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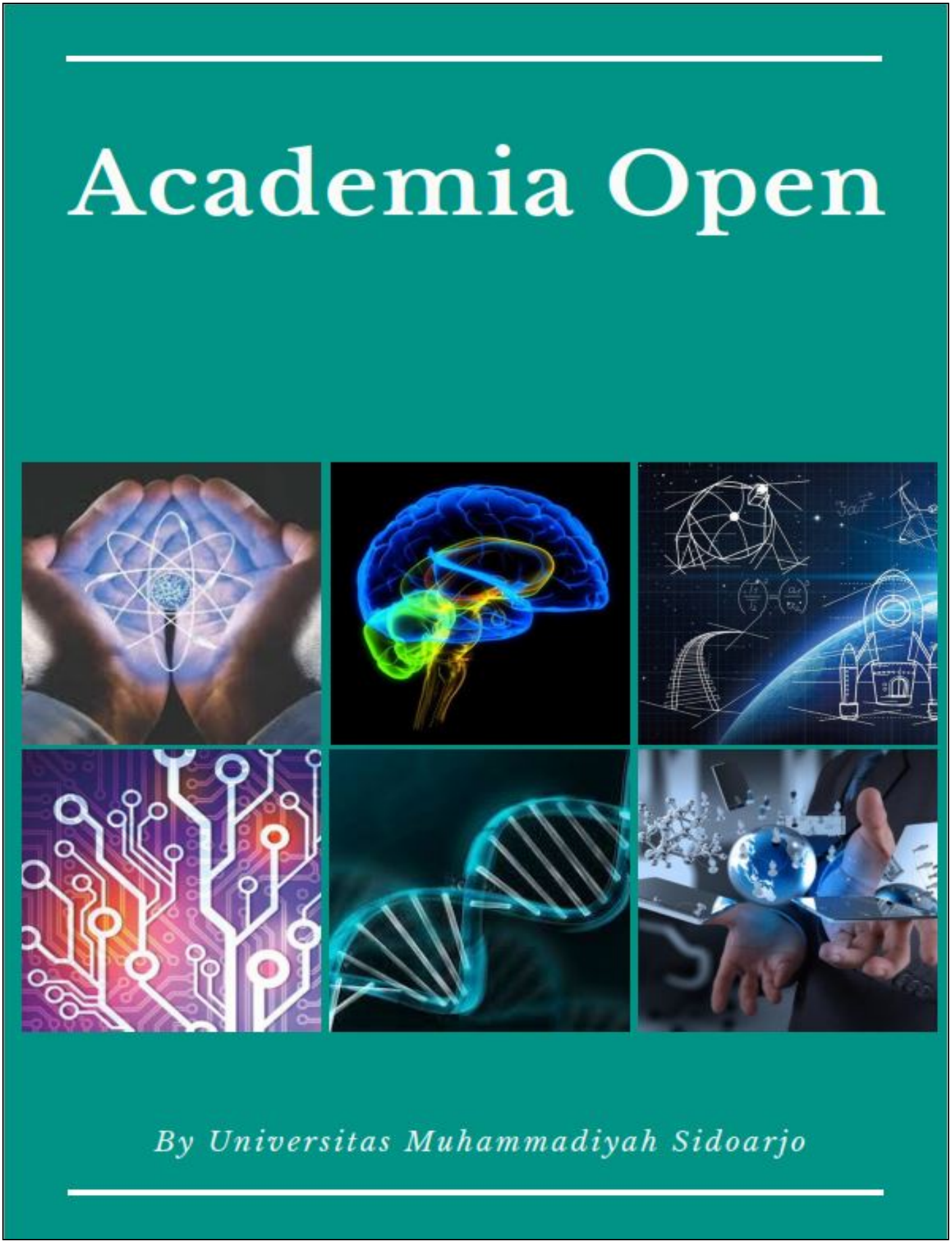

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Flavonoids from Verbascum thapsus Protect Against Nephrotoxicity in Rats

Flavonoid dari Verbascum thapsus Melindungi Terhadap Nefrotoksisitas pada Tikus

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Abstract

General Background: Nephrotoxicity induced by carbon tetrachloride (CCl4) poses significant health risks, prompting the exploration of natural antioxidants for renal protection. **Specific Background:** Verbascum thapsus, a plant known for its flavonoid content, has shown potential in mitigating oxidative stress, but its specific protective effects against CCl4-induced nephrotoxicity remain under-investigated. **Knowledge Gap:** While previous studies have indicated antioxidant properties of flavonoids, their efficacy in renal preservation in vivo has not been fully elucidated. **Aims:** The study evaluated the antioxidative and nephroprotective properties of flavonoids from Verbascum thapsus leaves in a rat model of CCl4-induced nephrotoxicity.**Results:** Rats were divided into three groups: control, CCl4-treated, and flavonoid plus CCl4-treated. Biochemical analyses revealed that CCl4 significantly elevated malondialdehyde (MDA), urea, creatinine, and uric acid levels while decreasing superoxide dismutase (SOD) and glutathione (GSH) levels. Notably, flavonoid administration markedly reduced MDA, urea, creatinine, and uric acid levels and enhanced SOD and GSH levels compared to the CCl4-only group. **Novelty:** This study uniquely highlights the protective role of flavonoids from Verbascum thapsus against nephrotoxicity, demonstrating both their antioxidative capacity and renal preservation in an experimental model. **Implications:** The findings support the potential use of Verbascum thapsus as a therapeutic agent in the management of kidney diseases, suggesting further investigation into its application in clinical settings to address nephrotoxicity and related renal disorders.

Highlights:

1. **Nephroprotective:** Flavonoids significantly protect against CCl4-induced kidney damage.
2. **Biochemical Enhancement:** Improved antioxidant levels; reduced harmful biochemical indicators.
3. **Therapeutic Potential:** Supports natural treatments for kidney diseases.

Keywords: Verbascum thapsus, flavonoids, nephrotoxicity, antioxidants, carbon tetrachloride

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Introduction

The kidneys are important organs that participate in multiple essential activities. Because of their elevated blood flow and impaired cellular transport pathways, they are particularly susceptible to toxic effects of drugs and toxins, these substances are then deposited in the nephron cells [1]. Typically, nephrotoxicity is caused by oxidative stress, which is stemmed from a lack of balance in the oxidant-antioxidant ratio that favors oxidants. This imbalance injures cells and produces excessive amounts of reactive oxygen species (ROS) and nitrogen species (RNS) [2]. Under typical physiological conditions, cells have a balanced redox system, which is generated and dissipates ROS and RNS. However, when this equilibrium is lost, the increase in ROS/RNS causes harmful changes to occur in cellular components [3]. This causes neutrophil inflammation, increased release of proteases, and the generation of various oxidative compounds, all of which have an important role in aging and disease [4]. Oxidative stress has been linked to multiple diseases, including cancer, diabetes, Alzheimer's disease, stroke, viral infections, neurodegenerative processes, infarction, cerebral edema, kidney problems, neurological diseases, and inflammation [5]. Carbon tetrachloride (CCl4) is considered to be one of the most toxic chemicals and is commonly used in scientific research to create experimental models that replicate the stress-induced pathophysiology in various diseases [6].

The mechanism of toxicity associated with CCl4 primarily hinges on the production of harmful radicals, specifically (CCl3) and (CCl3O2) [7]. These radicals inflict damage across various organs by binding to DNA, proteins, and lipids [8]. Cytochrome P450, the key enzyme responsible for nephrotoxicity induced by CCl4, is located in cortical tubule cells and enhances lipid peroxidation at the renal brush border [9]. Additionally, CCl4 influences mitochondrial activity in the kidneys, including the transport of calcium across mitochondrial membranes [9]. Antioxidants serve as protective agents against oxidative cellular damage by neutralizing free radicals, acting as barriers to oxidation, although they can be less effective at lower concentrations. These molecules fulfill various physiological roles in the body. Antioxidants function as free radical scavengers, engaging with reactive radicals to convert them into less active and less harmful forms [10].

Antioxidants exhibit a dynamic nature as they can readily provide electrons to free radicals, triggering a reaction that results in cellular loss. Once a free radical acquires an electron from an antioxidant, its desire to assault the mobile ceases, effectively stopping the cycle of oxidation reactions [11].

Verbascum represents a large genus within the closely related family Scrophulariaceae, encompassing approximately 2500 species. One notable species, Verbascum thapsus L., is commonly found growing wild in rocky terrains, barren landscapes, woodlands, clearings, and along roadsides.

V. thapsus is a perennial that is hardy and can be classified as either annual or biennial. It's characterized by a downward gaze and produces a smaller amount of plant material. The rosette is capable of growing to a height of up to 60 cm. The leaves are organized in a reverse pattern and may have a completely notched or sloping edge. The stems are solid and stiff, and range in height from 50 to 180 cm. The flowers are typically organized in a random fashion, typically forming a circle that is perpendicular to the axilla. The plants have a yellow color and are composed of five sepals, five petals, a two-celled ovary, and five stamens.[14] V. thapsus is renowned for its numerous medicinal properties, including pain relieving, anti-histaminic, anti-inflammatory, cancer fighting, antioxidant, antiviral, bacterial and fungal inhibitor, hypnotic, and sedative. The leaves, flowers, and roots are typically used for pain management, anti-inflammatory, antiseptic, and expectorant properties. Additionally, they are diuretic, emollient, and expectorant. Additionally, they have a nervine effect, and they are used for wound healing.[15]

The current investigation has examined the effects of flavanols derived from the leaves of mullein, their focus is on the antioxidant properties of these molecules and their potential to protect the kidneys

Methods

Plant Material

The grist leaf of of Verbascum thapsus was bought from the local market of Thi-Qar city.

Separation of flavonoids from the leaf of Verbascum thapsus

50gm of grist turned into extracted for n-hexane with the aid of soxhlet extraction process to strip the oil [16]. The defatted grist changed into dried. The flavonoids isolate have been received the usage of the subsequent method [17]:

Fifty grams of defatted grist had been jumbled with 250 mL of 80% methanol utilizing a stirring at temperature of the room to 24 hrs. The methanolic product was filtered and a precipitate was taken away, afterward 25 mL 1% lead acetate transformed into add into the leaky. Thereafter an aggregate got filtered, and the clear answer was

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excluded utilizing a buchner funnel. After 25 mL of acetone and 30 mL of the listen hydrochloric acid were mixed to precipitate, then a filter changed into introduced for the combination and a filtrate turned into evaporated to confer (zero.58) gm. This quantity transformed into go into answer in 25 mL of the distill water, subsequently extracted by ethyl acetate (3 x 50). The amounts of the ethyl acetate draw out were dried to supply (0.58) gram. Dry flavonoids were stored in a sealed box in the cooler.

Phytochemical Analysis

The chemical tests had been finished at the flavonoid extract to guarantee that extract have flavonoid

simplest using the same old methods to understand the additives, by way of changes of the colour as depict by [16].

Acute toxicity studies

The study of acute toxicity for the flavonoid extract was executed by using albino rats. The animals were abstain from food overnight before an experiment and kept under standard states. The extracts were administrated by orally in doses(1000,3000,5000 mg/kg) and animals were observe to 72 hours [18].

Experimental Animals

The Male rats weighing (150–250 g) have been utilized to test. The animals have been maintained at temperature of room (25 ºC) and granted access to the meals and the faucet water. Rats have been acclimatized to the laboratory conditions for 1 week before commencement of the trials. All animals have been treated carefully and humanely kept below degree ethical of notions as in accordance with the standards of university (UMS/IP7.Five/M3/ four-2012). A total of 18 male human rats have been divided into three businesses (6 rats /group) and treated with the follows [19] :

Group I (The control group): Received normal saline (1mL Kg) by utilizing orally.

Group II: Injected intra peritoneal (I.P) with CCl4 (1 mL/kg) every 72h for 10 days.

Group III: Received flavonoid extract (1000mg/kg) orally for 10 days plus CCl4 (1.0 mL/kg, I.P) delivered at each 72h.

A suspension of the extract turned into organized in ordinary saline and supplied to the animals by means of gastric gavage needle.

Blood transformed into collected by heart puncture the use of sterile syringes. Serum was received by centrifugation at 1500 rpm for 15 min and refrigerated at -20 ºC for further analysis

Estimation of Biochemical

Antioxidant parameters

Parameter serum malondialdehyde (MDA) become measured in step with the approach of [23], serum Superoxide dismutase (SOD) turned into measured in keeping with the method of [24], and serum Glutathione GSH became measured in step with the approach of [20].

Kidney function tests

Serum urea, creatinine, and uric acid ranges have been tested in the samples by use of a colorimetric approach [21,22,23] respectively.

Statistical Analysis

The consequences of the prevalent take a look at had been investigated by means of univariate analysis of variance. The data have been reported as suggest ±trendy deviation (suggest±SD). The least vast distinction test (LSD) was employed to examine the differentiation amongst manner (organizations) with the help of the utilization of a statistical program for social technology SPSS. P< 0.05 became deemed significant.

Result and Discussion

Phytochemical screening confirmed the presence of flavonoids exclusively as stated in table (1)

Phytochemicals	Verbascum thapsus Phytochemicals
Flavonoids	+

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Alkaloids	-
Tannins	-
Saponins	-
Amino Acids	-

Table 1. *The phytochemical content of the flavonoids extracted from the leaf of Verbascum thapsus*

The acute toxicity evaluation of the Flavonoid extract from the leaves of Verbascum japonicum showed that the extract was not harmful to animals. A dosage of 1,000 mg/kg was considered to be safe for rats.

The concentrations of serum MDA, SOD, and GSH are listed in Table 2. As is evident in Table 2, the serum MDA level increased while the SOD and GSH levels decreased in response to CCl4 administration compared to the control group. The degree of lipid peroxidation was determined by measuring the amount of MDA, which is a result of this process. [24,25] Additionally, superoxide dismutase (SOD) promotes the dissipation of oxygen and the conversion of superoxide ions (O2−) into hydrogen peroxide (H2O2), which is then detoxified into water (H2O) by catalase [26].

Also known as γ-glutamylcysteinylglycine, Glutathione is a tripeptide that contains the amino acids cysteine, glycine, and glutamic acid. This chemical functions as a non-enzymatic antioxidant in living cells, this facilitates the transportation and release of hydrogen. Low levels of glutathione are responsible for hemolysis, which is the destruction of red blood cells, this leads to anemia and stress oxidases. It's crucial to the intermediate metabolism pathway by providing sulfhydryl groups that are necessary for the detoxification of acetaminophen. Glutathione is directly involved in the detoxification of free radicals and oxygen species that are reactive. Additionally, it facilitates the metabolism of ascorbic acid and decreases the conversion of SH protein complexes to oxidized products [27]. Glutathione functions as a chelator for copper, this prevents the latter from participating in the Haber-Weiss process and maintains the reduced form of exogenous antioxidants, including vitamins C and E. Additionally, by directly binding, glutathione is crucial for the detoxification of a variety of chemicals, both organic and inorganic [28].

Lowered levels of glutathione compared to its antioxidant capacity cause an increase in free radicals, this is associated with the nephrotoxicity caused by CCl4 [29]. Additionally, the P450 enzyme system promotes the conversion of CCl4 into highly toxic trichloromethyl ions and begins to promote lipid peroxidation, this is considered to be a significant cause of the development of kidney injury [30]. As a result, glutathione is considered essential for preserving the kidneys from the deleterious effects of oxidants by removing hydrogen peroxide, hydrogen peroxide, and radical hydroxylation, this is done. Since SOD is a glutathione-based enzyme that is dependent on the level of glutathione, its activity will decrease as the level of glutathione decreases [31]. Table 2 further demonstrates that the administration of Flavonoids extracted from the plants at a dose of 1000 mg/kg to the animals decreased the increase in MDA caused by CCl4 and increased SOD and GSH. There were significant differences in the levels of MDA, SOD, and GSH between groups II and III ($p < 0.05$).

These flavonoids may consume free radicals that are produced by CCl4 via the P450 enzyme system, this will consequently lower the damage caused by oxidative stress. Additionally, the separated flavonoids may inhibit the production of free radical compounds by decreasing the amount of CCl4 present [32].

The final results demonstrate the effectiveness of flavonoids derived from the leaves of Mullein as anti-oxidants, this is in agreement with the results of [15].

Parameters	Groups				P value	
	N	Group I N.S	Group II CCl4 1ml/kg	Group III CCl4 (1ml/kg) + 1g/kg Flavonoid isolated	Group I vs Group II	Group II vs Group III
MDA(µmol\L)	6	4.548 ± 0.252	7.546 ± 0.181*	6.329 ± 0.269*	0.000	0.000
SOD (U\gm)	6	± 1.921 63.810	34. 090 ± 2.737 *	52.516 ± 1.398*	0.000	0.000
GSH (µmol\L)	6	598.193 ±6.419	373.156 ± 6.799*	± 6.772* 468.661	0.000	0.000

Table 2. *The effects of flavanols derived from mullein's leaves on the antioxidant properties of male rats*

Table (3) show that urea, creatinine and uric acid levels had been used as biochemical indication for the evaluation of kidney damage and these parameters were significantly higher ($P < 0.05$) in CCl4- treated animals as compared with the control group. The increasing ranges of urea and creatinine might be caused of a reduced glomerular filtration charge as a consequence of acute renal impairment [33]. Uric acid is the metabolic quit end products of

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purine metabolism [34]. The rise of such serum markers is a probable sign of renal damage which may be linked to modifications in tubular re-absorption and glomerular infiltration rate hampering the kidney's capacity eliminate these substances and generate higher serum amounts [25].

Moreover, Table 3 shows how treatment of the animals with a concentration1000 mg/kg of the flavonoid extract significantly lowered (P < 0.05) the CCl4-precipitated increases in urea, creatinine, and uric acid levels. This gives more support to the role of the flavonoid extract in eliminating waste products and as an antioxidant.

Parameters	Groups				P value	
	N	Group I N.S	Group II CCl4 1ml/kg	Group III CCl4 (1ml/kg) + 1g/kg Flavonoid isolated	Group I vs Group II	Group II vs Group III
Urea (mg/dL)	6	23.200 ±4.642	7.488 ± 4.283*	34.483 ±4.479*	0.000	0.000
Creatinine (mg/dL)	6	0.651± 0.027	1.146 ± 0.123 *	0.857 ± 0.032 *	0.000	0.000
Uric acid (mg/dL)	6	1. 872 ±0.109	578* 0. ± 4.983	2.8 15 ±0.473 *	0.000	0.000

Table 3. *Effect of flavonoids remoted the leaf of Verbascum thapsus on certain biochemical markers in male rats.*

N =wide variety of animals , Means ±SD, N.S = normal saline , * p< 0.05.

Conclusion

Flavonoid isolated from the leaf of Verbascum thapsus has sturdy antioxidant activities and tremendous shielding results towards CCl4 prompted nephrotoxicity in rats, this result supports the usage of the leaf of Verbascum thapsus to deal with kidney illnesses

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