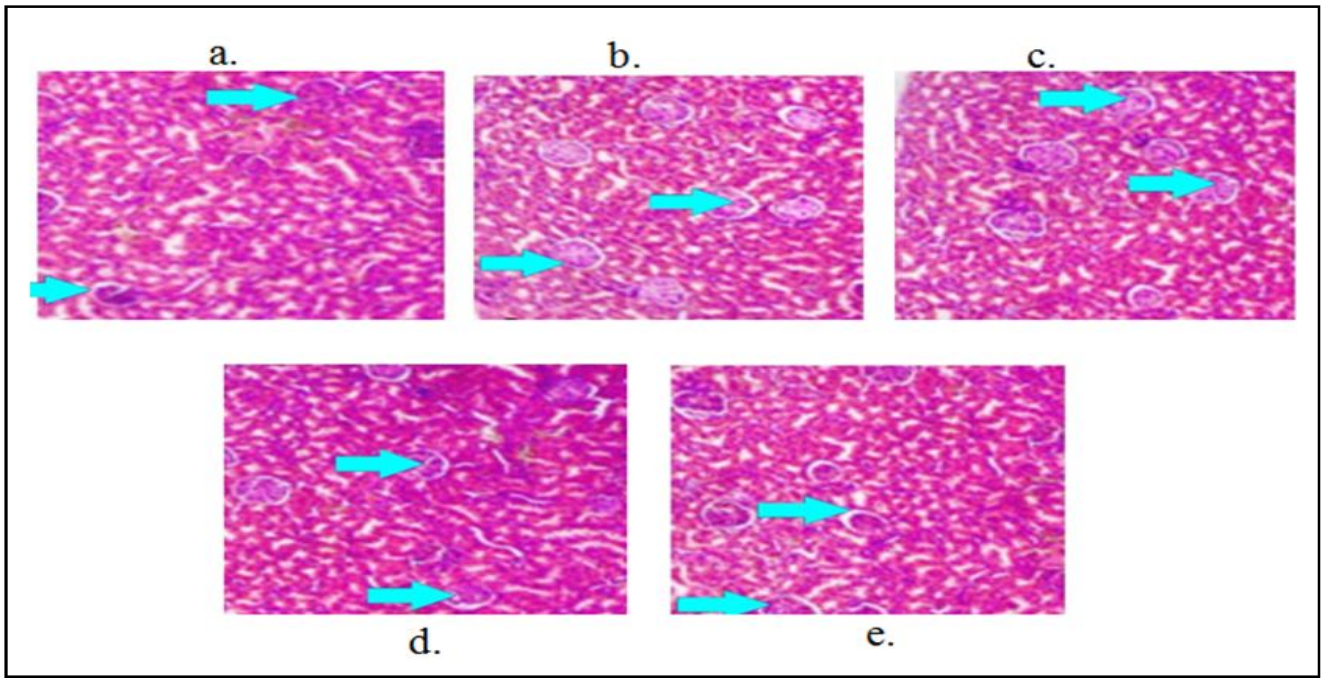


**Figure 3: Photomicrograph of a section through a kidney that has been stained with eosin using a microscope. Group (a) is normal, Group (b) High-salt diet (HSD), Group (c) HSD combined with 75 mg/kg body wt of PRE, Group (d) HSD+100 mg/kg body wt. of PRE, Group (e) HSD+150 mg/kg body wt. of PRE.**

**Table 3: Rats were given PRE and HSD concurrently, and the weight of their harvested kidneys was recorded**

| Group | Weight of harvested kidney (g) |
|---|---|
| **Group 1** (Rat Food) | 0.60 ± 0.02 |
| **Group 2** (HSD) | 0.96 ± 0.02 |
| **Group 3** (HSD + 75 mg/kg PRE) | 0.85 ± 0.01 |
| **Group 4** (HSD + 100 mg/kg PRE) | 0.70 ± 0.02 |
| **Group 5** (HSD + 150 mg/kg PRE) | 0.61 ± 0.01 |

## 5. Discussion

In the present investigation, it was shown that the ingestion of a high-salt diet (HSD) comprising eight percent of the total weight resulted in a 54.5% increase in adiposity associated with hypernatremia, as compared to the consumption of a standard diet (51.2%), in rats throughout an eight-week experimental period. The weight gain and increased kidney volume relative to body weight reported in the rats subjected to a high-salt diet (HSD) might perhaps be explained by the reduced impact of leptin, an adipocytokine, and adipsin hormones, which are recognized for their role in appetite regulation.

Furthermore, it was noted that the animals exclusively administered high-sucrose diet (HSD) consumed a greater quantity of water compared to the groups subjected to other dietary conditions; namely, the group provided with ordinary rat chow during the whole duration of the eight-week investigation. The prevailing belief attributed this phenomenon to the signal induction originating from aldosterone, a hormone produced in the kidneys. The weight reduction observed in different groups co-administered with varying doses of PRE (75, 100, and 150 mg/kg) body weight of the *M. recutita* whole plant is partially consistent with previous findings on the aqueous extract of the same plant. The observed reduction in body weight may be related to the efficacy of the many metabolites in alleviating mitochondrial and endoplasmic reticulum (ER) stress caused by hypernatremia. This stress is known to contribute to the production of fatty adipose polymers.

Plant concentrates that are rich in nutrients have the potential to stimulate the breakdown of fatty triglycerides into adenosine triphosphate (ATP) within the mitochondria and the structural components of the innermost layer of blood vessels. This process leads to a decrease in body fat, water retention, and the ratio of extracellular volume in the bloodstream. Over time, this phenomenon leads to an enhancement in the general health state of the groups receiving treatment, accompanied with a decrease in weight following co-administration.

Based on the results of this investigation, it was shown that HSD induced significant alterations in the structural integrity of the kidneys as well as the biomarkers associated with renal function, ultimately leading to the development of nephrosclerosis (Figure 3). As a result, there was a notable elevation in renal-related blood bio-molecules, including albumin, nitrogenous urea, uric acid, creatinine, and renal weight, by 60% in rats that were exclusively fed a high-salt diet (HSD) for 8 weeks, in comparison to the groups that were provided a regular chow diet. This phenomenon was seen in rats who were only fed a high-salt diet (HSD). The HSD indicated above caused damage to the integrity of the kidney, leading to disease that was dependent on the dosage. This pathology included hyperlipidemia and obesity, both of which were shown to be connected with depletion of globulin, as demonstrated by Tojo and Kinugasa (2012). The pathophysiology of high salt-induced nephrosclerosis involves excessive activation of the renin-angiotensin-aldosterone system (RAAS) in the adrenal gland of a compromised kidney, in response to inflammatory agonists. This leads to an increase in markers associated with nephrosclerosis and a gain in plasma volume within the kidney (Duarte and Cooper-Dehoff, 2010). Nevertheless, the simultaneous administration of PRE (at doses of 75, 100, and 150 mg/kg) body weight in a separate group of rats given a high-salt diet (HSD) exhibited a considerable and dose-dependent amelioration of the pathological conditions. Furthermore, it is plausible that PRE might potentially impede the renin response, bringing it closer to a state of normalcy.

In a more comprehensive context, the pressure natriuresis response (PRE) serves to inhibit the renal hyperactivity induced by high salt diet (HSD), as well as the stimulation of the adrenal gland renin hormone secretion (He and MacGregor, 2004). The PRE achieves this by counteracting the influx and electrochemical gradient of sodium and water, which are reabsorbed from the renal tubules back into the bloodstream (Tojo and Kinugasa, 2012). The phenolic-rich concentrates have been shown to enhance the attenuation effects on the excessive deposition of serum protein macromolecules. This in turn, leads to an eventual improvement in the credibility of creatinine clearance. This outcome is beneficial for the overall health of the individual. The observed homeostatic balance, as demonstrated by the PRE on HSD-induced kidney parenchyma dysfunction, is depicted in Figure 3. This balance is indicated by markers such as globulin levels and kidney weight. Additionally, the effectiveness of the phytochemicals in mitigating albuminuria, a kidney disease (Tojo and Kinugasa, 2012), is also supported. Notably, the co-treated groups showed improved physical performance (Elagib and Nabiela, 2012). The study conducted by Olaleye *et al.* (2014) demonstrated the effectiveness of dose-dependent administration of rich concentrates in mitigating the assaults on various groups. Additionally, the study found that the co-administration of gallic and tannic acids extracts resulted in a significant restoration of renal parameters in rats. This restoration was observed through the reduction of endoplasmic reticulum hyper inductivity and the restoration of nephron specificity, bringing these parameters close to their normal levels.

In addition, the hormone aldosterone, produced by the adrenal gland located in the kidney, has a distinctive function in the regulation of the sodium-potassium electrochemical equilibrium inside the kidney as well as the concentrations of plasma protein in the bloodstream. It is worth noting that individuals who are vulnerable to high salt diet (HSD) agonists have been reported to exhibit an excessive synthesis of aldosterone, along with the activation of enzymes referred to as Angiotensin converting enzymes (ACE) and endothelin converting enzymes (ECE). Furthermore, the plentiful extracts facilitated a notable recovery, perhaps impeding the activity of ACE and ECE enzymes, as well as the conversion of angiotensinogen into angiotensin I, a peptide with central effects. Consequently, this hindered the conversion of inactive angiotensin into its active form. The phenol-rich plant concentrates have the potential to enhance kidney functionality through various mechanisms, such as the activation of endothelial nitric oxide synthase (Philip, 2020), induction of hydrogen sulphide ($H_2S$) production (Boegehold, 2013), promotion of cardiac inotropism (Kass *et al.*, 1987), and simultaneous reduction in ACE-associated NADPH oxidase activity (Boegehold, 2013). These effects can lead to improvements in other renal parameters, volume gain, and ultimately bring the kidney function close to normal levels, thereby potentially preventing kidney dysfunction (Andonova *et al.*, 2022). Nevertheless, there is no overall reduction in protein content. Throughout the 8-week study, it was observed that the HSD fed group exhibited a notable disparity in

concentration levels. However, the administration of PRE demonstrated a dose-dependent reduction in high salt nephrotoxicity and albuminuria, as indicated by the photomicrograph (Figure 3). These findings suggest that PRE could potentially serve as a viable therapeutic option for the treatment of HSD-induced renal injury. The golgi apparatus is primarily responsible for the transportation of biolipids, which may be classified into four primary categories: high density lipoprotein (HDL), low density lipoprotein (LDL), triglycerides (TG), and very low-density lipoprotein (VLDL).

The observed rise in total cholesterol and low-density lipoprotein levels in rats subjected to a high-salt diet (HSD) might perhaps be attributed to the denaturation of tunica intima and hepatocytes induced by the agonist. The process of denaturalization may have resulted in the development of hyperlipidemia as a compensatory reaction, leading to the manifestation of increased body fat and the accumulation of fat in the liver. The statistically significant differences ($p < 0.05$) observed in the lipid profile, specifically total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL), between the group of rats treated with the HSD agonist and the group of rats fed a normal chow diet, may be attributed to a decline in hepatic lipase activity. However, the effectiveness of PRE in addressing hyperlipidemia in co-treated groups was reported to be successful. The findings of Ezeugwunne *et al.* (2018) suggest that the amelioration of hypernatremia-induced damage to the tunica intima and hepatocytes may be achieved through the formation of hydrogen, covalent, and/or ionic bonds. These bonds are commonly observed morphological manifestations of intracellular ameliorative mechanisms, which in turn enhance the anti-lipidemic efficacy of the affected constituents.

After the administration of the prescribed intervention, there was a notable elevation in the levels of high-density lipoprotein (HDL), which is widely acknowledged as a favorable variant of cholesterol. The observed elevation in high-density lipoprotein (HDL) levels promoted the transportation of low-density lipoprotein (LDL) from the tissues and endosomal lumen back to the hepatocytes, where it could be stored. As a result, this process helped to mitigate extracellular toxicity, reduce the likelihood of hepatocellular dysfunction, and decrease the availability of LDL. These factors are beneficial and significant, especially in the context of rats that were concurrently administered with concentrated plant extracts. Despite the fact that animals exposed to a high-salt diet (HSD) exhibited a notable disruption in the concentration of low-density lipoprotein (LDL) relative to other lipid parameters, potentially due to reduced reverse transport mediated by high-density lipoprotein (HDL), the administration of PRE was able to enhance the restoration and normalization of the comprehensive lipid profile in a dose-dependent manner. This process should have led to the liberation of lipid molecules and subsequent creation of atherosclerotic plaques. Additional potential processes include the over-induction of the sodium/potassium ATPase homeostatic threshold (Ching *et al.*, 1994), the derailment of inotropism (Kass *et al.*, 1987), and the promotion of atherogenesis on the vascular intima (Kass *et al.*, 2020). Furthermore, PRE shown the ability to function as a therapeutic agent in effectively alleviating all of the aforementioned symptoms in a compact timeframe among a practically normal population.

## 6. Conclusion

The present investigation successfully demonstrated the effectiveness of the phenol rich extract derived from the whole plant. Specifically, it confirmed that the extract is fatal at dosages over 5000 mg/kg/body weight, whereas doses of 75, 100, and 150 mg/kg/body weight were shown to be safe. The extract demonstrated properties that counteracted obesity, hyperlipidemia, and aided in the recovery of renal function in rats subjected to a high-salt diet. This is also highly relevant to the evaluation of chronic and acute toxicity of the aqueous extract, as shown in experimental rats. Based on the preceding information, researchers have shown interest in phenolic phytoconstituents obtained from *M. recutita*. These compounds have the potential to be utilized as a complementary alternative agent in the future for the purpose of managing adiposity driven by HSD, hyperlipidemia, and renal dysfunctionality.

## Conflict of interest

The authors declare no conflict of interest relevant to this article.

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