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سعادة أ. د. رئيس تحرير المجلة المصرية للدراسات المتخصصة المحترم  
جامعة عين شمس، كلية التربية النوعية، القاهرة، مصر  
تحية طيبة وبعد،،،

يسر معامل التأثير والاستشهادات المرجعية للمجلات العلمية العربية (أرسياف - ARCIF)، أحد مبادرات قاعدة بيانات "معرفة" للإنتاج والمحتوى العلمي، إعلامكم بأنه قد أطلق التقرير السنوي التاسع للمجلات للعام 2024.

ويسرنا تهنئكم وإعلامكم بأن المجلة المصرية للدراسات المتخصصة الصادرة عن جامعة عين شمس، كلية التربية النوعية، القاهرة، مصر، قد نجحت في تحقيق معايير اعتماد معامل "أرسياف Arcif" المتوافقة مع المعايير العالمية، والتي يبلغ عددها (32) معياراً، وللاطلاع على هذه المعايير يمكنكم الدخول إلى الرابط التالي: <http://e-marefa.net/arcif/criteria>

وكان معامل "أرسياف Arcif" العام لمجلتكم لسنة 2024 (0.4167).

كما صنفت مجلتكم في تخصص العلوم التربوية من إجمالي عدد المجلات (127) على المستوى العربي ضمن الفئة (Q3) وهي الفئة الوسطى، مع العلم أن متوسط معامل "أرسياف" لهذا التخصص كان (0.649).

وبإمكانكم الإعلان عن هذه النتيجة سواء على موقعكم الإلكتروني، أو على مواقع التواصل الاجتماعي، وكذلك الإشارة في النسخة الورقية لمجلتكم إلى معامل "أرسياف Arcif" الخاص بمجلتكم.

ختاماً، نرجو في حال رغبتكم الحصول على شهادة رسمية إلكترونية خاصة بنجاحكم في معامل "أرسياف"، التواصل معنا مشكورين.

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# **The Protective Effect of Foods Rich in Anthocyanins and Curcuma Against Carbon Tetrachloride-Induced Toxicity**

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## The Protective Effect of Foods Rich in Anthocyanins and Curcuma Against Carbon Tetrachloride-Induced Toxicity

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### Abstract

The present study is aimed to assess the protective effect of foods rich in anthocyanins and curcuma on the induced infection of carbon tetrachloride. Forty eight Albino rats (Sprague Dawley strain), weighing about  $190 \pm 10$  g was obtained from Agricultural Research Center, Giza, Egypt, the rats were divided into two groups, the first group ( $n = 6$  rats) was fed only on the basal diet as a negative control group (ve-). The second major group (42 rats) is randomly divided into seven sup groups (six animals each), as follows: group (2) the positive control group (ve+) received fed on basal diet, group (3) and (4) received were fed a basal diet containing hibiscus 10 %. and 20% respectively, group (5) received were fed a basal diet containing hibiscus +Curcuma 10%.

**Keywords:** Omega, Fish Oil, Heart Disease, Lipid profile , Cardio vacuolar disease , rats

### ملخص:

**العنوان :** الأثر الوقائي للأغذية الغنية بالأنثوسيانين والكرم على السمية المستحدثة برابع كلوريد الكربون

**المؤلفون :** أسامة السيد مصطفى ، زينب مصطفى ، هالة راشد عطايا ، إسراء جمال محمود تهدف الدراسة الحالية إلى تقييم التأثير الوقائي للأطعمة الغنية بالأنثوسيانين والكرم على الإصابة المستحدثة برابع كلوريد الكربون. تم الحصول على ثمانية وأربعين فأراً ألبينو، وزن حوالي  $190 \pm 10$  جم من مركز البحوث الزراعية بالجيزة، مصر، وتم تقسيم الفئران إلى مجموعتين، المجموعة الأولى (عددها = 6 فئران) تم تغذيتها على الغذاء الأساسي فقط كمجموعة ضابطة سالبه (-ve). المجموعة الرئيسية الثانية (42 فأراً) تم تقسيمها عشوائياً إلى سبع مجموعات فرعية (6 فأراً لكل منها)، على النحو التالي: المجموعة الضابطة الموجبة (+ve) التي تم تغذيتها على الغذاء الأساسي وتم تغذية المجموعة (3) و(4) على الغذاء الأساسي يحتوي على الكركديه 10% و20% على التوالي. المجموعة (5) والتي تم تغذيتها على الغذاء الأساسي ويحتوي على الكركديه + الكرم 10%،

**الكلمات الدالة :** الكركديه ، الكرم ، الأنثوسيانين ، رابع كلوريد الكربون ، انزيمات الكبد

## Introduction

Hibiscus sabdariffa is a shrub that belongs to the family Malvaceae. Over 200 different species of Hibiscus exist worldwide. Hibiscus sabdariffa has been noted to have a high nutritional potential, particularly in the leaves, calyces, and seeds. The Roselle calyx, which is used to make a variety of beverages, has been said to contain a significant amount of vitamins, minerals, flavonoids, protein, lipids, carbs, and other nutrients. There are reports on the plants' antioxidant, antihypertensive, hepatoprotective, nutritive, and antihyperlipemic qualities. (Onyeka et al., 2023).

Anthocyanin there is increasingly convincing scientific evidence that supports both a preventative and therapeutic role of anthocyanins towards certain chronic disease states. Many anthocyanin-based extracts and juice concentrates from crop and/or food processing waste have become commercially available as colorants and/or value-added food ingredients. There is a large and evolving peer-reviewed literature on how anthocyanin chemistry and concentration may affect their coloring properties in food. Equally as important is the food matrix, which can have large impacts on anthocyanin color expression, stability and degradation, particularly regarding the applications of anthocyanins as food colorants and their health-promoting properties. This Special Edition of Foods, titled "Anthocyanins in Foods," presents original research that extends our understanding of these exciting and complex compounds (Taylor et al., 2019).

Curcuma, derived from the rhizome curcuma longa, is one of the primary ingredients in turmeric and curry powders that are used as spices in Middle Eastern and Asian countries, especially on the Indian subcontinent. More recently, laboratory studies have demonstrated that dietary curcumin exhibits various biological activities and significantly inhibits colon tumorigenesis and tumor

size in animals. Curcuma displays both anti-inflammatory and antioxidant properties, giving it the potential to be considered in the development of cancer preventive strategies and applications in clinical research. Experimental studies have shown the biological activities of the compound, but much more information on pharmacokinetics, bioavailability, and food content are needed (Reema et al., 2006).

One of the most well-known and well-used experimental models of acute liver injury is CCl<sub>4</sub>. This model of chemical liver damage is used for the examination of the liver damage mechanisms and of the possible anti-hepatotoxic (hepatoprotective) activities of various synthetically generated substances or natural products (Dejan et al., 2019).

The Liver is an important organ which plays a central role in metabolic homeostasis. It also has an amazing regenerative capability after liver mass loss. Mechanism of CCL<sub>4</sub> induced hepatotoxicity, especially necrosis and fatty liver, has long been a challenging subject of many researchers from various fields over the past 50 years. Even though the mechanisms of tissue damages are different among chemicals and affected tissues, CCL<sub>4</sub> played a role as a key substance of tissue injury. A number of studies were conducted and various hypotheses were raised. As a result, several important basic mechanisms of tissue damages were emerged, involving metabolic activation, reactive free radical metabolites, lipid peroxidation, covalent binding and disturbance of calcium homeostasis (Hai et al., 2019).

**This study aimed** to assess the protective effect of foods rich in anthocyanins and curcumin on the induced infection of carbon tetrachloride.

## Materials and Methods:

### 3.1. Materials

- Hibiscus sabdariffa and curcuma longa were purchased



from the local market, Cairo, Egypt.

- Carbon tetrachloride obtained from El- Gomhoriya Company, Cairo, Egypt.
- Commercial kits used for determining. Malondialdehyde (MDA) and Serum total antioxidant capacity (TAC) were obtained from Bio-diagnostic Co. Dokki, Egypt. Serum ALT, AST, ALP, total proteins and albumin content kits were purchased from Spin-react, Germany.
- Antodin , Ibuprofen , casein, sucrose, corn oil, fibre (cellulose), mineral mixture, vitamin mixture, choline chloride, D-L methionine, and corn starch were obtained from were obtained from El-Gomhoreya Co., Cairo, Egypt.

### 3.2. Experimental animals

Forty eight Albino rats (Sprague Dawley strain), weighing about  $190 \pm 10$  g was obtained from Agricultural Research Center, Giza, Egypt. The animal groups were kept in an atmosphere of filtered, pathogen-free air, water, and a temperature of 20-25°C for 8 weeks, with a 12-hour light/dark cycle and a light cycle (8-20 h) and a relative humidity of 50%. For one week, all rats were fed a basal diet. The basal diet was designed to contain 14% casein, 10% sucrose, 4% corn oil, 5% fiber (cellulose), 3.5 percent mineral mixture, 1% vitamin mixture, 0.25 percent choline chloride, 0.3 percent D-L methionine, and 61.95 percent corn starch (**Reeves *et al.*, 1993**). Before starting the experiment for acclimatization. All the experimental procedures were carried out in accordance with international guidelines for the care and use of laboratory animals. The experiment was conducted at Agricultural Research Center, Giza, Egypt. Animals were fed a standard diet and provided water freely. They were left to acclimatize for one week before starting the experiment. The standard diet was formulated according to the American Institute of Nutrition 93 (AIN-93) (**Reeves *et al.*, 1993**). The salt mixture was prepared according to (**Hegsted *et al.*, 1941**). The vitamin mixture was prepared according to Association of Official

Analytical Chemists (**cmpbell, 1963**). The composition of the standard diet, vitamin mixture and mineral mixture was illustrated in Table (a), (b) and (c). respectively

**Table (a): Composition of standard diet (g / kg diet)**

Ingredients	Basal diet g/kg
Casein	140
corn starch	620
Sugar ( sucrose)	100
Cellulose	50
Corn oil	40
Mineral mixture	35
Vitamin mixture	10
L- Cystine	1.8
Choline bitartrate	2.5
Tert-Butylhydroquinone	0.008

(Reeves et al. 1993)

**Table (b): The vitamin mixture composition**

Vitamin	Amount	Vitamin	Amount
Vitamin A	200 IU	Panthothenic acid	0.40 mg
Vitamin D	100 IU	Choline chloride	200 mg
Vitamin E	10 IU	Isoitol	24 mg
Vitamin K	0.50 IU	Vitamin B12	2.00 mg
Thiamin	0.50 mg	Folic acid	0.02 mg
Pyridoxine	1.00 mg	Para-amino benzoic	0.02 mg
Niacin	4.00 mg	Para-amino benzoic	0.02 mg
Biotin	0.02 mg	Para-amino benzoic	0.02 mg

(Cmpbell, 1963).

**Table (c): The salt mixture composition**

Salt	Amount (mg)	Salt	Amount (mg)
CaCO <sub>3</sub>	600	Fe (C <sub>6</sub> H <sub>5</sub> O <sub>7</sub> ) <sub>2</sub> .H <sub>2</sub> O	55
K <sub>2</sub> HPO <sub>4</sub>	645	KI	1.6
CaHPO <sub>4</sub> .2H <sub>2</sub> O	150	MnSO <sub>4</sub> .4H <sub>2</sub> O	10
MgSO <sub>4</sub> .2H <sub>2</sub> O	204	ZnCl <sub>2</sub>	0.5
NaCl	334	CuSO <sub>4</sub> .5H <sub>2</sub> O	0.6

(Hegested *et al.*, 1941)

### 3.3 Methods:

#### 3.3.1 Preparation of hibiscus sabdariffa leaves powder

The leaves of hibiscus sabdariffa were washed; dried under room temperature and powdered using an electric blender according to the method of **Olayinka, (2023)**.

### **3.4. Chemical analysis**

#### **3.4.1. Proximate composition of hibiscus sabdariffa and curcuma longa**

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 – (moisture + protein + fat + ash)

### **3.5. Experimental design**

After an acclimation period of one week, the rats were divided into two groups. The first group (n= 6 rats) was fed only on the basal diet as a negative control group (ve-) The second major group (42 rats) is randomly divided into seven sub groups (six animals each), as follows: Group (2) the positive control group (ve+) received fed on basal diet. Group (3) received were fed a basal containing hibiscus powder 10 %. Group (4) received were fed a basal diet containing hibiscus powder 20 %. Group (5) received were fed a basal diet containing hibiscus +Curcuma 10%. Group (6) received were fed a basal containing Curcuma powder 10%. Group (7) received were fed a basal containing Curcuma 20%. While Group (8) received were fed a basal diet containing hibiscus +Curcuma 20% (**Erhan, 2020**)

### **3.6. Induction of hepatotoxicity**

After pretreatment for 14 days, in the second major group, were injected by carbon tetrachloride (CCL<sub>4</sub>) as 2 ml/kg body weight (**Ikponmwosa and Eromosele, 2019**).

At the end of the experiment ( 30 days ) , the rats were fasted overnight before sacrificed, the blood samples were

collected from hepatic portal vein for each rat in dry centrifuge tubes without anticoagulant and allowed to clot then, centrifuged at 3000 r.p.m for 10 minutes at room temperature to obtain the serum. Serum was carefully separated and transferred into dry clean tubes by Pasteur pipette and kept frozen at- 20 °C until analysis.

### 3.7. Biological evaluation

During the experiment 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)

$$\text{BWG\%} = [(\text{Final weight g} - \text{Initial weight g}) / (\text{Initial weight g})] \times 100$$

$$\text{FER} = \text{Gain in body weight} / \text{feed intake (g)}$$

$$\text{Relative organs weight ROW \%} = (\text{Organ weight} / \text{Final weight}) \times 100$$

### 3.8. Biochemical analysis

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

#### 3.8.1. Determination of the lipid profile:

Serum cholesterol was determined according to the method described by Allain *et al.*(1974).Serum triglycerides (TG) was determined according to the method described by Fossati and Principe (1982).High density lipoprotein cholesterol (HDL-c) was colorimetrically determined according to the method described by Burstein (1970).

Low density lipoprotein cholesterol (LDL-c) was colorimetrically determined according to the method described by

Friedwald *et al.* (1972). Very low density lipoprotein cholesterol (VLDL-c) was color metrically determined according to the method described by Friedwald *et al.* (1972).

### 3.8.2. 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)**.

### 3.8.3. Determination of serum total protein:

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

### 3.8.4. Determination of the kidney function:

Serum uric acid was determined by **Barham and Trinder, (1972)** using spectrophotometer (model DU 4700) adjusted at 510 nm. Serum urea nitrogen was determined according to the method described by **Batton and Crouch, (1977)** using spectrophotometer (model DU 4700) adjusted at 550 nm. Serum creatinine was determined by **Tietz, (1986)** using spectrophotometer (model DU 4700) adjusted at 510 nm.

### 3.8.5. 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.

## 3.9. 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. The least significant

difference (LSD) value was used to determine significant difference between means. Data was represented as Mean  $\pm$  SD. Values were considered significant at  $P \leq 0.05$ , otherwise were considered non-significant.

## Results and discussion:

### 4.1. Proximate chemical composition of Hibiscus sabdariffa and Curcuma longa leaves powder :

Roselle leaves are leaves of the Malvaceae plant Hibiscus sabdariffa, they are a common culinary ingredient as well as a traditional medicine in many countries including their role in treating various ailments and promoting overall health. These all stem from the bioactive components (anthocyanins, polyphenols, flavonoids, protocatechuic acid, malic acid, ascorbic acid, hibiscus acid contained in it **Great et al., (2023)**).

The data tabulated in table (1) Showed the proximate composition (g/100g) of hibiscus sabdariffa and curcuma longa leaves powder.

It can be noticed that, hibiscus sabdariffa leaves powder contained moisture, protein, fat, fiber, ash and carbohydrate with values (7.32, 24.82, 20.20, 9.88, 2.40 and 33.52 g/100g, respectively). While the values of curcuma longa leave powder were 6.52, 39.5, 2.47, 34.47, 13.81 and 44.74 g/100g, respectively.

These results of chemical composition for hibiscus sabdariffa and curcuma longa leaves powder revealed that carbohydrate recorded the highest average for both, but the content of protein was higher in curcuma longa than hibiscus sabdariffa leaves powder whereas the values of fat were higher in hibiscus sabdariffa than curcuma longa leaves powder.

**Mabrouk et al., (2016)** showed that the proximate chemical composition of indicated was the major component (28.47%) followed by crude protein (25.87%), crude ether extract (21.05%), crude fiber (18.95%) and finally ash (5.66%). Dried

*Hibiscus sabdarifa* calyxes collected from different markets in Uyo, Eastern Nigeria were evaluated for proximate composition revealed that the crude protein ranged from 8.34 – 9.97%, crude fibre (7.26 – 7.82%) and fat (8.51 – 9.26%). The moisture content ranged from 13.13 – 14.85% Adebayo-tayo and Samuel, (2009). It was relatively comparable with that found by **Samy (1980), Al Wandawi et al., (1984), El Adawy and Khalil (1994), Parkouda et al., (2008), Mahmoud et al., (2008), Nzikou et al., (2011), Mariod et al., (2013) and Elneairy (2014)**. The obtained data confirmed the aforementioned results of SEM; RS had a relatively high oil and protein and can be used as a source for edible oil production and meal for animal feeding and /or human use.

There is a slight difference of protein content between fresh and dried turmeric leaves. The protein content in fresh turmeric leaves was higher than that of dried turmeric leaves even though moisture content of both leaves was almost same. -is considered due to the degradation of protein by hot-air drying **Olayinka, (2023)**. focused that on the proximate, minerals and phytochemical composition of turmeric powder extracts moisture, dry matter, protein, fibre, ether extract, ash and carbohydrate content of 5.59, 94.41, 8.73, 7.06, 5.61, 5.06 and 67.95 % respectively. The results showed that the rhizome powder contains appreciable and high qualities crude protein and carbohydrates of 8.73% and 67.95% respectively. The presence of nutrients proves that turmeric powder can be used as food supplement. The result from this study agreed with the report of **Ikpeama et al., (2014)**, who reported that turmeric is an excellent source of carbohydrate and protein.

**Table (1): Proximate composition (g/100g) of *Hibiscus sabdariffa* and *Curcuma longa* leaves powder**

Constituents	Plants	
	<i>Hibiscus sabdariffa</i>	<i>Curcuma longa</i>
Moisture	7.32±0.12c	6.52± 0.21c
Crude Protein	24.82±0.25a	39.5±0.11a
Crude fat	20.20±0.21a	2.47±0.13c



Crude fiber	9.88±0.01b	34.47±0.12a
Total Ash	2.40±0.23c	13.81±0.11b
carbohydrates	33.52±0.12a	44.74±0.12a

\* Data are presented as means ± SDM ( $n=3$ ). a, b, c and d: Means with different letter in the same culom are significantly different ( $P \leq 0.05$ )

#### **4.2. Biological evaluation of Hibiscus sabdariffa and Curcuma longa leaves powder on experimental rat's induced liver injury by Carbon Tetrachloride:**

THE LEAVES OF HIBISCUS WERE WASHED; DRIED UNDER ROOM TEMPERATURE AND POWDERED USING AN ELECTRIC BLENDER ACCORDING TO THE METHOD OF THE EFFECT OF AQUEOUS ETHANOL (1:1) EXTRACT OF THE CALYX OF HIBISCUS ON CARBON TETRACHLORIDE (CCL<sub>4</sub>) INDUCED LIVER DAMAGE WAS INVESTIGATED. ORAL ADMINISTRATION OF THE EXTRACT FOLLOWING A SINGLE CCL<sub>4</sub> DOSE PROMOTED THE HEALING OF OXIDATIVE LIVER DAMAGE AS DETERMINED BY SERUM AMINOTRANSFERASES, ALT, AST, LEVELS AND LIVER THIOBARBITURIC ACID REACTIVE SUBSTANCES LEVELS. IT APPEARED FROM THE STUDY THAT THE EXTRACT OF HIBISCUS SABDARIFFA ENHANCES THE RECOVERY FROM HEPATIC DAMAGE INDUCED BY CCL<sub>4</sub>. DEJAN ET AL., (2019)

The mean values of initial weight, final weight, feed intake, body weight gain %, and feed efficiency ratio (FER) of experimental rats pretreated with hibiscus sabdariffa and curcuma longa leaves powder.

Data in Table (2) showed that, the initial weight of all groups had similar values to that of the control (-) group. The weights ranged from 207g to 209 g., where there was none statistically significant difference among groups.

The positive control group showed a significant ( $P \leq 0.05$ ) decrease in body weight gain %, feed intake, and feed efficiency ratio (FER) compared to the negative control group. In contrast,



all treatment groups pretreated with *Hibiscus sabdariffa* and *Curcuma* powder showed a significant ( $P \leq 0.05$ ) increase in these parameters compared to the positive control group. Notably, the Hibiscus 10% group (100 mg/kg BW) exhibited a significantly higher final weight compared to the positive control group."

Hibiscus aqueous extract is a rich source of anthocyanins. The results of the study implied Hs aqueous extract at 300 mg/kg is the dose which can the most weight reduction effect with no severe haematological and biochemical changes in all experimental animals. Keywords: Hibiscus sabdariffa, obese rats, roselle, aqueous extrac **Maizatul Hasyima et al., (2018)**

**Emmanuel et al., (2015)** revered that the decrease in the body weight of the treated rats in comparison with the control further attests to the antiobesity property of Hibiscus This result agrees with those of **Carvajal-Zarrabai et al., (2009)** who observed a drastic loss of weight among animals treated with various concentrations of H. Sabdariffa extracts. In contrast however, **Olatunji et al., (2005)** observed no significant decrease in the body weight of rats that were chronically treated with 25mg/kg and 50mg/kg body weight of Hibiscus extracts. **Carvajal-Zarrabai et al., (2009)** were of the opinion that such weight decreases might have been as a result of dietary palatability problem when Hibiscus concentration was increased. The loss of appetite in treated animal models due to daily administration of Hibiscus extracts had earlier been reported **Orisakwe et al., (2004)**. Hence, there is every reason to believe that the earlier observation of **Olatunji et al., (2005)** must have been occasioned by the low concentration of H. sabdariffa extract they used.

**Table (2): Mean body weight gain (g) of experimental rats which treated with Hibiscus sabdariffa and Curcuma longa leaves powder**

Groups	Body weight (g) /wk				
	IBW	FBW	FI	FER	BWG/wk %
Control (-ve)	207.33± 7.64a	220± 7.63a	23.3± 3.06a	5.91± 2.26a	29.0± 8.29a
Control (+ve)	208.50± 3.51a	243± 5.51d	17.0± 1.00c	1.69± 0.68c	6.28± 2.57d
Hibiscus 10 %	207± 2.00a	253± 4.58b	23.0± 1.00a	4.44± 0.51ba	22.2± 2.81ba
Hibiscus 20 %	207.33± 3.06a	240± 3.21c	21.6± 2.52ba	3.403± 0.24cba	15.9± 0.92cb
Hibiscus+Curcum 10%	209.67± 1.53a	228± 2.52dc	20.0± 2.00cba	2.106± 0.29cb	9.07± 1.78dc
Curcuma 10%	209.33± 5.69a	230± 9.45dc	22.0± 2.65ba	2.290± 1.77cb	10.32± 7.24dc
Curcuma 20%	209.67± 3.51a	207± 5.00a	17.6± 2.52cb	3.50± 1.87cba	12.6± 4.99dc
Hibiscus +Curcuma 20%	208.33± 2.08a	236± 12.58c	23.3± 3.06a	5.91± 1.44a	29.0± 3.44a

Data are presented as means  $\pm$  SDM ( $n=6$ ). a, b, c and d: Means with different letter among treatments in the same colum are significantly different ( $P \leq 0.05$ ) IBW= Initial body weight; FBW= Final body weight; BWG= Body Weight gain; WK: Week.

### 4.3. Relative organs weight of experimental rats:

Data presented in table (3) Showed the effect of pre-treatment hibiscus sabdariffa and curcuma longa leaves powder, their mix at level 10% and 20% on group rat's induced liver injury by carbon tetrachloride.

Positive control group showed significant increase in relative liver and kidney weight organs ( $3.63 \pm 0.17\%$  and  $0.63 \pm 0.05\%$  respectively) compared to negative control group ( $2.02 \pm 0.11\%$  and  $0.42 \pm 0.01\%$  respectively).

Pre-treatment with hibiscus sabdariffa and curcuma longa leaves powder, their mix at level 10% and 20 % on group rats, significantly decreased the liver weight and kidney weight when compared to positive control group. Interestingly, there was no significant difference of all treated groups for liver and kidney organs weight compared to negative control group.

*Curcuma longa* is a well-known medicinal plant with various health benefits. This study was designed to evaluate the administration of Indonesian *C. longa* maceration for its effect on promoting growth and development of the ovary and uterus before mating in female albino rats. Material and Methods: A total of 15 female Sprague Dawley rats in their dioestrous phase were assigned into three different groups: the Control group (mineral water); the Cur-Low group (mineral water with 1% *C. longa* maceration) and the Cur-High group (mineral water with 5% *C. longa* maceration). The treatments were given for 20 days. Serum concentrations of follicle-stimulating hormone, oestradiol and progesterone were determined. After the sacrifice of the rats, ovary and uterine relative weight, uterine cornua diameter and length, uterine gland diameter (by histology), the number of primary, secondary, tertiary, and Graafian follicles, the number of corpora lutea and vascular endothelial growth factor (VEGF) expression in the ovary were measured.

Uterine vascularisation was also evaluated. Results: Administration of *C. longa* maceration significantly improved the relative weights of the uterus and ovary; uterine cornua diameter, length and vascularisation; uterine gland diameter; and expression of VEGF in the ovary. It also increased the number of tertiary follicles and corpora lutea, albeit not significantly. Follicle-stimulating hormone serum concentrations were lower in the administered rats. Conclusion: Oestradiol and progesterone levels rose with *C. longa* maceration treatment. The maceration improved the reproductive organs of unmated rats and had potential to optimise the uterine environment for supporting pregnancy in order to produce high-quality offspring. **Andriyanto *et al.*, (2023)**

Data presented in table (3) Showed the effect of pre-treatment hibiscus sabdariffa and

**Ronice *et al.*, (2024)** showed that the effect of curcuma longa rhizome powder supplementation on liver weight and fat pad deposition the liver weights were significantly increased ( $p <$

0.05) in high fructose high-fat diet-fed groups ( $3.61 \pm 0.01$  g/100 g of body weight) compared to the base group ( $2.60 \pm 0.03$  g/100 g of body weight) of rats. Curcuma longa rhizome powder supplementation at 5% significantly decreased ( $p < 0.05$ ) the liver weights of high-fructose high-fat diet fed rats ( $3.05 \pm 0.21$  g/100 g of body weight). It appears that the wet weight of peritoneal and mesenteric fat deposits was significantly increased ( $p < 0.05$ ) in high-fructose high-fat diet-fed groups ( $2.18 \pm 0.02$  and  $2.96 \pm 0.03$  g/100 g of body weight, respectively), compared to the base group ( $1.46 \pm 0.06$  and  $1.82 \pm 0.10$  g/100 g of body weight, respectively). However, Curcuma longa rhizome powder supplementation at 10% in high-fructose high-fat diet-fed rats significantly reduced ( $p < 0.05$ ) deposition of peritoneal and mesenteric fat ( $1.35 \pm 0.14$  and  $1.65 \pm 0.02$  g/100 g body weight, respectively), compared to the control group ( $2.18 \pm 0.02$  and  $2.96 \pm 0.03$  g/100 g body weight, respectively).

**Table (3): Mean organ weight/body weight (%) of experimental rats which treated with Hibiscus sabdariffa and Curcuma longa leaves powder**

Groups	Organ's weight (%)	
	liver	kidney
Control (-ve)	$2.02 \pm 0.11$ b	$0.41 \pm 0.01$ b
Control (+ve)	$3.63 \pm 0.17$ a	$0.63 \pm 0.05$ a
Hibiscus 10 %	$2.41 \pm 0.14$ b	$0.49 \pm 0.08$ b
Hibiscus 20 %	$2.23 \pm 0.14$ b	$0.42 \pm 0.02$ b
Hibiscus +Curcuma 10%	$2.11 \pm 0.11$ b	$0.42 \pm 0.01$ b
Curcuma 10%	$2.41 \pm 0.05$ b	$0.47 \pm 0.05$ b
Curcuma 20%	$2.46 \pm 0.12$ b	$0.43 \pm 0.07$ b
Hibiscus +Curcuma 20%	$2.63 \pm 0.17$ a	$0.43 \pm 0.05$ a

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

#### 4.4. Biochemical analysis

##### 4.4.1. Effect of hibiscus sabdariffa and curcuma longa leaves powder on lipid profile of rat's induced liver injury by Carbon Tetrachloride.

Curcumin reduces the accumulation of deposits in the

arteries and has antibacterial properties. Hibiscus is also a beneficial drink for patients with cholesterol, and leads to a reduced risk of cardiovascular disease when consumed (**Dahiru et al., 2003** and **Geum et al., (2017)**).

Data presented in Table (4) , Showed the effect of pretreatment hibiscus sabdariffa and curcuma longa leaves powder, their mix at level 10% and 20% on the serum biochemical parameters (TC, TG, HDL, LDL and VLDL) in rats. In the current study, rats induced with carbon tetrachloride showed a significant increase ( $P \leq 0.05$ ) in total triglycerides (TG), low-density lipoprotein (LDL-c), and total cholesterol (TC), VLDL: very low-density lipoprotein. While HDL-c decreased significantly ( $P \leq 0.05$ ) compared to the positive control group.

On the other hand, the data in the table observed that, TC, TG, LDL, VLDL) below, were decreased significantly ( $P \leq 0.05$ ) in all treatment groups compared with control positive group, but increased significantly ( $P \leq 0.05$ ) in HDL compared with control positive group .The lowest result of the treatment group for TC and HDL were in Hibiscus+Curcuma 10% group, by average ( $202.00 \pm 5$  and  $47.33 \pm 0.81$ mg/dl), while group Curcuma 20%, were lowest in TG and VLDL by average ( $122.33 \pm 0.88$  and  $24.46 \pm 0.18$  mg/dl), and group Hibiscus+Curcuma 20% was in LDL by average ( $128.80 \pm 2.60$  mg/dl) compared with other treatment groups.

Tzu-Li et al., (2007) ,showed that the effects of the extracts of H. sabdariffa on serum cholesterol showed that the extracts of H. sabdariffa are capable of reducing the serum cholesterol levels of treated rats on a dose dependent fashion. This observation on the hypocholesterolemic ability of H. sabdariffa is consistent with several past reports. The consumption of Hibiscus sabdariffa extracts can significantly decrease serum cholesterol levels in human beings. This view was corroborated by the findings of **Lin et al., (2007)** who observed a significant decrease in the serum cholesterol level of treated men and women. Similarly, **Chen et al., (2003)** reported of its anti-atherosclerotic property, while

Mckay, (2010) noted its ability to lower the blood pressure of pre-hypertensive and mildly hypertensive adults.

**Ronice et al., (2024)**, showed that the effect of the curcuma longa rhizome powder supplementation on the lipid profiles of the high-fructose high-fat diet-fed rats, it can be serum triglyceride, total cholesterol, and LDL-cholesterol levels were significantly ( $p < 0.05$ ) increased in rats that consumed the high-fructose high-fat diet ( $185.35 \pm 5.37$  mg/dl,  $208.53 \pm 1.53$  mg/dl, and  $128.73 \pm 3.64$  mg/dl, respectively) compared to the base group rats ( $128.43 \pm 1.11$  mg/dl,  $124.40 \pm 4.63$  mg/dl, and  $39.26 \pm 3.78$  mg/dl, respectively). Curcuma longa rhizome powder supplementation at 5% in high-fructose high-fat diet-fed rats significantly ( $p < 0.05$ ) decreased the serum triglyceride, total cholesterol, and LDL-cholesterol level in high-fructose high-fat diet-fed rats ( $116.41 \pm 2.65$  mg/dl,  $119.94 \pm 3.86$  mg/dl, and  $20.44 \pm 3.61$  mg/dl, respectively) compared to the control group ( $185.35 \pm 5.37$  mg/dl,  $208.53 \pm 1.53$  mg/dl, and  $128.73 \pm 3.64$  mg/dl, respectively).

**Table (4): Lipid Profile (mg/dl) of all experimental rats which treated with Hibiscus sabdariffa and Curcuma longa leaves powder:**

Groups	Parameters				
	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Control (-ve)	$185.33 \pm 0.88f$	$95.66 \pm 1.20e$	$51.62 \pm 1.76a$	$114.53 \pm 0.99d$	$19.13 \pm 0.24e$
Control (+ve)	$238.66 \pm 0.88a$	$154.66 \pm 1.21a$	$35.65 \pm 1.45b$	$172.06 \pm 2.54a$	$30.93 \pm 0.25a$
Curcuma 10%	$208.33 \pm 0.88bc$	$127.66 \pm 1.20bc$	$50.31 \pm 2.72a$	$132.46 \pm 3.27bc$	$25.53 \pm 0.24bc$
Curcuma 20%	$204.33 \pm 0.88de$	$122.33 \pm 0.88d$	$48.32 \pm 0.84a$	$131.53 \pm 1.47bc$	$24.46 \pm 0.18d$
Hibiscus +Curcuma 10%	$204.00 \pm 1.15de$	$122.66 \pm 1.45d$	$50.64 \pm 1.45a$	$128.80 \pm 2.60c$	$24.53 \pm 0.29d$
Hibiscus 10 %	$210.66 \pm 1.76b$	$130.66 \pm 1.76b$	$48.00 \pm 1.52a$	$136.53 \pm 2.07b$	$26.13 \pm 0.35b$
Hibiscus 20 %	$207.00 \pm 1.15cd$	$130.66 \pm 0.88b$	$52.01 \pm 1.60a$	$128.86 \pm 0.75c$	$26.13 \pm 0.17b$
Hibiscus+Curcuma 20%	$202.00 \pm 0.57e$	$126.66 \pm 0.88c$	$47.33 \pm 0.81a$	$129.33 \pm 1.13c$	$25.33 \pm 0.18c$

Data are presented as means  $\pm$  SDM ( $n=6$ ). a, b, c and d: Means with different letter among treatments in the same culom are significantly different ( $P \leq 0.05$ ) TC: Total Cholesterol TG: Triglycerides HDL: High Density Lipoprotein. LDL: Low Density Lipoprotein. VLDL: Very Low Density Lipoprotein.

#### **4.4.2. Effect of hibiscus sabdariffa and curcuma longa leaves powder on liver functions of rat's induced liver injury by Carbon Tetrachloride.**

Curcuma longa has been shown to be a potent anti-inflammatory, antioxidant and anticarcinogenic agent. The present investigation aimed at examining the possible potential protective effect of curcuma against oxytetracyclin-induced fatty liver in an attempt to understand its mechanism of action, which may pave the way for possible therapeutic applications *Eman et al., (2011)*.

Data presented in Table (5) , showed the effect of pretreatment hibiscus sabdariffa and curcuma longa leaves powder, their mix at level 10% and 20% on the serum biochemical parameters (ALT, AST, and ALP) in rats.

As seen in Table (5) and Figure (5) aspartate transaminase (AST) and alanine transaminase (ALT) and alkaline phosphatase (ALP) activities were elevated significantly by carbon tetrachloride administration, the values were ( $38.66 \pm 0.88$ ,  $41.00 \pm 0.56$  and  $117.00 \pm 1.52$ , U/L respectively) compared to negative control ( $19.33 \pm 1.76$ ,  $21.00 \pm 1.73$  and  $86.33 \pm 1.20$  U/L respectively).

Also, there were , Significant ( $P \leq 0.05$ ) reduction was observed in activities of AST, ALT and ALP for all treatment groups pretreated with hibiscus sabdariffa and curcuma longa leaves powder, their mix at level 10% and 20% compared to positive control group.

**Emmanuel et al., (2015)** investigated that the effect of Hibiscus sabdariffa calyx aqueous extracts on the serum cholesterol, body weight and liver marker enzymes activities of normal albino rats. The aqueous extract was orally administered (100 – 800 mg/kg body weight) for 28 days to normal male albino



rats. Total cholesterol, body weight, aspartate aminotransferase (AST), alkaline phosphatase (ALP) and alanine aminotransferase (ALT) levels were measured. Hibiscus sabdariffa administration significantly reduced serum cholesterol and body weight in a dose and duration dependent pattern. AST, ALP and ALT levels were significantly elevated in a dose and duration dependent pattern. The significant increase in the levels of the liver enzymes tends to suggest dysfunction in the coordinating of the liver activity. The extract ability to lower the total cholesterol level and body weight suggests its usefulness as a hypocholesterolemic and anti-obesity agent. **Prommetta *et al.*, (2006)** observed that doses ranging from 250 – 1000 mg/kg/day, did not elicit any adverse effect on several important organs such as liver, kidney and the blood system. Similarly, reports abound on the hepato-protective effects of H. sabdariffa extracts **Farombi (2003)** as well as chemo-preventive and anti-oxidative effects **Usuh *et al.*, (2005)**. In an attempt to explain the observed increases in the activities of these marker enzymes following the administration of Hibiscus sabdariffa extracts, a unique adaptation by the liver to the assault from the plant extract or as a result of fresh synthesis of the enzyme molecules following extract administration. It seems also plausible that the effect of the extracts on the activities of Aspartate and Alanine Aminotransferase may be the case of organ chain reactions **Orisakwe *et al.*, (2004)**.

**Ronice *et al.*, (2024)** showed that the result may be due to the presence of phytochemical compounds in the Curcuma longa rhizome powder that inhibited the digestive enzymes in a consequence of the utilization of fats stored in adipose tissue for the production of energy necessary for the functioning of the organism. This leads to the reduction of peritoneal and mesenteric fats as well as the weight of the liver. These results are in line with those of **Manikandan *et al.*, (2013)**, the hepatic damage was evaluated by measuring the activities of the liver enzyme markers, Serum ALT and AST increase when hepatic damage occurs and the activities of these enzymes were measured in all groups.



**Akiyama *et al.*, (1996)** study showed that the serum AST and ALT levels increased in high-fructose high-fat diet-fed rats compared to the base group. Hepatocyte damage caused by the consumption of a high-fructose high-fat diet is evident in most experimental models and patients with clinical conditions **Timbrell *et al.*, (1996)**, reverred that the enzymes are considered markers of hepatic dysfunction. Generally, hepatocyte damage causes these enzymes to be transported in the serum. Induction of metabolic disorder results in the beginning of oxidative stress in several tissues, including the liver. This leads to the peroxidation of membrane lipids, altering hepatic lipid prolle, which consequently cause cellular damage and membrane rupture, with the release of AST and ALT into the bloodstream **Erukainure *et al.*, (2013)** and **Njapndounke *et al.*, (2021)**.

**Table (5): Liver enzymes of all experimental rats which treated with *Hibiscus sabdariffa* and *Curcuma longa* leaves powder**

Groups	Parameters		
	AST(U/L)	ALT(U/L)	ALP (U/L)
Control (-ve)	19.33± 1.76e	21.00± 1.73f	86.33± 1.20e
Control (+ve)	38.66± 0.88a	41.00± 0.56a	117.00± 1.52a
Curcuma 10%	32.66± 1.45b	36.00± 1.15b	107.66± 0.88c
Curcuma 20%	24.66± 1.30d	26.66± 1.20e	102.66± 0.91d
Hibiscus +Curcuma 10%	27.33± 1.20d	28.66± 0.88de	104.66± 1.45cd
Hibiscus 10 %	34.00± 0.60b	37.00± 1.15b	112.66± 1.45b
Hibiscus 20 %	31.00± 0.57bc	32.33± 1.20c	108.00± 1.52c
Hibiscus +Curcuma 20%	28.00± 0.59cd	30.33± 0.89cd	105.66± 0.89cd

Data are presented as means ± SDM ( $n=6$ ). a, b, c and d: Means with different letter among treatments in the same culom are significantly different ( $P \leq 0.05$ ) AST: aspartate amino transferase ALT: alanine amino transferas ALP: alkaline phosphatase.

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#### **4.4.3. Effect of hibiscus sabdariffa and curcuma longa leaves powder on kidney functions of rat's induced liver injury by Carbon Tetrachloride.**

renal system consists of the kidney, ureters, and the urethra. The overall function of the system filters approximately 200 liters of fluid a day from renal blood flow which allows for toxins, metabolic waste products, and excess ion to be excreted while keeping essential substances in the blood. The kidney regulates plasma osmolarity by modulating the amount of water, solutes, and electrolytes in the blood. It ensures long term acid-base balance and also produces erythropoietin which stimulates the production of red blood cell. It also produces renin for blood pressure regulation and carries out the conversion of vitamin D to its active form. The renal development, the process of urine production and excretion, and the clinical significance of the renal system will be the focus. *Ifeanyichukwu et al., (2023)*.

Data presented in table (6), Showed the effect of hibiscus sabdariffa and curcuma longa leaves powder, their mix at level 10% and 20% on group rats induced liver injury by carbon tetrachloride. The serum urea, uric acid and creatinine were elevated significantly by carbon tetrachloride administration, the values were ( $43.66 \pm 1.76$ ,  $6.22 \pm 0.14$  and  $1.76 \pm 0.07$ mg/dl , respectively) compared to negative control group ( $25.00 \pm 1.73$ ,  $3.57 \pm 0.05$  and  $1.04 \pm 0.03$ mg/dl, respectively). While, there was significant ( $P \leq 0.05$ ) decrease in serum urea, uric acid and creatinine for all treatment groups pretreated with hibiscus sabdariffa and curcuma longa leaves powder, their mix at level 10% and 20% compared to positive control group

**Table (6): Kidney function (mg/dl) of experimental rats which treated with Hibiscus sabdariffa and Curcuma longa leaves powder**

Groups	Parameters		
	Urea (mg/dl)	Uric Acid (mg/dl)	Creatinine (mg/dl)
Control (-ve)	25.00± 1.73e	3.57± 0.05f	1.04± 0.034d
Control (+ve)	43.66± 1.76a	6.22± 0.14a	1.76± 0.074a
Curcuma 10%	33.00± 1.73bc	5.21± 0.04cd	1.12± 0.023cd
Curcuma 20%	31.00± 1.52bcd	5.12± 0.09cde	1.09± 0.017cd
Hibiscus +Curcuma 10%	29.00± 1.15cde	5.37± 0.02bc	1.14± 0.012bcd
Hibiscus 10 %	34.66± 1.76b	5.59± 0.11b	1.23± 0.017b
Hibiscus 20 %	34.66± 0.88b	5.01± 0.09de	1.16± 0.014bc
Hibiscus +Curcuma 20%	26.66± 0.88de	4.86± 0.02e	1.12± 0.011cd

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

#### **4.4.4. Effect of hibiscus sabdariffa and curcuma longa leaves powder on oxidative stress of rat's induced liver injury by Carbon Tetrachloride:**

As seen in Table (7), Serum total antioxidant capacity TAC activities were decreased significantly by carbon tetrachloride administration, while, there was significant elevated in serum malondialdehyde MDA compared to negative control group.

The results showed enhancement for all treatment groups pretreated with hibiscus sabdariffa and curcuma longa leaves powder, their mix at level 10% and 20%, back to significant increase in serum TAC activities and significant decreased in serum malondialdehyde MDA compared to positive control.

The effect of aqueous ethanol (1:1) extract of the calyx of Hibiscus sabdariffa on carbon tetrachloride (CCl<sub>4</sub>) induced liver damage was investigated. Oral administration of the extract

following a single CCl<sub>4</sub> dose promoted the healing of oxidative liver damage as determined by serum aminotransferases, ALT, AST. **Dahiru et al., (2003)**

**Ronice et al., (2024)** showed that the effect of curcuma longa rhizome powder supplementation on serum and liver levels of oxidative stress markers in high-fructose high-fat diet-fed rats. It can be observed that serum and liver levels of malondialdehyde (MDA) and nitric oxide (NO) were significantly ( $p < 0.05$ ) increased in high-fructose high-fat diet fed rats (MDA:  $67.54 \pm 2.48$  nmol/ml and  $142.54 \pm 6.15$  nmol/mg and NO:  $27.83 \pm 1.44$  nmol/ml and  $83.92 \pm 3.77$  nmol/mg, respectively) compared to the base group rats (MDA:  $36.02 \pm 1.90$  nmol/ml and  $83.28 \pm 5.30$  nmol/mg and NO:  $16.94 \pm 2.16$  nmol/ml and  $25.81 \pm 4.69$  nmol/mg, respectively). Curcuma longa rhizome powder supplementation at 10% significantly decreased ( $p < 0.05$ ) the MDA and NO levels in the serum and liver in high-fructose high-fat diet-fed rats (MDA:  $39.75 \pm 1.02$  nmol/ml and  $92.71 \pm 2.98$  nmol/mg and NO:  $19.36 \pm 1.28$  nmol/ml and  $37.22 \pm 4.34$  nmol/mg, respectively) compared to the control group rats (MDA:  $67.54 \pm 2.48$  nmol/ml and  $142.54 \pm 6.15$  nmol/mg and NO:  $27.83 \pm 1.44$  nmol/ml and  $83.92 \pm 3.77$  nmol/mg, respectively).

Metabolic disorders have become a major and growing global health problem, so finding potentially novel solutions with fewer harm is favourable to solving this problem. Thus, this research aimed to determine the effect of a diet supplemented with Curcuma longa rhizome powder on markers of oxidative stress as well as biochemical and haematological parameters of rats with diet-induced metabolic disorders. **Dangang et al., (2024).**

**Table (7): TAC and MDA of experimental rats which treated with Hibiscus sabdariffa and Curcuma longa leaves powder**

Groups	Parameters	
	TAC (u/ml)	MDA (nmol/ml)
Control (-ve)	10.53± 0.69c	76.61± 1.45b
Control (+ve)	0.633± 0.06d	117.01± 2.08a
Curcuma 10%	14.01± 0.08a	55.22± 0.57cd
Curcuma 20%	14.38± 0.03a	50.12± 0.59e
Hibiscus +Curcuma 10%	14.56± 0.09a	54.62± 1.45cd
Hibiscus 10 %	12.69± 0.13b	57.33± 0.88c
Hibiscus 20 %	14.08± 0.12a	52.31± 0.81de
Hibiscus +Curcuma 20%	14.81± 0.02a	52.63± 0.84de

Data are presented as means ± SDM (n=6). a, b, c and d: Means with different letter among treatments in the same culom are significantly different ( $P \leq 0.05$ ) TAC: total antioxidant capacity MAD: Malondialdehyde.

## Conclusion:

From this study it is concluded that, Feeding rats on hibiscus and Curcuma had the ability to reduce high levels of uric acid, urea beside improved also the lipid profile and the liver enzymes, MDA and TAC enzymes in rats induced of carbon tetrachloride, and the ratios used for them did not result in any negative effects

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