

**Protective Effect of *Gymnema  
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Officinalis* Leaves Against  
Hepatorenal Toxicity of  
Paracetamol in Experimental  
Rats**

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## Protective Effect of *Gymnema Sylvestre* and *Rosmarinus Officinalis* Leaves Against Hepatorenal Toxicity of Paracetamol in Experimental Rats

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### Abstract

This study aimed to investigate the protective effect of *Gymnema sylvestre* and Rosemary (*Rosmarinus Officinalis*) leaves to protect against paracetamol induced hepatorenal toxicity. Thirty-six female Albino rats were randomly divided into two main group as follow: The first group (n= 6 rats) was fed only on the basal diet as a negative control group (ve-) normal (Group1). The second major group is randomly divided into five sup groups (six rats each), as follows: Group (2) the Positive control group (ve+) received orally (2 ml/ kg BW) distilled water/day/rat. Group (3) pretreated orally with 10% / diet /day from rosemary leaves powder. Group (4) pretreated orally with (10 % / diet /day) from *G sylvestre* leaves powder. Group (5) pretreated orally with (5% *G sylvestre* +5% rosemary leaves powder / diet /day). While group (6) the reference drug group received orally (100 mg/kg BW) Silymarin suspended in distilled water. After pretreatment for 14 days, induced liver injury in the second major group, Paracetamol at a dose of 2 g/kg BW for 7 days suspended in water was taken orally. At the end of the experiment, the rats were fasted overnight before sacrificed, the blood samples were collected then, centrifuged to obtain the serum for biochemical analysis. The results showed that phytochemical analyses of gymnema and rosemary leaves powder recorded the presence of tannins, saponins and flavonoids. Pre-treatment with rosemary, gymnema, their mix at level 10% and silymarin at level 100 mg/kg BW on group rats attenuated the adverse effects in biological and biochemical alterations that caused by Paracetamol PCM administration, where the pretreatment caused significant increase in body weight gain %, feed intake and feed efficiency ratio (FER) and significant reduction the liver weight and kidney weight when compared to the PCM-intoxicated group. Moreover, Pre-treatment on treated group rats caused significant reduction in PCM-induced increase levels of ALT, AST, ALP, serum urea, uric acid, creatinine & MDA and significant

elevation of total protein , CAT &SOD activity. This reversal against PCM-induced effects was also seen of histopathological examination of liver and kidney. In conclusion: The clear improvement in all the treated groups demonstrates the importance of the gymnema and rosemary leaves and their mix for protection against paracetamol-induced toxicity in rats' liver and kidney and this effect could be attributed to their antioxidant activity.

**Keywords:** Rosemary, Gymnema, Paracetamol, Liver, Kidney, toxicity, Rats.

التأثير الوقائي لأوراق الجيمنيميا و إكليل الجبل ضد السمية الكلوية للباراسيتامول في  
فئران التجارب

### الملخص العربي:

تهدف هذه الدراسة الى معرفة التأثير الوقائي لأوراق نبات الجيمنيميا وإكليل الجبل (الروزماري) للوقاية من السمية الكلوية الكلوية التي يسببها الباراسيتامول. تم تقسيم ستة وثلاثين من إناث الفئران البيضاء عشوائيا إلى مجموعتين رئيسيتين على النحو التالي: تم تغذية المجموعة الأولى (ن = 6 فئران) فقط على النظام الغذائي القاعدي كمجموعة طبيعية (المجموعة ١) . تم تقسيم المجموعة الرئيسية الثانية عشوائيا إلى خمس مجموعات فرعية (ستة فئران لكل منها) ، على النحو التالي: المجموعة (٢) المجموعة الضابطة الإيجابية التي تناولت بالفم (٢ مل / كجم من وزن الجسم) ماء مقطر / يوم / فأر. مجموعة (٣) معالجة عن طريق الفم مع ١٠٪ / حمية / يوم من مسحوق أوراق إكليل الجبل. المجموعة (٤) المعالجة عن طريق الفم مع (١٠٪ / حمية / يوم) من مسحوق أوراق الجيمنيميا. مجموعة (٥) معالجة فمويا ب (٥٪ جم مسحوق الجيمنيميا + ٥٪ أوراق إكليل الجبل / حمية / يوم) بينما تلقت المجموعة (٦) مجموعة الأدوية المرجعية عن طريق الفم (١٠٠ مل/كجم من وزن الجسم) سيليمارين معلقا في الماء المقطر. وبعد المعالجة المسبقة لمدة ١٤ يوما، تم إصابة الكبد المستحثة في المجموعة الرئيسية الثانية بتناول الباراسيتامول بجرعة ٢ جم/كجم من وزن الجسم لمدة ٧ أيام معلقة في الماء بالفم . في نهاية التجربة ، تم صيام الفئران طوال الليل قبل الذبح ، ثم تم جمع عينات الدم للحصول على المصل للتحليل الكيميائي الحيوي. أظهرت النتائج أن التحليلات الكيميائية النباتية لمسحوق أوراق الجيمنيميا وإكليل الجبل سجلت وجود التانينات والصابونين والفلافونويد. خففت المعالجة المسبقة بمسحوق إكليل الجبل و الجيمنيميا ومزيجهما عند المستوى ١٠٪ والسيليمارين عند المستوى ١٠٠ مل/كجم من وزن الجسم على مجموعة الفئران من الآثار الضارة في التغيرات البيولوجية والكيميائية الحيوية التي يسببها إعطاء باراسيتامول PCM ، حيث تسببت المعالجة المسبقة في زيادة كبيرة في زيادة وزن الجسم، وتناول الطعام ونسبة كفاءة الغذاء وكذلك انخفاض

كبير في وزن الكبد ووزن الكلى بالمقارنة مع المجموعة الإيجابية. علاوة على ذلك ، ادت المعالجة المسبقة الى انخفاض كبير في مستويات الزيادة التي يسببها PCM من ALT و AST و ALP واليوريا وحمض اليوريك والكرياتينين و MDA وارتفاع كبير في كل من البروتين الكلي ونشاط CAT و SOD . ولوحظ هذا الانعكاس ضد التأثيرات التي يسببها PCM أيضا في الفحص النسيجي المرضي للكبد والكلى. في الختام: التحسن الواضح في جميع المجموعات المعالجة يوضح أهمية أوراق الجيمينيما وإكليل الجبل ومزيجها للحماية من السمية المستحثة بالباراسيتامول في كبد الفئران والكلى ويمكن أن يعزى هذا التأثير إلى نشاطها المضاد للأكسدة.

## Introduction

Paracetamol, also known as acetaminophen, is a widely used anti-pyretic that has long been established to cause liver toxicity once above therapeutic levels. Hepatotoxicity from paracetamol overdose, whether intentional or non-intentional, is the most common cause and remains a global issue. Given the increased prevalence of combination medications in the form of pain relievers and antihistamines, paracetamol can be difficult to identify and remains a significant cause of acute hepatotoxicity, as evidenced by its contribution to over half of all acute liver failure cases. Hepatocytes metabolize paracetamol produces reactive oxygen species, originally thought to be the ultimate cause of liver injury in paracetamol overdose. Mitochondrial dysfunction has instead been attributed as the main source of free radicals and oxidative stress in paracetamol hepatotoxicity (Walaa *et al.*, 2014 and Laura and Nikolaos, 2020).

Herbal drug therapy is considered a common practice adopted in traditional and alternative medicine and had been used since ancient times for the treatment of human ailments (Hozayen, 2012). Among these culinary herbs are *Gymnema sylvestre* and rosemary (*Rosmarinus Officinalis*). *Gymnema* is a green leafy vegetable used in the Northern Thai cuisine which has antioxidant activities and may be applicable for preventing oxidative stress and aging-related disease. *Gymnema* leaves were potentially composed of phenolics, quinic acids, flavonoids, and triterpenoid saponins. *Gymnema* with high contents of phenolics, flavonoids, quercetin, and kaempferol showed significant relation to antioxidation and protection in endothelial cell death suppressed by reactive nitrogen species. Ultimately, *Gymnema* leaves with high phenolic compounds are a promising raw material to develop as an antioxidant functional food (Onanong *et al.*, 2024).

*Rosmarinus officinalis*, a common spice used worldwide for culinary and medicinal purposes (Sasakia *et al.*, 2013). Various pharmacological studies demonstrated the analgesic, anti-inflammatory, antioxidative,

antitumor, antibacterial, and hepatoprotective properties of rosemary (Minaiyan *et al.*, 2011). Therefore, the current investigation has been conducted to investigate the protective effect of gymnema and rosemary leaves against hepatorenal toxicity of paracetamol in experimental rats.

## Materials and Methods

### Materials:

*Gymnema sylvestre* and rosemary (*Rosmarinus Officinalis*) leaves were obtained from the Orman botanic garden in Giza, Egypt. Samples were identified and authenticated at Cairo University Research Park (CURP). The kits used for analysis were obtained from Bio-diagnostic Co. Dokki, Egypt. Paracetamol (PC) and Silymarin were obtained from El-Gomhoreya Co., Cairo, Egypt. Casein (85% protein), cellulose, dextrose, choline chloride, DL-methionine, vitamins and salt mixture were obtained from Cairo Company Chemical Trading, Cairo, Egypt. Corn oil was obtained from the local market. Corn starch was obtained from Starch and Glucose Co. Helwan, Cairo, Egypt. Thirty-six female Albino rats (Sprague Dawley strain), weighing about  $150 \pm 20$  g was obtained from Agricultural Research Center, Giza, Egypt.

### Methods:

#### Preparation of *Gymnema sylvestre* leaves powder.

*Gymnema sylvestre* (gudmar) leaves powder was prepared as per the method given by Farzana *et al.*, (2019).

#### Preparation of rosemary *R. officinalis* leaves powder

The leaves of *R. officinalis* were washed, shade dried under room temperature and powdered using an electric blender according to the method of Kalinda and Rioba, (2020).

### Chemical analysis

#### A. Proximate composition of *Gymnema sylvestre* and *R. officinalis*

Moisture, protein, fat, crude fiber and ash were determined according to the method of AOAC, (2015) and then the carbohydrate content was calculated by difference (Fernandes *et al.*, 2015). All determinations were made in triplicate.

Total carbohydrates =  $100 - (\text{moisture} + \text{protein} + \text{fat} + \text{ash})$

#### B. Characterizations of phytochemical composition of *Gymnema sylvestre* and *R. officinalis* (rosemary)

1 g and 0.5 g of crude extract were measured for both *M. spicata* and *R. officinalis* respectively and constituted in 10ml of DCM, 10 ml methanol and 10 ml distilled water in their respective concentrations. 1ml of the *M. spicata* - and *R. officinalis* -dichloromethane, methanol and distilled filtrate was added to vials. The veils were labelled with the phytochemicals under analysis as saponins, flavonoids, glycosides, alkaloids and tannins respectively for each organic solvent used. The

respective phytochemicals were tested as per the standard procedures with slight modifications as described by **Harboene, (1973)**.

### **Diet Preparation and Experimental Animal Design**

The basal diet was prepared according to AIN-93M diet (**Reeves et al., 1993**). Thirty-six female Albino rats were housed in well conditions and fed on basal diet in Research Labs, Agricultural Research Center, Giza, Egypt. After one week of acclimatization, the rats were randomly divided into two main group as follow:

The first group (n= 6 rats) was fed only on the basal diet as a negative control group (ve-) normal (Group1). The second major group is randomly divided into five sup groups (six animals each), as follows: Group (2) the Positive control group (ve+) received orally (2 ml/ kg BW) distilled water/day/rat by epi-gastric tube. Group (3) pretreated orally with 10% / diet /day from rosemary leaves powder. Group (4) pretreated orally with (10 % / diet /day) from *G sylvestre* leaves powder. Group (5) pretreated orally with (5% *G sylvestre* +5% rosemary leaves powder / diet /day). While group (6) the reference drug group received orally (100 mg/kg BW) Silymarin suspended in distilled water (**Erhan, 2020**).

### **Induction of hepatotoxicity**

After pretreatment for 14 days, induced liver injury in the second major group, Paracetamol at a dose of 2 g/kg BW for 7 days suspended in water was taken orally (**Ikponmwosa and Eromosele, 2019**).

During the experiment period, the quantities of diet, which were consumed and/or waste, were recorded every day. Water and basal diet had been introduced under hygienic conditions. At the end of the experiment, the rats were fasted overnight before sacrificed, the blood samples were collected from hepatic portal vein for each rat in dry centrifuge tubes then, centrifuged to obtain the serum for biochemical analysis.

### **Biological evaluation**

Feed intake was recorded every day and body weight was recorded every week. Biological evaluation of the different diets was carried out by calculating of body weight gain% (BWG %), feed efficiency ratio (FER) and organs weight as a percent of total body weight according to **Chapman et al., (1959)** using the following equations: -

Feed intake (FI) = Initial weight of diet (g) – Left over diet weight (g)

BWG% = [(Final weight g - Initial weight g) / (Initial weight g)] X 100

FER= Gain in body weight / feed intake (g)

Relative organs weight ROW % = (Organ weight / Final weight) X 100

## Biochemical analysis

After the serum prepared, serum samples were analyzed by bio diagnostic kits:

### A. Determination of the activity of liver enzymes:

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were determined calorimetrically using spectrophotometer (model DU 4700) at 505 nm according to the method of **Reitman and Frankel, (1957)**. Alkaline phosphatase (ALP) activity was determined calorimetrically using spectrophotometer (model DU 4700) at 510 nm according to the method by **Belfield and Goldberg, (1971)**.

### B. Determination of serum total protein:

Serum total protein was determined at 550 nm according to the method described by **Gornal et al., (1949)**.

### C. Determination of the kidney function:

Serum uric acid was determined by **Barham and Trinder, (1972)**. Serum urea nitrogen was determined according to the method described by **Batton and Crouch, (1977)**. Serum creatinine was determined according to **Tietz, (1986)**.

### D. Determination of the oxidative stress:

Serum Catalase (CAT), Superoxide Dismutase (SOD) activity and malondialdehyde (MDA) were determined according to (**Beutler et al., 1963; Kakkar et al., 1984 and Draper and Hadly, 1990**) respectively.

## Histopathology Technique

The tissues sample from liver and kidney were fixed immediately after dissection in 10% neutral formalin for 24 h, then collected and dehydration was done on concentration of alcohol, cleaned in xylene and embedded in paraffin wax. Tissues were sectioned at a thickness of 3 micron and stained with hematoxylin and eosin stains (**Banchroft et al., 1996**).

## Statistical Analysis

The data obtained from the present study was statistically subjected to K analysis of variance (ANOVA) according to **Snedecor and Cochran (1980)** by the computerized program SPSS software, version "20" for Windows. Data was represented as Mean  $\pm$  SD. Values were considered significant at  $P \leq 0.05$ , otherwise were considered non-significant.

## Results and Discussion

### Proximate chemical composition of *gymnema sylvestre* and rosemary leaves powder

The data tabulated in Table (1) showed the Proximate composition (g/100g) of *gymnema sylvestre* and rosemary leaves powder. It can be

noticed that *gymnema sylvestre* leaves powder contained moisture, protein, fat, fiber, ash and carbohydrate with values (7.35, 10.88, 5.80, 11.42, 9.43 & 55.12, respectively). While the values of rosemary leaves powder were 7.64, 4.15, 19.95, 18.93, 8.27 & 41.06 respectively.

These results of chemical composition for *gymnema sylvestre* and rosemary leaves powder revealed that carbohydrate recorded the highest average for both, but the content of protein was higher in *gymnema* than rosemary leaves powder whereas the values of fat were higher in rosemary than *gymnema* leaves powder.

**Table (1): A proximate composition (g/100g) of *gymnema sylvestre* and rosemary leaves powder**

Constituents	Plants	
	<i>Gymnema sylvestre</i>	<i>Rosemary</i>
Moisture	7.35±0.04 <sup>cd</sup>	7.64±0.05 <sup>c</sup>
Crude Protein	10.88±0.06 <sup>b</sup>	4.15±0.03 <sup>cd</sup>
Crude fat	5.80±0.05 <sup>d</sup>	19.95±0.05 <sup>b</sup>
Crude fiber	11.42±0.19 <sup>b</sup>	18.93±0.04 <sup>b</sup>
Total Ash	9.43±0.04 <sup>bc</sup>	8.27±0.02 <sup>c</sup>
Carbohydrates	55.12±0.01 <sup>a</sup>	41.06±0.03 <sup>a</sup>

Data are presented as means ± SDM ( $n=3$ ). a, b, c and d: Means with different letters among treatments.

in the same column are significantly different ( $P \leq 0.05$ )

### Phytochemical analyses of *gymnema* and rosemary leaves powder

Data presented in Table (2) showed the phytochemical analyses of *gymnema sylvestre* and rosemary leaves powder recorded the presence of tannins, saponins and flavonoids. The results noticed that alkaloids were undetected in *gymnema* but glycosides were undetected in rosemary leaves powder.

By comparing these results with some other studies, it is clear that *gymnema* is a green leafy vegetable has antioxidant activities and may be applicable for preventing oxidative stress and aging-related disease. GI leaves were potentially composed of phenolics, quinic acids, flavonoids, and triterpenoid saponins (Onanong *et al.*, 2024). Plant phenolics have been acknowledged as strong natural antioxidant agents and are considered a vital human dietary component (Lin *et al.*, 2016, Cefalo *et al.*, 2019 and Ecevit *et al.*, 2022).

*Gymnema* leaf phytochemicals are composed of triterpene saponins (Srinuanchai *et al.*, 2021) pregnane glycosides (Trang *et al.*, 2021), phenolic compounds (Dedvisitsakul and Watla, 2022), vitamins, carotenes and xanthophylls (Chanwitheesuk *et al.*, 2005).

The quantification of substances in rosemary with recognized antioxidant action revealed the presence of phenols and flavonoids (Natalia *et al.*, 2023). In this regard, many secondary metabolites have been isolated and



identified from *Rosmarinus* spp., including essential oils, flavonoids, tannins, terpenes, and phenolic acids (Bouyahya *et al.*, 2017 and Borges *et al.*, 2019). In addition, carnosol, carnosic acid, and rosmarinic acids, which are reported to be the main components of rosemary, account for many of its biological activities (Bai *et al.*, 2010, Jordan *et al.*, 2014, Mohaddeseh *et al.*, 2020 and Ibrahim *et al.*, 2022).

Table (2): Phytochemical analyses of *gymnema sylvestre* and rosemary leaves powder

Constituents (%)	Plants	
	<i>Gymnema sylvestre</i>	rosemary
Alkaloids	-	+
Glycosides	+	-
Tannins	+	+
Saponins	+	+
Flavanoids	+	+

+: contains the tested substances. -: undetect the tested substances

### Biological evaluation of rosemary and gymnema leaves powder on experimental rats induced liver injury by Paracetamol:

Data in Table (3) showed that the initial weight of all groups had similar values to that of the control (-) group. The weights ranged from 156.7 to 166.2 g., where there was no statistically significant difference among groups. The positive control group had significant ( $P \leq 0.05$ ) decrease in final weight, body weight gain (g), body weight gain %, feed intake and feed efficiency ratio (FER) compared with control negative group, while all treatment groups that pretreated with rosemary, gymnema leaves powder and their mixture at 10% concentration showed significant increase in these parameters compared to positive control group. Whereas there was significant difference for silymarin group at (100 mg/kg BW) which recorded higher significant just to final weight and FER compared to positive control group. These data agree with Ahmed *et al.*, (2010) that reported ethanol extract of this plant cause increase in the weight of the whole body, liver, pancreas in Wistar rats.

Table (3): Mean body weight gain (g), body weight gain %, feed intake and feed efficiency ratio (FER) of experimental rats which pretreated with rosemary and gymnema leaves powder

Groups	Parameters					
	Initial weight (g)	Final weight (g)	Weight gain (g)	Weight gain (%)	Feed intake(g)	FER
Control -ve	164.2±6.30 <sup>a</sup>	208.5±12.26 <sup>a</sup>	44.3±3.7 <sup>a</sup>	26.99±2.6 <sup>a</sup>	17.07±0.5 <sup>a</sup>	0.093±0.007 <sup>a</sup>
Control +ve	156.7±9.77 <sup>a</sup>	169.9±9.08 <sup>c</sup>	13.2±1.6 <sup>c</sup>	8.42±1.1 <sup>cd</sup>	15.11±0.19 <sup>c</sup>	0.030±0.001 <sup>d</sup>

10%Rosemary	164.2±4. 11 <sup>a</sup>	197.5±11. 8 <sup>ab</sup>	33.3±3.1 b	20.19±1. 9 <sup>b</sup>	16.85±0. 35 <sup>b</sup>	0.070±0.0 08 <sup>b</sup>
10%G sylvestre	166.2±5. 56 <sup>a</sup>	205.7±10. 05 <sup>a</sup>	39.55±3.5 b	23.72±2. 1 <sup>b</sup>	15.99±0. 41 <sup>b</sup>	0.088±0.0 06 <sup>ab</sup>
5% rosemary+ 5% G sylvestre	158.7±6. 39 <sup>a</sup>	196.7±10. 1 <sup>ab</sup>	38.05±3.6 b	23.96±2. 2 <sup>b</sup>	16.54±0. 52 <sup>b</sup>	0.082±0.0 05 <sup>ab</sup>
Silymarin (100 mg/kg BW)	159.5±7. 23 <sup>a</sup>	177.1±9.5 9 <sup>b</sup>	17.4±2.3 c	10.89±1. 8 <sup>c</sup>	15.22±0. 42 <sup>bc</sup>	0.041±0.0 02 <sup>c</sup>

Data are presented as means ± SDM (n=6). a, b, c and d: Means with different letter in the same column are significantly different ( $P \leq 0.05$ )

### Relative organs weight of experimental rats pretreated with gymnema and rosemary leaves powder:

Data presented in Table (4) showed the effect of pre-treatment rosemary, gymnema, their mix at level 10% and silymarin at level 100 mg/kg BW on group rats induced liver injury by paracetamol. Positive control group showed significant increase in relative liver and kidney weight organs compared to negative control group. These results agree with Zainul *et al.*, (2020) that reported effect of paracetamol PCM-intoxicated rats body weight (BW), liver weight (LW) and their ratio (LW/BW). Rats from PCM-induced hepatotoxic control group (Group +) showed a significant increase in the LW when compared with the normal control group (Group ve -).

Pre-treatment with rosemary, gymnema, their mix at level 10% and silymarin at level 100 mg/kg BW on group rats, significantly decreased the liver weight and kidney weight when compared to the PCM-intoxicated group. Interestingly, there was no significant difference of all treated groups for liver and kidney organs weight compared to negative control group. The results are agreed with Zainul *et al.*, (2020) that reported of pre-treatment with 200 mg/kg silymarin also caused significant reduction in the LW/BW ratio, thus, indicating their ability to attenuate PCM-induced liver damage.

**Table (4): Relative organs weight of experimental rats which pretreated with rosemary and gymnema leaves powder**

Groups	Organ's weight (%)	
	liver	kidney
Control (-ve)	2.01±0.11 <sup>b</sup>	0.52±0.01 <sup>b</sup>
Control (+ve)	3.97±0.17 <sup>a</sup>	0.93±0.05 <sup>a</sup>
10% Rosemary	2.51±0.14 <sup>b</sup>	0.59±0.08 <sup>b</sup>
10%G sylvestre	2.33±0.14 <sup>b</sup>	0.52±0.02 <sup>b</sup>
5% rosemary+ 5% G sylvestre	2.51±0.05 <sup>b</sup>	0.57±0.05 <sup>b</sup>
Silymarin (100 mg/kg BW)	2.48±0.12 <sup>b</sup>	0.53±0.07 <sup>b</sup>

Data are presented as means  $\pm$  SDM ( $n=6$ ). a, b, c and d: Means with different letter in the same column are significantly different ( $P \leq 0.05$ )

### Biochemical analysis

#### Effect of rosemary and gymnema leaves powder on liver functions of rats induced liver injury by Paracetamol:

As seen in Table (5) serum aspartate transaminase (AST) and alanine transaminase (ALT) and alkaline phosphatase (ALP) activities were elevated significantly by Paracetamol administration, while, there was significant decrease in serum total proteins compared to negative control. In agreement with our results, many studies found that the activities of AST, ALT, and ALP increased in paracetamol treated rats (Yousef *et al.*, 2010, Hurkadale *et al.*, 2012, Kiran *et al.*, 2012 and Datta *et al.*, 2013). In addition, Zainul *et al.*, (2020) found that, liver damage induced by PCM (Group+) caused significant increased in serum liver marker enzymes (ALT, AST and ALP).

Elevated levels of activities of these enzymes in serum suggested that paracetamol induced hepatocellular damage (Yousef *et al.*, 2010). An obvious sign of hepatic injury is the leaking of cellular enzymes into the plasma because of the disturbance caused in the transport functions of the hepatocytes (Sabir *et al.*, 2008). When the liver cell is damaged, a variety of enzymes located normally in the cytosol is released into the blood, thereby causing increased enzyme levels in the serum (Sadasivan *et al.*, 2006). These findings can be clarified by Laura and Nikolaos, (2020) that reported drug induced liver injury is a common cause of acute liver injury. Paracetamol, also known as acetaminophen, is a widely used anti-pyretic that has long been established to cause liver toxicity.

Significant reduction was observed in serum AST, ALT and ALP for all treatment groups pretreated with rosemary, gymnema leaves powder and their mixture at 10% concentration and also, silymarin group at (100 mg/kg BW) compared to positive control group. Whereas there was significant increase of total protein for all treatment groups compared to positive control group. Furthermore, these results showed an improvement in all the treated groups compared to negative control groups for liver functions. These results agree with Zainul *et al.*, (2020) and Maimonah *et al.*, (2023).

Sherif *et al.*, (2009) indicated that liver enzymes increased after liver damage because of increased membrane permeability or because of liver cell necrosis and cytosol leakage into the serum. The pretreatment of rats with gymnema or rosemary leaves and their combination improved the liver functions. The ameliorative effect of rosemary may be due to its antioxidant properties in combating free radical induced oxidative stress and tissue injury.

Regarding, the results of pretreatment with gymnema leaves are agreement with **Raheel et al., (2023)** which reported that the rats were fed with the extract and powder of the gymnema leaves, both ALT, AST and ALP level of rats were declined. These results may be due to gymnema have antioxidant, antibiotic, anti-inflammatory, antiviral, gastro and hepatoprotective due to the presence of phytochemicals, such as gurmarin, gymnemic acid as well as gymnema saponins (**Farzana, et al., 2019**). The results in current study agreed with **Natalia et al., (2023)** that found the treatment of rats with *Rosmarinus officinalis L.* (rosemary) resulted in inhibition of lipid peroxidation of the liver and the prevention of hepatotoxicity, maintaining alanine and aspartate aminotransferase (ALT/AST) levels equal to those of the normal, non-treated rats. These findings highlight the potent antioxidant activity of rosemary, which can protect mitochondria from oxidative damage in vitro, and effects such as the antioxidant and hepatoprotective effects observed in vivo.

Moreover, **Walaa et al., (2014)** and **Nevien (2018)** reported that the effect of rosemary on serum liver functions in rats with induced toxicity was indicated that the positive control group had a significant increase in serum levels of AST, ALT and ALP compared to the healthy group. Supplementation with rosemary significantly decreased the elevated levels of serum ALT, AST and ALP compared to the positive control group.

The mean values of total protein illustrated in table 5 agree with **Walaa et al., (2014)** and **Nevien (2018)** that reported the total protein of intoxicated rats had significant reduction compared to the negative control. The decreased production of proteins by paracetamol is due to damage to the hepatocytes. The supplementation with rosemary significantly increased serum total protein compared to the positive control group. These reflect the hepatoprotective activity of rosemary.

**Table (5): Liver enzymes of all experimental rats which treated with rosemary and gymnema leaves powder**

Groups	Parameters			
	AST(U/L)	ALT(U/L)	ALP (U/L)	TOTAL PROTEINs(g/dl)
Control (ve-)	112.25±6.08 <sup>bc</sup>	57.75±4.46 <sup>b</sup>	83.9±3.92 <sup>c</sup>	7.15±0.73 <sup>ab</sup>
Control (ve+)	150.31±9.92 <sup>a</sup>	72.31±5.94 <sup>a</sup>	171.18±9.84 <sup>a</sup>	3.75±0.31 <sup>c</sup>
10% Rosemary	98.52±8.18 <sup>c</sup>	46.75±4.65 <sup>c</sup>	152.63±8.45 <sup>b</sup>	7.83±0.29 <sup>a</sup>
10% <i>G sylvestre</i>	115.51±7.06 <sup>b</sup>	54.25±4.57 <sup>b</sup>	154.72±9.99 <sup>b</sup>	7.95±0.41 <sup>a</sup>
5% rosemary+ 5% <i>G sylvestre</i>	93.52±7.05 <sup>c</sup>	53.52±5.84 <sup>b</sup>	120.19±7.56 <sup>bc</sup>	7.33±0.49 <sup>ab</sup>

Data are presented	Silymarin (100 mg/kg BW)	116.75±6.84 <sup>b</sup>	50.11±4.31 <sup>bc</sup>	147.78±6.38 <sup>b</sup>	7.17±0.36 <sup>d</sup>
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as means  $\pm$  SDM ( $n=6$ ). a, b, c and d: Means with different letter in the same column are significantly different ( $P \leq 0.05$ ) AST: aspartate amino transferase ALT: alanine amino transferase ALP: alkaline phosphatase.

### Effect of rosemary and gymnema leaves powder on kidney functions of rats induced liver injury by Paracetamol

As seen in Table (6) serum urea, uric acid and creatinine were elevated significantly by paracetamol administration, the values were (56.5±4.89, 3.975±0.21 and 0.823±0.07, respectively) compared to negative control group (36.25±2.08, 2.125±0.40 and 0.685±0.07, respectively). These results agree with **Walaa et al., (2014)** that found Paracetamol-administered rats induced a highly significant increase in the levels of serum urea and creatinine when compared with normal rats. The observed increase in urea and creatinine levels is an indication for renal impairment. While there was significant ( $P \leq 0.05$ ) decrease in serum urea, uric acid and creatinine for all treatment groups pretreated with rosemary, gymnema leaves powder and their mixture at 10% concentration and also, silymarin group at (100 mg/kg BW) compared to positive control group. Furthermore, these results showed an improvement in all the treated groups compared to negative control group for kidney functions. These results of pretreatment with *gymnema sylvestre* leaves are in agreement with **Raheel et al., (2023)** which reported that the rats were fed with the extract and powder of the *gymnema sylvestre* leaves, serum urea and creatinine of rats was declined.

*R. officinalis* and its main compounds, in particular, carnosic acid, rosmarinic acid, and carnosol to reveal the antidotal and protective effects of rosemary against biological toxins and chemical toxic agents. Rosemary has protective effects against a broad range of biological toxins including mycotoxins, snake venoms, and bacterial toxins through fungistatic and fungicidal actions, anti-hemorrhagic and anti-edematogenic activities, as well as the inhibition of nitrite formation. Also, rosemary has a protective role against chemical toxic agents such as metals, pesticides, cardiotoxic, neurotoxic, hepatotoxic, and nephrotoxic agents. These effects of rosemary and its components are mostly mediated through different mechanisms including antioxidant, radical scavenging, anti-apoptotic properties, anti-inflammatory effects, and the regulation of the renal, hepatic, and cardiac enzymes (**Mohaddeseh et al., 2020**).

Our results agree with **Walaa et al., (2014)** that found the treatment of paracetamol-administered rats with rosemary extract

produced a highly significant decrease in serum urea, creatinine. Moreover, **Azab et al., (2014)** reported that rosemary ameliorated the nephrotoxicity and improved the structural changes in the kidney and declined the blood urea, creatinine and uric acid. The protective effects of rosemary and its components are mostly mediated through different mechanisms such as the inhibition of oxidative stress, reduction of inflammatory mediators including tumor necrosis factor-alpha (TNF- $\alpha$ ), interleukin-6 (IL-6), interleukin-17 (IL-17), cyclooxygenase-2 (COX-2) and nuclear factor  $\kappa$ B (NF- $\kappa$ B) as well as the modulation of apoptosis and mitogen-activated protein kinase (MAPK) signaling pathways (**Mohaddeseh et al., 2020**).

**Table (6): Kidney functions of experimental rats which treated with rosemary and gymnema leaves powder**

Groups	Parameters		
	Urea (mg/dl)	Uric Acid (mg/dl)	Creatinine (mg/dl)
Control (ve-)	36.25 $\pm$ 2.08 <sup>c</sup>	2.13 $\pm$ 0.40 <sup>b</sup>	0.685 $\pm$ 0.07 <sup>c</sup>
Control (ve+)	56.51 $\pm$ 4.89 <sup>a</sup>	3.98 $\pm$ 0.21 <sup>a</sup>	0.823 $\pm$ 0.08 <sup>a</sup>
10%Rosemary	44.75 $\pm$ 4.26 <sup>b</sup>	2.47 $\pm$ 0.56 <sup>b</sup>	0.718 $\pm$ 0.03 <sup>b</sup>
10% <i>G sylvestre</i>	40.52 $\pm$ 3.26 <sup>bc</sup>	2.18 $\pm$ 0.36 <sup>b</sup>	0.693 $\pm$ 0.02 <sup>c</sup>
5% rosemary+ 5% <i>G sylvestre</i>	42.11 $\pm$ 6.68 <sup>bc</sup>	2.13 $\pm$ 0.28 <sup>b</sup>	0.673 $\pm$ 0.09 <sup>c</sup>
Silymarin (100 mg/kg BW)	48.21 $\pm$ 4.57 <sup>b</sup>	1.95 $\pm$ 0.06 <sup>bc</sup>	0.685 $\pm$ 0.06 <sup>c</sup>

Data are presented as means  $\pm$  SDM ( $n=6$ ). a, b, c and d: Means with different letter in the same column are significantly different ( $P \leq 0.05$ )

#### **Effect of rosemary and gymnema leaves powder on oxidative stress of rats induced liver injury by Paracetamol:**

As seen in Table (7) serum catalase CAT and superoxide dismutase SOD activities were decreased significantly by Paracetamol administration, while, there was significant elevated in serum malondialdehyde MDA compared to negative control.

The results showed enhancement for all treatment groups pretreated with rosemary, *gymnema sylvestre* leaves powder and their mixture at 10% concentration and also, silymarin group at (100 mg/kg BW), back to significant increase in serum catalase CAT and superoxide dismutase SOD activities and significant decreased in serum malondialdehyde MDA compared to positive control group. The best results showed of 5% rosemary+ 5% *G sylvestre* group compared to negative control group.

In agreement with our results, many studies found that the rosemary enhanced the toxic impacts of on liver. This was showed by decrease of

the level of MDA and increment CAT and SOD. The obtained results about oxidative stress are in concurrence with **Nevien, (2018) and Abd El-Ghany et al., (2012).**, who reported that treatment with rosemary leaves prevents oxidative stress due to carnosic corrosive (CA), carnosol and other phenolic acids in rosemary CA can avert lipid peroxidation and it avoids the disruption of the biological membrane by searching free radicals.

Gynmena is a green leafy vegetable has antioxidant activities and may be applicable for preventing oxidative stress and aging-related disease. Its materials with high contents of phenolics, flavonoids, quercetin, and kaempferol showed significant relation to antioxidation and protection in endothelial cell death suppressed by reactive nitrogen species (**Onanong et al., 2024**).

**Table (7): Serum catalase, superoxide dismutase and malondialdehyde of intoxicated rats which treated with rosemary and gymnema leaves powder**

Groups	Parameters		
	CAT ( $\mu$ /L)	SOD ( $\mu$ /dl)	MDA (ng/ml)
Control (ve-)	96.15 $\pm$ 2.08 <sup>a</sup>	92.13 $\pm$ 3.51 <sup>a</sup>	10.16 $\pm$ 0.11 <sup>c</sup>
Control (ve+)	43.51 $\pm$ 1.89 <sup>d</sup>	39.11 $\pm$ 2.45 <sup>d</sup>	42.45 $\pm$ 2.71 <sup>a</sup>
10% Rosemary	81.22 $\pm$ 3.76 <sup>b</sup>	77.98 $\pm$ 3.16 <sup>b</sup>	22.18 $\pm$ 1.04 <sup>b</sup>
10% <i>G sylvestre</i>	80.12 $\pm$ 3.26 <sup>b</sup>	71.23 $\pm$ 2.91 <sup>b</sup>	23.19 $\pm$ 1.05 <sup>b</sup>
5% rosemary+ 5% <i>G sylvestre</i>	91.35 $\pm$ 4.86 <sup>a</sup>	86.25 $\pm$ 3.67 <sup>ab</sup>	17.23 $\pm$ 1.02 <sup>bc</sup>
Silymarin (100 mg/kg BW)	70.21 $\pm$ 3.15 <sup>bc</sup>	61.25 $\pm$ 2.15 <sup>bc</sup>	27.51 $\pm$ 1.14 <sup>b</sup>

Data are presented as means  $\pm$  SDM ( $n=6$ ). a, b, c and d: Means with different letter in the same column are significantly different ( $P \leq 0.05$ ).

CAT: catalase SOD: Superoxide dismutase MDA: malondialdehyde

### Histopathological Examination:

Liver and kidney sections of rats in different experimental groups was examined and the photomicrographs are illustrated in **Photo (1 to 4)**

#### 1. Histopathological examination of liver:

Microscopic examination of liver sections of rats from Control (-ve) group revealed the normal histological architecture of hepatic lobule (**Photo 1**). In adverse, liver of rats from Control (+ve) group showed hepatocellular vacuolar degeneration, fibroplasia in the portal triad and newly formed bile ductulus (**Photo 1**). Meanwhile, liver of rats from 10% Rosemary group exhibited hepatocellular vacuolar degeneration of some hepatocytes, slight

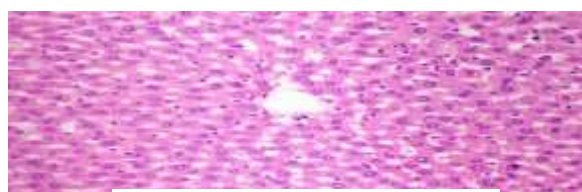
proliferation of Kupffer cells and slight portal fibroplasia (**Photo 2**). On the other hand, liver of rats from 10% *G sylvestre* group revealed mild changes described as slight proliferation of Kupffer cells (**Photo 2**). Otherwise, liver of rats from (5% rosemary+ 5% *G sylvestre*) group showed no histopathological changes in hepatic lobule (**Photo 2**). Also, examined sections from Silymarin (100 mg/kg BW) group (standard drug) exhibited no histopathological changes except slight proliferation of Kupffer cells (**Photo 2**).

In the present study, the hepatotoxicity of paracetamol was confirmed by histopathological examination of the liver tissue. Paracetamol induced hepatocellular vacuolar degeneration, fibroplasia in the portal triad and newly formed bile ductulus. These results are in agreement with many reports, showing that the histopathology analysis of the liver revealed signs of toxicity after administration of paracetamol and hydropic degeneration in the hepatocytes, associated with severe congestion and dilatation in the portal vein and fibrosis in the periductal tissue surrounding the dilated bile ducts (**Ratnasooriya and Jayakody , 2000, Liu et al., 2011, Yousef et al., 2010, Hurkadale et al., 2012, Kiran et al., 2012 and Datta et al., 2013**).

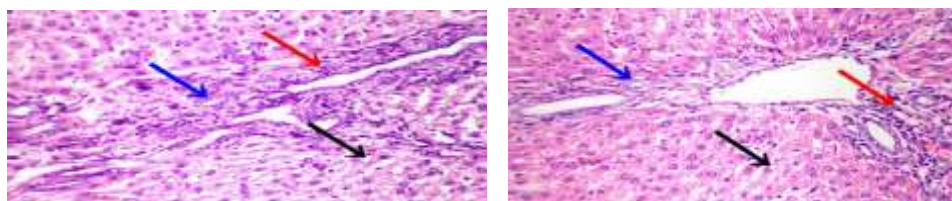
However, the enhancement of pretreatment groups in our results agree with (**Sotelo-Fe´lix et al., 2002**) that found administration of rosemary extract (125 mg/kg) can protect the liver from paracetamol-induced injury effectively. These results are in accordance with the results of (**Amin and Hamza, 2005**) who stated that rosemary pretreatment had shown protective effects by the marked recovery of normal liver histological architecture against azathioprine-induced toxicity in rats. **Natalia et al., (2023)** reinforced the antioxidant activity of rosemary exhibited a potent hepatoprotective effect against acetaminophen toxicity in vivo. Rosemary and its constituents including carnosic acid, rosmarinic acid, and carnosol have a lot of benefits such as anti-inflammatory, antioxidant, anti-mutagenic, anti-bacterial, antiviral, antinociceptive, and neuroprotective activities (**Mohaddessin et al., 2020**).

*G sylvestre* with its macronutrients and micronutrients profile is also rich in the polyphenolic compounds which all have an amazing property against different ailments. Liver tissue, adipose tissue and muscle cells are chief metabolic tissues that manage glucose and lipid homeostasis (**Tiwari et al., 2014**).



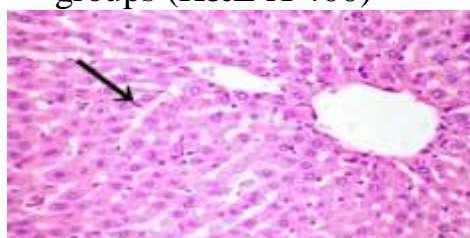


Control (-ve) group.

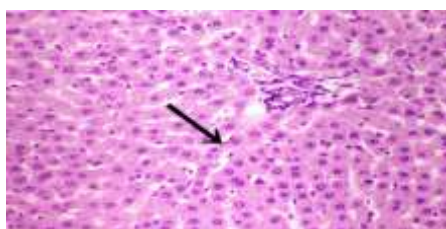


Control (+ve) group

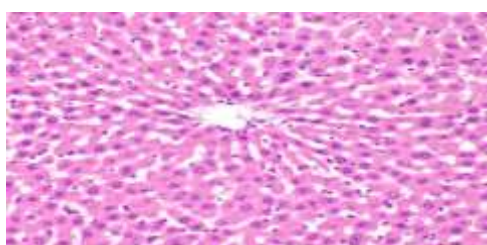
**Photo (1):** Photomicrograph of Liver in control (-ve) and control (+ve) groups (H&E X 400)



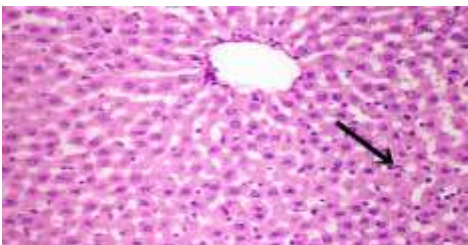
10% Rosemary group



10% G sylvestre group



(5% rosemary+ 5% G sylvestre) group



Silymarin group

**Photo (2):** photomicrographs of liver in all treated experimental groups (H&E X 400)

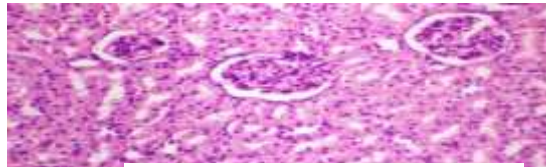
## 2. Histopathological examination of Kidney:

Microscopically, kidneys of rats from control (-ve) group revealed the normal histological structure of renal parenchyma (**Photo 3**). In contrariwise, kidneys of rats from control (+ve) group described vacuolar degeneration of epithelial lining renal tubules, congestion of intratubular renal blood vessel and proteinaceous cast in the lumen of renal tubules (**Photo 3**). Examined sections from 10% Rosemary and 10% *G sylvestre* groups revealed vacuolar degeneration of epithelial lining some renal tubules and slight congestion glomerular tuft (**Photo 4**). Otherwise, kidneys of rats from (5% rosemary+ 5% *G sylvestre*) group exhibited no histopathological (**Photo 4**). Meanwhile,

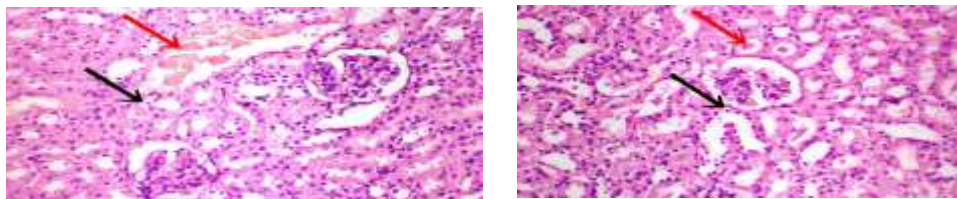
kidneys of rats from Silymarin (100 mg/kg BW) group demonstrated slight vacuolar degeneration of epithelial lining some renal tubules and proteinaceous cast in the lumen of renal tubules (**Photo 4**).

In the present study, the histological appearance of renal tissues confirmed the previous results. In paracetamol administered rats, vacuolar degeneration of epithelial lining renal tubules, congestion of intratubular renal blood vessel and proteinaceous cast in the lumen of renal tubules. This agrees with the study by **Lim et al., (2010)** who found that paracetamol (500 mg/kg) induced mild vascular and inflammatory changes with signs of vascular congestion, tubular necrosis, and glomerular atrophy, which is a degenerative phenomenon. Interstitial inflammation was observed in the kidney of paracetamol-administered rats (**Kumar et al., 2010**). It was stated that histological analysis of the kidney samples showed only minor evidence of renal damage (**Melo et al., 2006**). **Walaa et al., (2014)** found that paracetamol-induced renal damage was accompanied by an acute reduction in the glomerular filtration rate. Administration of rosemary with paracetamol normalized these defects in the histological architecture of the kidney. These agree with the study by **Walaa et al., (2014)** who found that pre-administration and post-administration of rosemary (100 mg/kg) with cisplatin dose on the 5th day revealed predominant normal kidney morphology with only occasional degenerating tubules.

**In conclusion:** This study suggests the potent role of rosemary, gymnema leaves powder and their mixture in management of liver and kidney toxicity -induced by paracetamol and this effect could be attributed to their antioxidant activity.

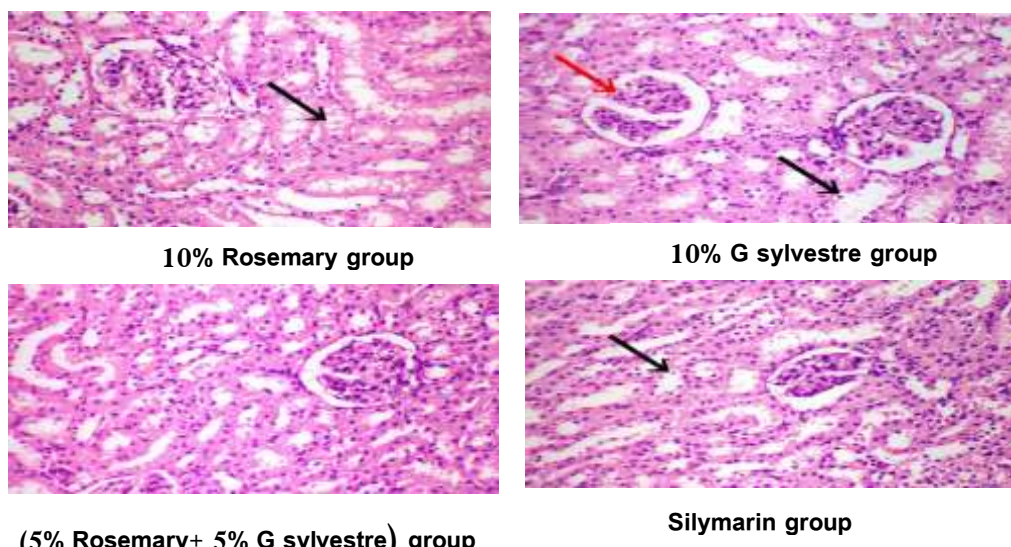


Control (-ve) group



Control (+ve) group

**Photo (3):** Photomicrograph of kidney in control (-ve) and control (+ve) groups (H&E X 400)



**Photo (4):** photomicrographs of kidney in all treated experimental groups (H&E X 400)

#### References

- Abd El-Ghany, MA., Motawee, M. and El-Kewawy, HE., (2012).** Biological effects of yoghurt with rosemary on injured liver rats. *Aust. J. Basic Appl. Sci.* 6:525–532.
- Ahmed AB., Rao A. and Rao M., (2010).** In vitro callus and in vivo leaf extract of *Gymnema sylvestre* stimulate  $\beta$ -cells regeneration and anti- diabetic activity in Wistar rats. *Phytomedicine* 17 (13), 1033–1039. 10.1016/j.phymed.2010.03.019 [PubMed] [CrossRef] [Google Scholar].
- Amin A and Hamza AA., (2005).** Hepatoprotective effects of Hibiscus, Rosmarinus and Salvia on azathioprine-induced toxicity in rats. *Life Sci.*77:266–278.
- A.O.A.C. (2012):** Association of official analytical chemistry international, 19thed., Gaithersburg, Maryland, USA.
- Azab A., Fetouh F., and Albasha, M., (2014).** Nephro-protective effects of curcumin, rosemary and propolis against gentamicin induced toxicity in Guinea pigs: Morphological and biochemical study. *American Journal of Clinical and Experimental Medicine*, 2(2), 28–35.
- Bai N., He K., Roller M., Lai C., Shao X., Pan M. and Ho C., (2010).** Flavonoids and Phenolic Compounds from *Rosmarinus officinalis*. *J.Agric. Food Chem.*; 58:5363–5367. doi: 10.1021/jf100332w.

- Banchroft JD., Sevens A. and Turner DR., (1996).** Theory and practice of histological techniques. Fourth ed. Churchill Livingstone, New York, London, San Francisco, Tokyo.
- Barham D. and Trinder P., (1972):** *Analyst*.97:142.
- Batton CJ. and Crouch SR., (1977):***Anal.Chem.*, 49:464-469.
- Belfield, A. and Goldberg, D.M., (1971).** Colorimetric Determination of Alkaline Phos-phatase Activity. *Enzyme*, 12, 561-568. <https://doi.org/10.1159/000459586>
- Beutler E.; Dubon B. and Kelly M., (1963):** Improved method for determination of blood Catalase. *J. Lab. Clin. Med.*, 61: 882-888.
- Borges RS., Ortiz B., Pereira A., Keita H. and Carvalho J., (2019).** *Rosmarinus officinalis* Essential Oil: A Review of Its phytochemistry, Anti-Inflammatory Activity, and Mechanisms of Action Involved. *J. Ethnopharmacology.*; 229:29–45.[doi: 10.1016/j.jep.2018.09.038](https://doi.org/10.1016/j.jep.2018.09.038).
- Bouyahya A., Et-Touys A., Bakri Y., Talbau A., Fella H., Abrini J., and Dakka N. (2017).** Chemical Composition of *Menthapulegium* and *Rosmarinus officinalis* Essential Oils and Their Antileishmanial, Antibacterial and Antioxidant Activities. *Microb. Pathog.* 2017; 111:41–49. [doi: 10.1016/j.micpath.08.015](https://doi.org/10.1016/j.micpath.08.015).
- Cefalo F., Cinti S., Moffa F., Impronta GP., Sorice T., Mezza A., Pontecorvi A., Giaccari and Sotagliflozin, (2019).** The first dual SGLT inhibitor: current outlook and perspectives, *Cardiovasc Diabetol.* 18 (1) 20, .
- Chanwitheesuk A. Teerawutgulrag N. and Rakariyatham, (2005).** Screening of antioxidant activity and antioxidant compounds of some edible plants of Thailand, *Food Chem.* 92: 491–497, .
- Chapman, DG., Gastilla, R. and Campbell, JA., (1959).** Evaluation of protein in food I.A. Method for the determination of protein efficiency ratio. *Can. J. Biochem Physiol*, 37:679-686.
- Datta S, Dhar S, Nayak SS and Dinda SC., (2013).** Hepatoprotective activity of *Cyperus articulatus* Linn. against paracetamol induced hepatotoxicity in rats. *J Chem Pharm Res* 2013; 5:314–319.
- Dedvisitsakul, K. and Watla, L., (2022).** Antioxidant activity and antidiabetic activities of Northern Thai indigenous edible plant extracts and them phytochemical constituents, *Heliyon* 8 (9), e10740, <https://doi.org/10.1016/j.heliyon.2022.e10740>. [doi: 10.1038/s41598-022-16592-7](https://doi.org/10.1038/s41598-022-16592-7).
- Draper, H. and Hadley, M. (1990).** Malondialdehyde determination as index of lipid peroxidation. *Methods Enzymol*, 186: 421-431.

- Ecevit AA., Barros JM., Silva RL. and Reis, (2022).** Preventing microbial infections with natural phenolic compounds, *Future Pharmacol* 2 (4). 460–498, <https://doi.org/10.3390/futurepharmacol2040030>.
- Erhan Sahin, (2020).** Silymarin attenuated nonalcoholic fatty liver disease through the regulation of endoplasmic reticulum stress proteins GRP78 and XBP-1 in mice. *Journal of Food Biochemistry* Volume 44, Issue 6 e13194.
- Farzana C and Muhammad HR., (2010).** Isolation and characterization of gymnemic acid from Indigenous gymnema sylvestr. *Journal of Applied Pharmaceutical science*,; 3(2):60-65.
- Farzana K., Moklesur RS., Long CM., Isa NM., Chao Z., Bassem Y, S., Hiew FT. and Mohammad AR. (2019).** Comprehensive Review on Phytochemicals, Pharmacological and Clinical Potentials of *Gymnema sylvestre*. *J. Pub Medical*, Oct 29:10:1223. doi:10.3389/fphar.2019.01223.
- Fernandes, M. C., Dumas, B. K., Show, C. J., and Ongóá, I. M. (2015).** Gender and Development: A Literature Review. LicenseCC BY-NC-SA 4.0. Retrieved March,10,2020,[https://www.researchgate.net/publication/281378258\\_Gender\\_and\\_Development\\_A\\_Literature\\_Review](https://www.researchgate.net/publication/281378258_Gender_and_Development_A_Literature_Review).
- Gornall AG, Bardawill CJ and David MM (1949).** Determination of serum proteins by means of the biuret reaction. *J. Biol. Chem.*, 177:751-66.
- Harboene, JB., (1973).** “Phytochemical methods”, Chapman and Hall Ltd. London, Pp. 49-188.
- Hozayen WG., (2012).** Effect of hesperidin and rutin on doxorubicin induced testicular toxicity in male rats. *Int J Food Nutr Sci*; 1:31–42.
- Hurkadale PJ, Shelar PA, Palled SG, Mandavkar YD and Khedkar AS., (2012).** Hepatoprotective activity of *Amorphophallus paeoniifolius* tubers against paracetamol-induced liver damage in rats. *Asian Pac J Trop Biomed*; 2:238–242.
- Ibrahim N., Abbas H., El-Sayed NS. and Gad HA., (2022).** *Rosmarinus officinalis* L. Hexane Extract: Phytochemical Analysis, Nanoencapsulation, and in Silico, in Vitro, and in Vivo Anti-photoaging Potential Evaluation. *Sci. Rep.*; 12:13102.
- Ikponmwosa EO. and Eromosele, (2019).** Comparative Evaluation of Biochemical Effects of Date Palm (*Phoenix dactylifera*) Fruit Extracts on Paracetamol-induced Hepatotoxicity in Wistar rats. *J. Appl. Sci. Environ. Manage.* Vol. 23 (4) 641-646 April 2019.

- Jordán MJ., Castillo J., Bañón S., Martínez-Conesa C., and Sotomayor JA., (2014).** Relevance of the Carnosic Acid/Carnosol Ratio for the Level of Rosemary Diterpene Transfer and for Improving Lamb Meat Antioxidant Status. *Food Chem.*; 151:212–218. doi: 10.1016/j.foodchem. 11.068.
- Kakkar, P., Das, B. and Synder, PN., (1984).** A modified spectrophotometric assay of superoxide dismutase. *Ind. J. Biochem. Biophys*, 21: 131-132.
- Kalinda SR and Rioba BN., (2020).** Phytochemical Analysis and Efficacy of Rosemary (*Rosmarinus Officinalis*) and Mint (*Mentha Spicata*) Extracts Against Fall Armyworm (*Spodoptera Frugiperda*) on Baby Corn (*Zea Mays*). *Acta Chemica Malaysia (ACMY)*, Vol. 4 (2): 66-71.
- Kiran PM., Raju A. and Rao BG., (2012).** Investigation of hepatoprotective activity of *Cyathea gigantea* (Wall. ex. Hook.) leaves against paracetamol-induced hepatotoxicity in rats. *Asian Pac J Trop Biomed*; 2:352–56.
- Kumar G., Hota D., Saikia UN. and Pandhi P., (2010).** Evaluation of analgesic efficacy, gastrotoxicity and nephrotoxicity of fixed-dose combinations of nonselective, preferential and selective cyclooxygenase inhibitors with paracetamol in rats. *Exp Toxicol Pathol* 2010; 62:653–662.
- Laura R. and Nikolaos P., (2020).** Liver injury induced by paracetamol and challenges associated with intentional and unintentional use. *World J Hepatol.* Apr 27; 12(4): 125–136. Published online 2020 Apr 27. doi:10.4254/wjh. v12.i4.125
- Lim AY., Segarra I., Chakravarthi S., Akram S. and Judson JP., (2010).** Histopathology and biochemistry analysis of the interaction between sunitinib and paracetamol in mice. *BMC Pharmacol*; 10:14.
- Lin M., Xiao J., Zhao Z., Li B., Xing X., Li M., Kong L., Li Q., Zhang Y., Liu H., Chen W., Qin H., Wu S., and Chen, (2016).** An overview of plant phenolic compounds and their importance in human nutrition and management of type 2 diabetes, *Molecules* 21 (10) 1374.
- Liu YT., Lu B. and Peng J., (2011).** Hepatoprotective activity of the total flavonoids from *Rosa laevigata* Michx fruit in mice treated by paracetamol. *Food Chem*; 125:719–725.
- Maimonah F., Mahmoud Z., Mohamed E., Bassem R., Riyad A., Shakir I., Mohammad A., Hiba S. and Safaa Y., (2023).** Antioxidative and Anti- Inflammatory Protective Effects of

- Fucoxanthin against Paracetamol- induced Hepatotoxicity in Rats. J. Marine Drugs, 21(11), 592; <https://doi.org/10.3390/md2111059>
- Melo DAS, Saciura VC, Poloni JAT, Oliveira GSA, JCFA Filho, and Padilha RZ., (2006).** Evaluation of renal enzymuria and cellular excretion as a marker of acute nephrotoxicity due to an overdose of paracetamol in wistar rats. Clin Chim Acta; 373:88–91.
- Minaiyan M, Ghannadi AR, Afsharipour M. and Mahzouni P.,(2011).** Effects of extract and essential oil of Rosmarinus officinalis L. on TNBS-induced colitis in rats. Res Pharm Sci; 6:13–21.
- Mohaddeseh S. A., Sahar F., Mahboobeh G. R., Soghra M., and Hossein H. (2020).** An updated review of protective effects of rosemary and its active constituents against natural and chemical toxicities. Phytotherapy, 35 (3): 1313-1328.
- Natalia SS., Vycória SR., Laura FL., Rayssa ML., Gabriel SA., Luís GP., Ricardo RS., Iseli LN., Debora CD., Norberto PL., and Tiago R., (2023).** Rosemary (*Rosmarinus officinalis* L.) Glycolic Extract Protects Liver Mitochondria from Oxidative Damage and prevents Acetaminophen-Induced Hepatotoxicity. Antioxidants (Basel), Mar.12(3):628
- Nevien S. Esmail, (2018).** Effect of Thyme and Rosemary on Liver Functions in Toxicated Rats by Aflatoxins J. Food and Dairy Sci., 3rd Mansoura International Food Congress (MIFC) October: 59 – 65
- Onanong N., Wanwisa S., Chaisak C., Uthaiwan S., Piya T., Nitra N., and Uracha R., (2024).** Relationship of phytochemicals and antioxidant activities in *Gymnema inodorum* leaf extracts. Heliyon , 10 : e23175. Contents lists available at ScienceDirect Heliyon journal homepage: [www.cell.com/heliyon](http://www.cell.com/heliyon) <https://doi.org/10.1016/j>.
- Raheel N., Ayesha NC., Asif M., Fizza T., and Muhammad TS., (2023).** Effect of *Gymnema sylvestre* extract on Liver and Kidney functioning: In-vivo study. Life Sci J ;20(9)1-11]. ISSN 1097-8135 (print); ISSN 2372-613X (online).
- Ratnasooriya WD. and Jayakody JRAC., (2000).** Long-term administration of large doses of paracetamol impair the reproductive competence of male rats. Asian J Androl; 2:247–255.
- Reeves, PG.; Nielsen, FH. and Fahey, GC., (1993).** AIN-93 purified diets for laboratory rodents: Final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN- 76A rodent diet. J. Nutr., 123(11):1939-1951.
- Reitman, A. and Franklel, S., (1957).** Amer.J.Clinic.Path, 28:56.

- Sabir SM and Rocha JBT., (2008).** Antioxidant and hepatoprotective activity of aqueous extract of *Solanum fastigiatum* (false 'Jurubeba') against paracetamol induced liver damage in mice. *J Ethnopharmacol.*120:226–232
- Sadasivan S, Latha PG, Sasikumar JM, Rajashekaran S, Shyamal S. and Shine VJ. (2006).** Hepatoprotective studies on *Hedyotis corymbosa* (L.) Lam. *J Ethnopharmacol*; 106:245–249.
- Sasakia K, El Omria A, Kondoa S, Hana J. and Isodaa H., (2013).** *Rosmarinus officinalis* polyphenols produce anti-depressant like effect through monoaminergic and cholinergic functions modulation. *Behav Brai Res*; 238:86–94.
- Sherif, SO.; Salama, EE. and Abdel-Wahhab, MA., (2009).** Mycotoxins and child health: the need for health risk assessment. (Review) *Int. J.Hyg. Environ. Health*, 212: 347 368.
- Snedecor GW. and Cochran, WG., (1980).** *Statistical Methods*, 7 th. IBIT Public. Co, Oxford.
- Sotelo-Fe´lix JI, Martinez-Fong D, Muriel P, Santilla´n RL, Castillo D. and Yahuaca P., (2002).** Evaluation of the effectiveness of *Rosmarinus officinalis* (Lamiaceae) in the alleviation of carbon tetrachloride-induced acute hepatotoxicity in the rat. *J Ethnopharmacol*; 81:145–154.
- Srinuanchai, R. Nooin, P. Pitchakarn, J. Karinchai, U. Suttisansanee, C. Chansriniyom, S. Jarussophon, P. Temviriyankul, O. Nuchuchua, (2021).** Inhibitory effects of *Gymnema inodorum* (Lour.) Decne leaf extracts and its triterpene saponin on carbohydrate digestion and intestinal glucose absorption, *J. Ethnopharmacol.* 266, 113398, .
- Tietz NW., (1986).** *Textbook of clinical chemistry* .WB. saunders, Philadelphia, pp 1271-1281.
- Tiwari P., Mishra BN. and Sangwan NS., (2014).** Phytochemical and pharmacological properties of *Gymnema sylvestre* important medicinal plant. *Biomed Research International.*40:20-22.
- Trang DT., Yen NT., Cuong LT., Anh NT., Hoai BH., Tai VV., Doan PH., Yen TH., Quang NX. , Nhiem CV., Minh PV. and Kiem A., (2021).** Pregnane glycosides from *Gymnema inodorum* and their  $\alpha$ -glucosidase inhibitory activity, *Nat. Prod. Res.* 35 (13): 2157–2163, <https://doi.org/10.1080/14786419.2019.1663517>
- Walaa G. Hozayen, Hanan AE. and Ekram MD., (2014).** Protective role of aqueous rosemary extract against hepatorenal toxicity of paracetamol in male rats *Medical Research Journal* · June 2014 Vol 13 No 1: 2090-6242



- Yousef MI., Omar S., El-Guendi MI., Abdelmegid LA., (2010).**  
Potential protective effects of quercetin and curcumin on paracetamol-induced histological changes, oxidative stress, impaired liver and kidney functions and haematotoxicity in rats. Food Chem Toxicol 2010.48:3246–3261.
- Zainul A Z., Farah HK., Teh LK., and Mohd ZS., (2020).**  
Hepatoprotective and antioxidant activities of Dicranopteris linearis leaf extract against paracetamol-induced liver intoxication in rats. Pharm Biol.; 58(1): 478–489. Published online 2020 Jun 1. doi: 10.1080/13880209.2020.1764058.