Potential Effects of Saussurea costus and Withania somnifera Roots on Liver Disorders in Rats

التأثيرات المحتملة لجذور القسط الهندي والأشواجاندا على اضطرابات الكبد في الفئران

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Abstract: Liver diseases represent a significant global health problem due to the liver's crucial role in detoxification and metabolism of compounds that generate free radicals (FR). Consequently, liver toxicity can be minimized by promoting factors like antioxidants. *S. costus* and *W. somnifera* (Ashwagandha) roots are among the most medicinal plants with high antioxidant content. Therefore, the present study was designed to investigate the probable effect of *Saussurea costus* (SCR) and Ashwagandha roots (AR) on rats suffering from liver diseases. Forty male albino rats (Sprague Dawley Strain) weighing (150±10g) were divided into two main groups. The first main group (5 rats) was fed on a basal diet as a control negative group. The second main group was treated with CCl4 to induce acute damage in the liver, then it was divided into seven subgroups (5 rats each). The first subgroup fed on basal diet as a positive control group. The second and third subgroups fed on basal diet containing 2.5% and 5% SCR, respectively. The fourth and fifth subgroups fed on a basal diet containing 2.5 % and 5% AR, respectively). The sixth subgroup fed on a basal diet containing 2.5% (SCR+AR). The seventh subgroup fed on a basal diet containing 5% (SCR+AR). The results indicated significant improvements in nutritional, chemical and biological parameters in groups of rats suffering from liver disease when treated with different levels of *Saussurea Costus* (SCR) and ashwagandha roots and their combination improved the body weight, organs weight/body weight, kidney functions (uric acid, urea nitrogen, and creatinine), lipid profile (cholesterol, triglycerides, HDL-c, LDL-c and VLDL-c), liver function tests (AST, ALT, ALP, Bilirubin,, LDH, GGT, and AFP), oxidative enzymes activity (GPX, SOD and CAT); when compared to the positive control group. The most pronounced improvements were in the two treated groups that received the combination of (SCR+ AR) and the best one of them was the high ratio of their combination, 5%. The histological examination of the liver confirmed a gradual improvement in all treated groups. Sensory evaluation of pan bread showed that all samples obtained a higher than 75% in overall acceptability. In conclusion, in addition to their many health advantages, *Saussurea Costus* and Ashwagandha roots have potential hepatoprotective properties and may be used in regular drinks and other nutritional applications to treat liver problems.

Key words: *Saussurea costus* Roots, Ashwagandha Roots, liver functions, serum lipid profile, kidney function, antioxidant enzymes.

Introduction

Liver is one of the most important and largest internal organs in the human body **(Reddy and Apparao, 2024)**; it has a wide range of functions, including protein synthesis, the production of biochemicals necessary for digestion and detoxification **(Zhang *et al*., 2014).** The liver also plays a vital role in maintaining health and at the same time is highly susceptible to disease and injury **(Cubero and Nieto, 2006 and Ajith *et al*., 2007)**. Liver injury and disease threaten human health and quality of life **(Xu *et al*., 2021).** Liver diseases are a major cause of illness and death worldwide **(Cubero and Nieto, 2006 and Ajith *et al*., 2007).** According to **WHO (2018)** Egypt ranks first in the world in liver disease deaths (68,866, representing 12.40% of total deaths) **(El Gizawy *et al*., 2022)**. In addition, the liver is the most vulnerable organ attacked by chemical toxic agents **(Bhondave *et al*., 2014 and Meng *et al*., 2019).** CCl4 is one of the hepatotoxins widely used to induce acute and chronic toxic liver injury in a wide range of laboratory animals **(Kaneko *et al*., 2013; Ma *et al*., 2014; Li *et al*., 2019; Sachan and Singh, 2023).** Numerous studies have revealed that CCl4-induced liver injury is mainly attributed to the highly reactive free radical (CCl3• and CCl3O2) of CCl4 metabolized by cytochrome P450 **(Al-Harbi *et al*., 2014).** The accumulated free radicals can lead to oxidative stress and activate liver macrophages to secret inflammatory cytokines, thereby causing liver injury **(Zhao *et al*., 2019).** So, it is imperative to protect the liver and prevent liver injury which may influence liver microenvironment and further drive severe liver diseases **(Wang *et al*., 2019)**. Recovering liver function or ameliorating the severity of liver pathologies is crucial to improving the health and life expectancy of patients with such diseases **(Luangmonkong *et al*., 2018).**

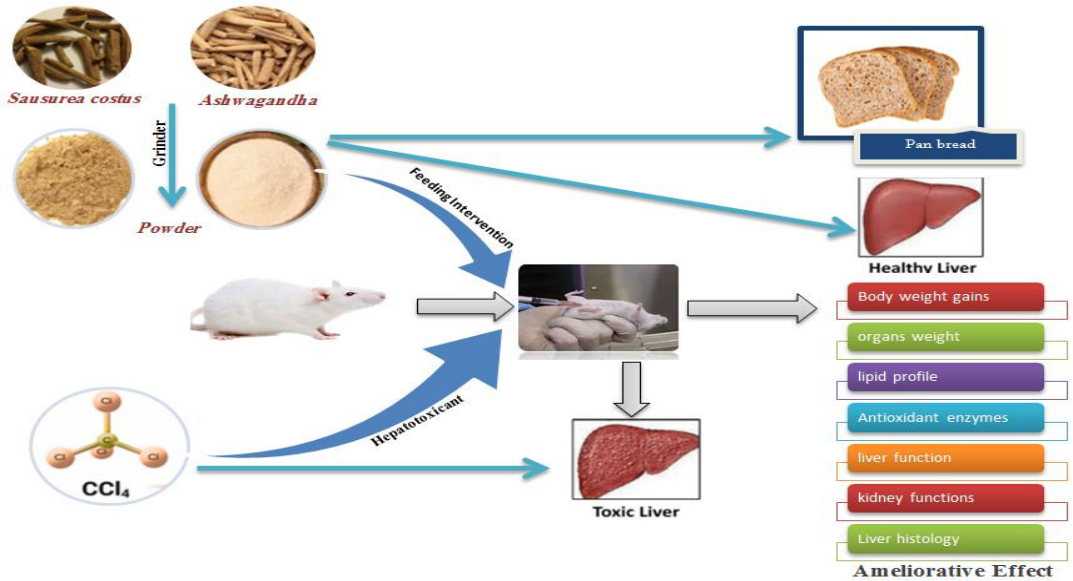

Fig (1): Graphical summary showing the effect of *S. costus* and *W. somnifera* roots on rats suffering from liver disorders.

Treatments for liver disease are often viewed with suspicion, and many patients often seek alternative therapies for their liver disorders **(Saab *et al.*, 2014)**. Plants act as a natural source of drugs and are considered to be an alternative to modern medicine, especially in developing countries for various reasons, including their lack of side effects or the presence of only mild ones, ease of availability, and low cost **(Abdallah *et al*. 2017and Nadda *et al.*, 2020).** In recent years, Indian Ayurveda gained more attention and popularity because of its safety and efficacy in treating liver and its associated diseases **(Afzal *et al.*, 2013 and Sharma *et al.*, 2021).** *Saussurea costus* (syn. *Saussurea lappa*, *Asteraceae*) is considered one of the most important traditional Chinese medicinal plants **(Ahmed *et al.*, 2016)**. It is well known in Islamic medicine **(Deabes *et al.*, 2021)**. The therapeutic value of *S. costus* lies in some active biochemical constituents that produce different physiological effects on the body. These biochemically active substances mainly include flavonoids, alkaloids, sesquiterpenes, phenolic compounds, tannins, carbohydrates, and glycosides **(Abdelwahab *et al*. 2019)**. Furthermore, costunolide, dehydrocostus lactone, and cynaropicrinin are the major constituents of the *S. costus* roots **(Elshaer *et al.*, 2021and Rathore *et al.*, 2021).** These compounds possess antioxidant, antimicrobial, anti-inflammatory, anti-cancer, anti-ulcer, antidiabetic and hepatoprotective properties **(Al Otibi *et al.*, 2020 and Behera *et al.*, 2021).**

Ashwagandha (*Withania somnifera*, family *Solanaceae*) is one of the most important herbs of the Ayurveda system of medicine in India. It is utilized widely as possessing different health useful **(Singh *et al.*, 2011 and Azab *et al.*, 2022)**. The roots of Ashwagandha have several bioactive chemical compounds, such as flavonoids, N-containing compounds, steroidal lactones, saponins, tannin and alkaloids **(Lopresti *et al.*, 2019; Paul *et al.*, 2021; Nile *et al.*, 2022 and Ramli *et al.*, 2023).** The major chemical components of Ashwagandha roots are steroidal lactones and alkaloids **(Jayaprakasam *et al.*, 2003)**. Withanine is the primary constituent of the numerous alkaloids. Other alkaloids include somniferine, msomnine, somniferinine, withananine, pseudo-withanine, tropine, pseudo-tropine, 3-a-gloyloxytropine, choline, cuscohygrine, isopelletierine, anaferine, and anahydrine **(Chen *et al.*, 2011and Bharti *et al.*, 2016)**. It has proven that, among others, ashwagandha has anti-stress, anti-inflammatory, antimicrobial, anti-cancer, anti-diabetic, anti-obesity, cardioprotective, hypolipidemic properties and antioxidant. **(Wiciński *et al.*, 2023).** It may have free radical scavenging activity and thereby can be used for the prevention and treatment of liver damage **(Sultana *et al.*, 2012)**. Ashwagandha roots were found to be devoid of any toxic effect in acute and sub-acute toxicity studies **(Durg *et al.*, 2020).** Extracts didn't cause any mortality, nor showed any changes in normal behavior in rats **(Mukherjee *et al.*, 2020).** Considering all the reasons mentioned above, incorporating these herbs into food products will be extremely beneficial to its users. Therefore, the current study was conducted to evaluate the potential effects of *Saussurea Costus* (SCR) and Ashwagandha roots (AR) on rats suffering from liver diseases

Material and Methods:

Materials:

- Ashwagandha roots was purchased from local market of medicinal plants in Cairo, Egypt, and was scientifically identified by Institute of Food Technology, Giza, Egypt.
- *Saussurea costus* (*S. costus*) was purchased from Medicinal and Aromatic Plant Research Department, Horticulture Research Institute, Agriculture Research Center (Giza, Egypt).
- Casein, vitamins, minerals, choline bitartrate, cellulose, Carbon tetrachloride (CCl4) and paraffin oil were obtained from ElGomhoria Company for Trading Drugs, Chemicals and Medical instruments, Cairo, Egypt
- All ingredients used in pan bread formulation (wheat flour, dry yeast, salt, sugar, skim milk powder, corn oil and starch) were obtained from the local market from Damietta Governorate, Egypt.

Animals: Forty adult male albino rats weighting (150 ± 10g) were obtained from a laboratory animal colony, Ministry of Health and Population, Helwan, Cairo, Egypt.

Chemicals: All chemicals, reagents and solvents were of analytical grade and purchased from Al-Gomhoryia Company for Trading Drugs, Chemicals and Medical Instruments, Cairo, Egypt.

Methods:

Preparation of *S. costus* and *W. somnifera* roots

Roots were sorted to remove foreign materials, cleaned thoroughly, and ground using an electric grinder into a fine powder that could pass through a 20-mesh sieve. The powder was then packaged in polyethylene bags and stored in a refrigerator at 4±1°C until used in biological experiments.

Preparation of Pan bread

Regarding Pan bread, it was prepared according to a standard formulation using *Saussurea costus*, Ashwagandha roots powder with two different levels and their mixture . Data in **Table (1)** shows the ingredients utilized in the Pan bread. Overall, Pan bread prepared by straight dough method as described in **A.A.C.C (2002)** as follows:

Ingredients were mixed for 4 minutes at slow speed (30 r.p.m) and for an additional 6 minutes at a fast speed (60rpm) The resulting dough was let to rest for 20 min at 28- 30 ˚C (first proofing) then divided, rolled and molded automatically in a molding machine. Each piece was put in baking molds and let to ferment for 60 min at 36 ˚C (final proofing) then the baking process was carried out in electrical oven at 210-220 ˚C for 15-20 min. After baking, bread allows it to cool at room temperature. Pan bread samples then cut them into slices by using an electric knife before.

Table (1): Formulas composition of pan bread

Ingredients (g)	Control	Pan Bread Formula (g)					
		2.5% SCR	5% SCR	2.5% AR	5% AR	2.5% (SCR+AR)	5% (SCR+AR)
Wheat flour	200g	195g	190g	195g	190g	195g	190g
SCR	.....	5g	10g	……	…..	2.5g	5g
AR	….	.....	……	5g	10g	2.5g	5g
dry yeast	5g	5g	5g	5g	5g	5g	5g
Sugar	10g	10g	10g	10g	10g	10g	10g
Salt	2g	2g	2g	2g	2g	2g	2g
corn oil	10g	10g	10g	10g	10g	10g	10g
Water	110g	110g	110g	110g	110g	110g	110g
skim milk powder	4g	4g	4g	4g	4g	4g	4g

SCR: *Saussurea costus* roots, **AR:** Ashwagandha roots

Sensory evaluation

Sensory evaluation was performed by invited eleven staff panelists from Home Economic Department, Faculty of Specific Education, Damietta University, Damietta, Egypt. Each panelist was asked to evaluate Pan bread (7 samples) according to color, odor, Texture, Taste, and general acceptability. The evaluation was carried out according to the method of **(Abd El – latif, 1990)**.

Biological experiments design

The biological studies were carried out in accordance with the National Research Council's Institute of Laboratory Animal Resources, Commission on Life Sciences regulations **(NRC, 2011).** Forty healthy adult male albino rats Sprague Dawley Strain, each weighing 150±10g. The rats were preserved in individual stainless-steel cages under healthy conditions and fed (one week) on a basal diet for adaptation. The basal diet was prepared according to the following formula as mentioned by **Reeves *et al*., (1993)**. The used vitamins mixture component was recommended by **Campbell, (1963),** while the salts mixture used was formulated according to **Hegested *et al*., (1941).** After that, the experimental animals were divided into two main groups. The first main group (5 rats) was fed on basal diet, as a control negative group, while the second main group consisted of (35 rats) treated with CCl4 in paraffin oil (50% v/v 4ml/kg) by single subcutaneous injection to induce acute damage in the liver **(Jayasekhar *et al*., 1997)**. Rats in the second main group were divided into 7 subgroups (n= 5) rats as follows:

- *Subgroup (1):* Fed on basal diet as positive control group
- *Subgroup (2)*: Fed on basal diet containing 2.5% SCR
- *Subgroup (3)*: Fed on basal diet containing 5% SCR
- *Subgroup (4)*: Fed on basal diet containing 2.5% AR
- *Subgroup (5)*: Fed on basal diet containing 5% AR
- *Subgroup (6)*: Fed on basal diet containing 2.5% (SCR+ AR)
- *Subgroup (7)*: Fed on basal diet containing 5% (SCR+ AR)

At the end of the experiments, all rats were fasted up to 12 hours and then sacrificed. Blood samples were collected from the aorta. The blood samples were centrifuged, separated, and stored frozen at -20°C until further analysis.

Biochemical analysis of serum

Serum total cholesterol, TG and HDL-C were determined according to the method described by **(Allain *et al*., 1974)**, **(Fossati and Principe, 1982)** and (**Burstein, 1970)**, respectively. Serum LDL-C and VLDL-C were determined according to the method described by **(FriedWald *et al*., 1972)**. Serum uric acid, urea nitrogen and Creatinine were determined by **(Fossati *et al*., 1980)**, (**Patton and Crouch, 1977)** and (**Bohmer, 1971)**, respectively. Aspartate amine transaminase (AST), Alanine amine transaminase (ALT) and alkaline phosphatase (ALP) activities were measured according to the method described by **(Reitman and Frankel, 1957) & (Bergmeyer and Brent, 1974**), respectively. Bilirubin **(Oser, 1965),** Lactate Dehydro-genase (LDH) **(Howell and Coll, 1979)**, ´Y-Glutamyl transferase (GGT) **(Rosalki, 1975)** and Alpha fetoprotein (AFP) were determined according to the method described by **(Mizejewski, 2001).** The activity of the antioxidant enzymes, Catalase (CAT) was measured according to the method of **Aebi *et al*., (1995).** Superoxide dismutase (SOD) was measured by **Janknegt *et al*., (2007).** Glutathione Peroxidase (GPX) was measured by **Paglia and Valentine, (1967).**

Histopathological Examination

Specimens from liver tissues were taken immediately after sacrificing an animal and fixed in 10% buffered neutral formalin solution. The fixed specimens were then trimmed, washed and dehydrated imbedded in paraffin, cut into sections of 46 microns thickness and stained with haematoxylin and eosin stain, according to **(Sheehan and Hrapechak, 1980).**

Statistical Analysis

The data in the current study were statistically analyzed by SPSS computer software SPSS 2000. The results were expressed as mean ± standard deviation "SD" and tested for significance using a one-way analysis of variance "ANOVA" test, according to **Armitage and Berry, (1987)**.

Results and Discussion:

Nutritional Parameters

Data in Table (2) showed a significant decline ($p \leq 0.05$) of body weight gain (BWG) and feed intake (FI) in positive control group, in comparison with negative control group and all treated groups. Regarding feed efficiency ratio (FER), it was found from data of the same table that, non-significant differences in all rats poisoned by CCL4 (positive control group) and treating groups fed on S. costus, Ashwagandha roots at both doses and their combination when compared to the control negative group. These results were in line with those found by **Hamzawy *et al*., (2013), Sarfo-Antwi *et al*., (2018)** and **Abd El-Rahman, (2021)** who showed that body weight and feed intake were decreased in rats that received CCl4. Also, the study stated by **El-Hashash *et al*., (2020)** concluded that injection of CCl4 is linked to reduced nutrient metabolism and absorption due to poor bile secretion as well as hunger loss

that results in weight loss. On the other side, **Jeyanthi and Subramanian, (2009)** found that the diets fortified with W. somnifera at different levels dose-dependently increased the body weight. As for results brought about by **Ali and Sabry (2023)** there was a substantial decline in BWG, FI, and FER for the positive group as contrasted with the negative control group, but feeding with S. costus roots powder at 2.5% and 5% showed significant increases in values of BWG%; FI and FER contrasted with the positive control group. The investigation documented that providing hepatotoxicity S. costus root powder at 2.5% and 5% in the curative and protective groups increased body weight gain, feed intake, and feed efficiency ratio on the hepatotoxicity of male albino rats.

The changes in the total body and relative liver weights are simple and efficient indicators for studying the pathological and toxic effects of diseases and chemicals on the liver **(Del Genio *et al.*, 2009).** The same Table (2) revealed that the relative weight of some internal organs (liver and kidney) decreased significantly in the CCl4-treated group compared to the normal group. Moreover, rats treated with SCR, AR, and their combination after receiving CCl4 showed a significant elevation in relative liver weight compared to those who received only CCl4. The data also showed that differences between all treatment groups in liver weight indices were minimal. However, no statistically significant differences were observed in all groups receiving *Saussurea costus*, Ashwagandha roots, and their combination among all treatment groups except the 2.5% SCR-treated group in relative kidney weight. These results are consistent with those obtained by **Elzamzamy and Elkewawy (2021)** who showed that the differences between all groups in both food utilization and liver weight index were insignificant. Also, **Tejaswi *et al.*, (2018)** found that the *S. costus* causes a significant reduction in the weight of the liver compared to toxicant groups. Besides, **Ichikawa *et al.*, (2006)** found earlier that the Ashwagandha roots contain withanolides, which have anti-inflammatory properties and thus may help protect against liver damage and can decrease its weight.

Table 2: Effect of *S. costus* and Ashwagandha Roots and their mixture on Nutritional Parameters of rats with Liver Diseases

Groups	Parameters				
	B.W.G%	Feed Intake g/day/rat	FER	Liver Weight/ Body Weight%	Kidney Weight/ Body Weight%
Control (-)	35.060 ^{a} ±5.56	40	139.086 ^{a} ±29.04	3.000^{a} ±0.25	0.900^{a} ±0.00
Control (+)	20.780 ^{b} ±2.09	23	148.003 ^{a} ±17.66	2.000^{c} ±0.14	0.500^{d} ±0.00
2.5%SCR	29.120 ^{a} ±3.44	37	128.715 ^{a} ±15.49	2.360^{bc} ±0.31	0.600^{c} ±0.00
5%SCR	28.520 ^{a} ±7.61	37	122.732 ^{a} ±30.91	2.720^{ab} ±0.34	0.800^{b} ±0.00
2.5%AR	32.180 ^{a} ±2.54	37	137.614 ^{a} ±5.07	2.640^{ab} ±0.15	0.800^{b} ±0.00
5%AR	28.080 ^{a} ±6.64	37	119.754 ^{a} ±28.75	2.800^{a} ±0.18	0.800^{b} ±0.00
2.5%(SCR+AR)	31.480 ^{a} ±3.14	40	123.014 ^{a} ±11.66	2.867^{a} ±0.30	0.800^{b} ±0.00
5% (SCR+ AR)	32.920 ^{a} ±5.02	40	124.513 ^{a} ±15.93	2.925^{a} ±0.49	0.800^{b} ±0.00

*(**SCR**) Saussurea costus roots, (**AR**) Ashwagandha roots, (**B.W.G**) body weight gain.*

Means under same column have the different letters are significant different at p≤0.05.

Liver Enzymes Activity

Alkaline phosphatase (ALP) and transaminases (AST and ALT), which measure the liver's functional state and hence signify hepatocyte damage, significantly increased when mice were treated with the hepatotoxic toxin carbon tetrachloride **(Perveen *et al*., 2018 and Afzal *et al*., 2013).** The data presented in Table (3) illustrates the effect of Saussurea costus, ashwagandha roots, and their combination on liver enzymes (ALT, AST, and ALP) in rats. Results indicated that CCL4 resulted in a significant increase ($P \leq 0.05$) in serum ALT, AST, and ALP levels relative to the control negative group (152.260, 275.360, and 558.260 vs. 14.500, 49.740, and 128.960, respectively). Results observed that *S. costus*, Ashwagandha roots, and their combination considerably reduced the level of the elevated enzyme markers in all treated groups, especially with the high mixture (5%) compared with a positive control group. These results are harmonized by those published by **Arafa (2021)** who found that the mean values (u/l) of serum AST and ALT were significantly lower in experimental groups treated with Ashwagandha roots as compared to the positive control and reduced the pathological damage caused to the liver. In addition, the study by **Altay *et al*., (2019)** who administered ashwagandha at a dose of 500 mg/kg for 6 weeks to rats in which liver fibrosis induced by CCl4 had been induced, showed a significant decrease in liver enzymes. Also, **Sultana *et al*., (2012)** reported that there were significantly lower levels of serum AST and ALT which come to almost normal levels in the Ashwagandha pretreated and gentamicin-treated rats. On the other hand, several authors reported that oral administration of *S. costus* reduced lipid peroxidation and improved liver function markers because of the wide range of phenolic components and natural products observed in it **(Ahmed, 2017; Alnahdi *et al*., 2017and Tejaswi *et al*., 2018)**.

Table 3: Effect of *S. costus*, Ashwagandha Roots and their mixture on Liver Enzymes of rats with Liver Diseases.

Groups	Parameters		
	Alt	AST	ALP
	(U/l)		
Control (-)	14.500^{f} ±1.28	49.740^{g} ± 3.75	128.960^{f} ±3.57
Control (+)	152.260^{a} ±10.89	275.360^{a} ± 19.98	558.260^{a} ±32.67
2.5%SCR	147.680^{ab} ±13.93	247.880^{b} ± 13.13	519.780^{b} ±13.03
5%SCR	95.300^{c} ± 4.19	195.240^{d} ± 11.79	376.200^{c} ±14.62
2.5%AR	139.160^{b} ± 9.42	211.280^{c} ± 11.02	512.840^{b} ±26.96
5%AR	68.660^{d} ± 4.65	173.220^{e} ± 11.62	347.240^{d} ±18.38
2.5% (SCR+AR)	74.380^{d} ± 4.38	173.560^{e} ± 13.05	267.000^{e} ±17.28
5% (SCR+ AR)	54.080^{e} ± 6.40	154.060^{f} ± 7.10	262.240^{e} ±29.41

*(**SCR**) Saussurea costus roots, (**AR**) Ashwagandha roots.* ***Means under same column have the different letters are significant different at p≤0.05.***

Serum Liver Functions tests

The injection of CCl4 was observed to cause liver cirrhosis in rats and resulted in elevated levels of the enzymes gamma-glutamyl transpeptidase, alanine aminotransferase, and bilirubin **(Gutierrez *et al*., 2010)**. The finding in Table (4) presented the effect of SCR, AR and their combination on serum liver function tests of rats suffering from liver diseases. The mean values of serum Bilirubin (0.940±0.05), Lactate Dehydrogenase (317.240±9.84), α-Fetoprotein (16.240±2.12) and ΄Y-Glutamyl transferase (35.240±3.60) increased significantly ($p \leq 0.05$) for the C+ group, as compared to the C- group. It could be observed that injected rats that were suffering from liver diseases and received basal diets with two different concentrations of SCR, AR and their combination had a significant decrease ($p \leq 0.05$) in serum Bilirubin, Lactate Dehydrogenase, α-Fetoprotein and-Glutamyl transferase, as compared to the PC group. In particular, the greatest results for these parameters were obtained with a 5% mixture treated with CCl4. This study is in line with the findings of those who revealed that **Elberry *et al*., (2010)** observed that Ashwagandha had significant antihepatotoxic against the carbon tetrachloride (CCl4) effect by reducing the levels of AST, ALT, and lactate dehydrogenase (LDH). This effect may be attributed, at least in part, to the antioxidant activities of these extracts. **Mansour and Hafez (2012)** who found that *W. somnifera* reduced serum GGT levels, it might protect against oxidative stress by modulating GGT levels in serum mediated through antioxidant mechanisms, Whereas **Sultana *et al*., (2012)** observed a significantly lower bilirubin level in an Ashwagandha-treated group compared to the untreated control group. Additionally, **El-Naggar *et al*., (2017)** reported that the administration of CCl4 to rats caused a significant ($P \leq 0.05$) increase in serum Gamma-glutamyl transferase (GGT), Alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) activities, but administration of the *S. lappa* simultaneously with CCl4 to albino rats caused a significant ($P \leq 0.05$) decrease in these parameters. **Arafa (2015)** showed that consumption of Costus maintains the integrity of the liver and protects it against damage caused by carbon tetrachloride, in addition to improving the enzymes gamma-glutamyl transpeptidase, alanine aminotransferase, and bilirubin.

Table 4: Effect of *S. costus*, Ashwagandha Roots and their mixture on Bilirubin, Lactate Dehydrogenase, α-Fetoprotein and ΄Y-Glutamyl transferase of rats with Liver Diseases.

Groups	Parameters			
	Bilirubin	Lactate Dehydrogenase	α-Fetoprotein	΄Y-Glutamyl transferase
	mg/dl	U/L	ng/ml	(U/L)
Control (-)	0.100 ^{f} ±0.00	123.120 ^{e} ±4.40	4.660^{f} ±0.35	9.020 ^{f} ±0.52
Control (+)	0.940 ^{a} ±0.05	317.240 ^{a} ±9.84	16.240^{a} ±2.12	35.240 ^{a} ±3.60
2.5%SCR	0.700 ^{b} ±0.00	296.800 ^{b} ±19.85	13.600^{b} ±0.44	30.540 ^{b} ±1.84
5%SCR	0.500 ^{c} ±0.00	249.520 ^{c} ±26.15	10.320^{c} ±0.78	21.600 ^{c} ±1.66
2.5%AR	0.680 ^{b} ±0.04	288.360 ^{b} ±10.04	11.120^{c} ±0.34	28.480 ^{b} ±3.43
5%AR	0.400 ^{d} ±0.00	246.660 ^{c} ±11.78	8.060^{d} ±0.77	17.360 ^{d} ±0.72
2.5 (SCR+AR)	0.440 ^{d} ±0.05	248.040 ^{c} ±13.92	6.760^{e} ±0.33	18.480 ^{d} ±1.56
5%(SCR+AR)	0.200 ^{e} ±0.00	195.680 ^{d} ±13.31	6.060^{e} ±0.18	11.740 ^{e} ±0.90

*(**SCR**) Saussurea costus roots, (**AR**) Ashwagandha roots.*

Means under same column have the different letters are significant different at p≤0.05.

Antioxidant enzyme activities

Results of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) levels in blood were reported in Table (5). Results indicated that CCL4 resulted in a significant decrease ($P \leq 0.05$) in serum SOD, CAT and GPx levels relative to the control negative group (23.600, 0.70, and 0.880 vs. 64.660, 1.68, and 3.380, respectively). Conversely, animals treated with two different concentrations of SCR, AR and their combination recorded significant increases and improvements ($P \leq 0.05$) in SOD and GPx, except for the group treated with 2.5% SCR, as compared to the positive control group. Non-significant changes in the mean value of SOD and GPx were observed between the groups (2.5% SCR and positive control group). Whereas the results from the CAT were increased in all treatments. The lowest catalase values in the group fed on 2.5% SCR and 2.5% AR could be noted. The highest value was recorded in the 5 % mixture form of SCR and AR. According to **Zhang *et al.* (2016) and Li *et al.* (2020),** prolonged oxidative stress in the liver affects liver cells either directly or indirectly. Interestingly, enhancement of the activities of some antioxidant enzymes such as catalase (CAT), glutathione reductase (GR), glutathione peroxidase (GPx), and superoxide dismutase (SOD) can inhibit oxidative stress and thereby delay the development of liver fibrosis **(Wu *et al*., 2019)**. These results are consistent with the study of **Ozturk et al. (2009)** which saw a significant decrease in antioxidant enzymes, including catalase, superoxide dismutase, and glutathione peroxidase activities in the CCl4 group when compared with the control group, and which indicated increased oxidative stress. **Singh *et al*. (2010)** reported that Ashwagandha root contains sitoindosides VIIX and withaferin A, which have antioxidant activity by enhancing the free radical scavenging enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx). **Singh and Singh (2019)** also reported that *W. somnifera* is responsible for increasing the amount of superoxide dismutase, catalase, glutathione peroxidase. **Arafa (2021)** indicated that the administration of *W. somnifera* for 4 weeks resulted in a significant increase in levels of antioxidant enzymes like GPx, SOD and CAT. On the other side, previous studies by **Jayasri *et al.* (2009), Choi *et al.* (2009), Jha *et al.* (2010), and Chang *et al.* (2012)** looked at the antioxidant activity of the plant costus and found that its leaves and rhizomes had high antioxidant activity, which may relate to its active ingredients, namely flavonoids, anthraquinone, and various terpenes. Therefore, costus may delay or provide protection for living organisms from damage caused by uncontrolled production of reactive oxygen species and the concomitant lipid peroxidation, protein damage, and DNA strand breaking. Their findings suggested that the costus plant could be employed as a natural antioxidant source.

Table (5): Effect of *S. costus*, Ashwagandha Roots and their mixture on antioxidant enzyme liver of rats with Liver Diseases.

Groups	Parameters		
	SOD activity (% inhibition)	Catalase (nM/ml/min)	Gpx activity (U/ml)
Control (-)	64.660^{a} ±3.87	1.68^{a} ±0.19	3.380^{a} ±0.34
Control (+)	23.600^{d} ±1.96	0.70^{f} ±0.00	0.880^{g} ±0.04
2.5%SCR	24.020^{d} ±2.46	0.92^{e} ±0.04	1.100^{g} ±0.10
5%SCR	35.180^{c} ±1.52	1.08^{d} ±0.10	1.900^{e} ±0.15
2.5%AR	33.260^{c} ±1.00	0.92^{e} ±0.04	1.380^{f} ±0.13
5%AR	39.720^{b} ±3.20	1.12^{cd} ±0.04	2.140^{d} ±0.05
2.5% (SCR+AR)	39.920^{b} ±3.88	1.24^{bc} ±0.11	2.460^{c} ±0.21
5% (SCR+ AR)	41.320^{b} ±2.47	1.26^{b} ±0.08	2.800^{b} ±0.12

SOD:** Superoxide dismutase **Gpx**: glutathione peroxidase **(SCR)** Saussurea costus roots, **(AR)** Ashwagandha roots. **Means under same column have the different letters are significant different at p≤0.05.

Serum kidney functions

Data tabulated in Table (6) showed the mean values of kidney function (urea, uric acid, and creatinine) levels of rats fed on various diets. It could be observed that the mean values of urea, uric acid, and creatinine of the control (+) group was higher than that of the control (-) group, being 17.740, 2.400, and 0.86 vs. 11.980, 1.640, and 0.38, showing a significant difference with a percent increase of 48.08%, 46.34%, and 126.31% of the C+ group as compared to the C- group. Concerning urea and creatinine levels, all rats fed on a basal diet with SCR, AR, and their mixture showed significant differences in mean values as compared to the PC group, except the group treated with 2.5% SCR and 2.5% AR because these treatments recorded non-significant changes in this parameter as compared to the PC group. As for uric acid, all rats fed on various diets from SCR, AR and their combination showed significant differences in mean values as compared to the C+ group. Numerically, the best treatment for urea, uric acid, and creatinine levels was recorded for the group fed on a 5% mixture treated with CCl4. These results corresponded with those of **Sultana *et al.*, (2022**), who were estimated serum creatinine, urea and uric acid and the result showed that the highest levels of serum creatinine, uric acid and urea were exhibited in the rats of CCl4 Group. Also, **Ali and Sabry, (2023)** discovered that giving Carbon tetrachloride caused hepatotoxicity by increasing biochemical indicators for the kidney not the liver. These results revealed a significant difference between the positive control group and the other groups (negative control, curative, and protective groups) fed CS roots powder at 2.5% and 5%. While rats fed 2.5% and 5% CS powder had significantly lowered their creatinine, uric acid, and urea levels. The observed improvement of kidney function biomarkers is attributed to the antioxidant activity of *S. costus* which is rich in bioactive components **(Ayaz, 2017)**. In addition, **Shimmi *et al.*, (2011)** found that the mean values of serum urea nitrogen and uric acid were significantly lower in the experimental group treated with the Ashwagandha roots as compared to the positive control. **Vasavan *et al.*, (2020)** indicated that treatments with

ashwagandha ameliorate creatinine, urea levels, and pathological damage caused to liver and renal tissue. **Harikrishnan *et al*., (2008)** indicated that Ashwagandha can improve kidney functions by increasing the glomerular filtration rate which may be due to some of its active components like phenolic compounds and flavonoids.

Table (6): Effect of *S. costus*, Ashwagandha Roots and their mixture on Kidney Functions of rats with Liver Diseases.

Groups	Parameters		
	Urea	U.A.	Creat.
	(mg/dl)		
Control (-)	11.980^{d} ±1.11	1.640^{d} ±0.11	0.38^{d} ±0.04
Control (+)	17.740^{a} ±0.55	2.400^{a} ±0.15	0.86^{a} ±0.05
2.5%SCR	17.760 ^{a} ±1.29	2.060^{b} ±0.18	0.86^{a} ±0.05
5%SCR	15.760^{b} ±0.66	1.880^{bc} ±0.13	0.68^{b} ±0.04
2.5%AR	17.580^{a} ±0.98	1.980^{b} ±0.08	0.80^{a} ±0.00
5%AR	14.020^{c} ±1.28	1.700^{cd} ±0.10	0.56^{c} ±0.08
2.5% (SCR+AR)	14.160^{c} ±1.29	1.600^{d} ±0.07	0.52^{c} ±0.04
5% (SCR+ AR)	13.040^{cd} ±1.64	1.520^{d} ±0.24	0.50^{c} ±0.00

*(**SCR**) Saussurea costus roots, (**AR**) Ashwagandha roots.* ***Means under same column have the different letters are significant different at p≤0.05.***

Serum lipid fractions

The findings in Table (7) presented the effect of *Saussurea costus*, Ashwagandha roots and their combination on lipid fractions of rats suffering from liver disease. The mean values (mg/dl) of serum TC (91.216±2.26), TG (52.080±2.36), LDL-c (68.880±2.64) and VLDL-c (10.416±0.47) increased significantly (p≤0.05), while HDL-c (11.920±1.17) decreased significantly (p≤0.05) for the C+ group, as compared to the C- group. Moreover, the administration of *Saussurea costus*, Ashwagandha roots, and their combination markedly modulated the deviations in these parameters compared with the counterpart group (p<0.05). However, HDL-c rose considerably in all treatment groups as compared to the PC group, while the positive control group's mean HDL-c value was significantly lower than that of the negative control group. The group treated with a 5% combination of SCR and AR had the best lipid profile results. These results were compatible with **Gopal and Sengottuvelu, (2008)** who reported that the Administration of CCl4 to rats produced hepatotoxicity and showed a significant increase in the serum levels of serum triglycerides and cholesterol in comparison to the control group. **Anwer *et al*. (2017)** reported that *W. Somnifera* (200 and 400 mg/kg) was administered orally once a day for 5 weeks, resulting in a significant reduction in TC, TG, LDL-c, VLDL-c levels with significant elevation of HDL-c levels. **Jha and Paul, (2020)** reported that *W. Somnifera* at the dose of 1000 mg/Kg b.w. was orally administered for 4 weeks and showed significant normalization in the lipid profile levels. On the other hand, the regulatory effects of costus on lipid profile levels have been previously confirmed by **Ali and Sabry, (2023)** who observed that rats that were given a diet enriched with *S. costus* root powder at 2.5 and 5 % simultaneously with CCl4 showed a significant decrease in both LDL-C, VLDL-C, TC, and TG on average when contrasted with the positive

control group. All treatment groups (curative and protective) with enriched meals including various levels of SC demonstrated greater mean HDL-C values when contrasted with the positive control group; these agree with the current results.

Table 7: Effect of *S. costus*, Ashwagandha Roots and their mixture of Lipid Profile of rats with Liver Diseases.

Groups	Parameters				
	Chol.	T.G.	HDL	LDL	VLDL
	(mg/dl)				
Control (-)	$75.036^{cd} \pm 3.31$	$35.880^{e} \pm 3.85$	$22.040^{a} \pm 1.15$	$45.820^{de} \pm 2.94$	$7.176^{e} \pm 0.77$
Control (+)	$91.216^{a} \pm 2.26$	$52.080^{a} \pm 2.36$	$11.920^{e} \pm 1.17$	$68.880^{a} \pm 2.64$	$10.416^{a} \pm 0.47$
2.5%SCR	$85.300^{b} \pm 3.86$	$51.900^{a} \pm 2.42$	$13.260^{de} \pm 0.98$	$61.660^{b} \pm 3.40$	$10.380^{a} \pm 0.48$
5%SCR	$76.136^{c} \pm 3.09$	$45.180^{bc} \pm 3.02$	$15.920^{c} \pm 1.16$	$51.180^{c} \pm 2.38$	$9.036^{bc} \pm 0.60$
2.5%AR	$84.372^{b} \pm 3.12$	$48.660^{ab} \pm 1.65$	$13.760^{d} \pm 1.88$	$60.880^{b} \pm 1.54$	$9.732^{ab} \pm 0.33$
5%AR	$74.256^{cd} \pm 2.75$	$42.780^{cd} \pm 4.84$	$17.060^{c} \pm 0.98$	$48.640^{cd} \pm 2.15$	$8.556^{cd} \pm 0.96$
2.5% (SCR+AR)	$72.664^{cd} \pm 1.19$	$42.320^{cd} \pm 1.83$	$15.980^{c} \pm 0.77$	$48.220^{cd} \pm 0.98$	$8.464^{cd} \pm 0.36$
5% (SCR+ AR)	$71.916^{d} \pm 1.41$	$40.880^{d} \pm 2.05$	$19.980^{b} \pm 0.82$	$43.760^{e} \pm 1.58$	$8.176^{d} \pm 0.41$

Chol: *cholesterol,* ***TG:*** *Triglyceride,* ***LDLc:*** *Low density lipoprotein cholesterol,* ***HDLc:*** *High density lipoprotein cholesterol,* ***VLDLc:*** *Very low-density lipoprotein cholesterol (****SCR)*** *Saussurea costus, roots, (****AR)*** *Ashwagandha roots.*

Means under same column have the different letters are significant different at p≤0.05.

Histopathological examination of liver

Microscopically, the liver of rats from group 1 (normal control) revealed normal hepatocytes with normal radial arrangements around hepatic cords (H&E, x400) **(photo 1)**. On the contrary, the livers of rats from group 2 (positive control) subjected to carbon tetrachloride (CCl4) exhibited severe damage upon microscopic examination. Characterized by centrilobular necrosis, inflammatory infiltrate, steatosis, and fibrosis, these findings indicate a complex hepatocellular response to CCl4-induced hepatotoxicity. (H&E, x400) **(photo 2).** Livers of rats treated with 2.5% SCR, 2.5% AR and 2.5 % (SCR+AR) respectively showed slight enhancement in hepatocytes and hepatic architecture (H&E, x400). **(photo 3, 5 and 7)**, whereas, the livers of rats treated with 5% SCR, and 5 % AR respectively showed moderate enhancement in hepatocytes and hepatic architecture. (H&E, x400) **(photo 4 and 6).** Moreover, Liver of rats treated with 5% (SCR+AR) showed the best enhancement in hepatocytes and hepatic architecture (H&E, x400) **(Photo 8).**

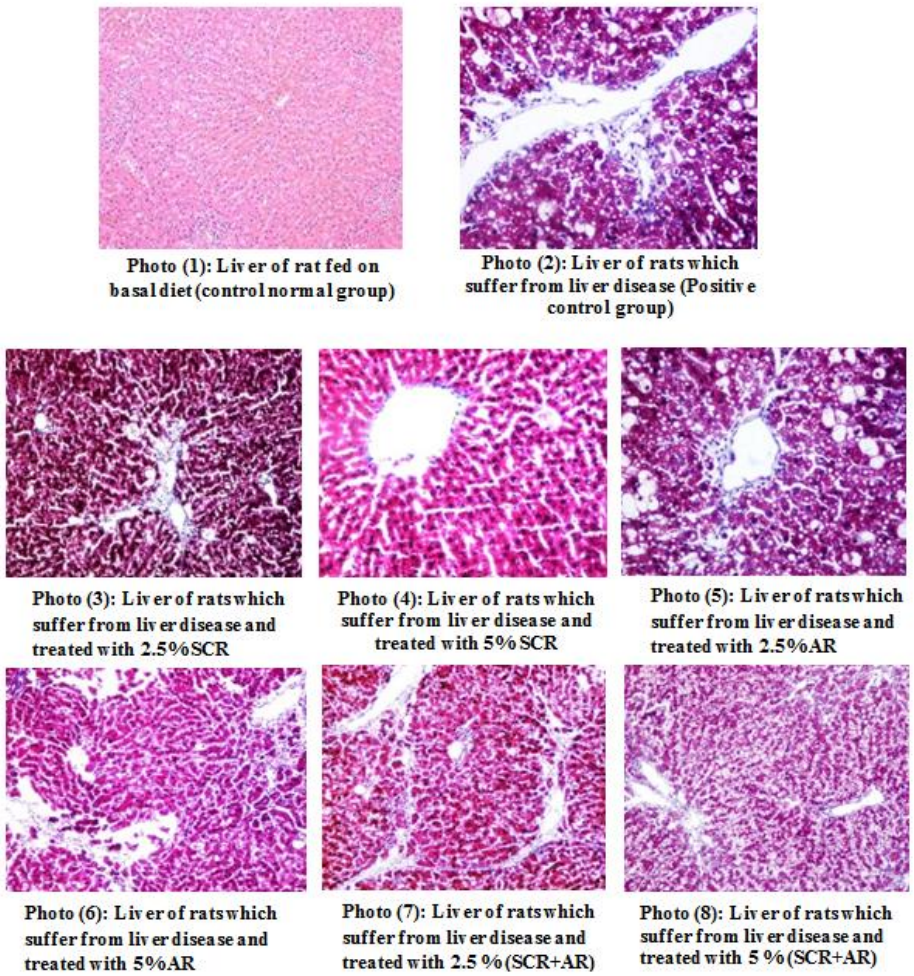

Fig (2): Histological examination of liver

Sensory evaluation

Results of sensory evaluation are presented in Table 8. The pan bread sensory properties observed were color, smell, taste, texture, and generally acceptable. Pan bread produced from whole-wheat flour and 2.5% SCR and 2.5% AR substitution was the highest rated among the panelists and statistically significant ($P \leq 0.05$) in color, smell, taste, and generally acceptable. On the other hand, the mean values of color, smell, taste, and generally acceptable decreased gradually with the increase in the level of *S.Costus* and Ashwagandha roots powder and their combination, as compared to the control sample. Furthermore, adding 2.5% from SCR to pan bread showed no significant differences ($p \leq 0.05$) in texture, as compared to the control. In general, results from overall acceptability showed that all samples obtained higher than 75%, which means that *S. costus* and Ashwagandha roots powder is an acceptable herb and can be used in the preparation of pan bread which is appropriate for liver patients.

Table (8): Sensory evaluation of pan bread fortified with *S. costus*, Ashwagandha roots and their mixture

Groups	Color	Smell	Taste	Texture	General	Total
Control	20.00 [a] ± 0.00	20.00[a] ± 0.00	19.90 [a] ± 0.30	19.90 [a] ± 0.30	19.90 [a] ± 0.30	99.72 [a] ± 0.90
2.5%SCR	19.00 [b] ± 0.74	18.87 [b] ± 1.00	18.05 [b] ± 1.2	19.09[ab] ± 1.02	18.85 [b] ± 0.88	93.87 [b] ± 3.85
5%SCR	17.94[c] ± 0.83	17.78 [c] ± 0.93	16.81[cd] ± 1.45	18.18[bc] ± 1.05	18.00[bc] ± 0.86	88.72[cd] ± 4.17
2.5%AR	18.85 [b] ± 0.75	18.21[bc] ± 1.19	18.14 [b] ± 1.49	18.69 [b] ± 1.58	18.45 [b] ± 1.10	92.36[bc] ± 5.55
5%AR	18.34[bc] ± 1.27	17.67 [c] ± 1.37	17.49[bc] ± 1.38	18.01[bc] ± 1.78	18.23[bc] ± 1.47	89.76[cd] ± 6.44
2.5%(SCR+AR)	17.94[c] ± 1.10	17.50 [c] ± 0.80	16.87[cd] ± 1.10	18.05[bc] ± 1.14	17.40 [cd] ± 1.11	87.78 [d] ± 4.66
5% (SCR+ AR)	17.00[d] ± 1.07	16.45 [d] ± 1.19	16.09 [d] ± 0.91	17.27 [c] ± 1.25	17.00 [d] ± 0.86	83.81 [e] ± 4.13

*(**SCR**) Saussurea costus roots, (**AR**) Ashwagandha roots.*

Means under same column have the different letters are significant different at p≤0.05.

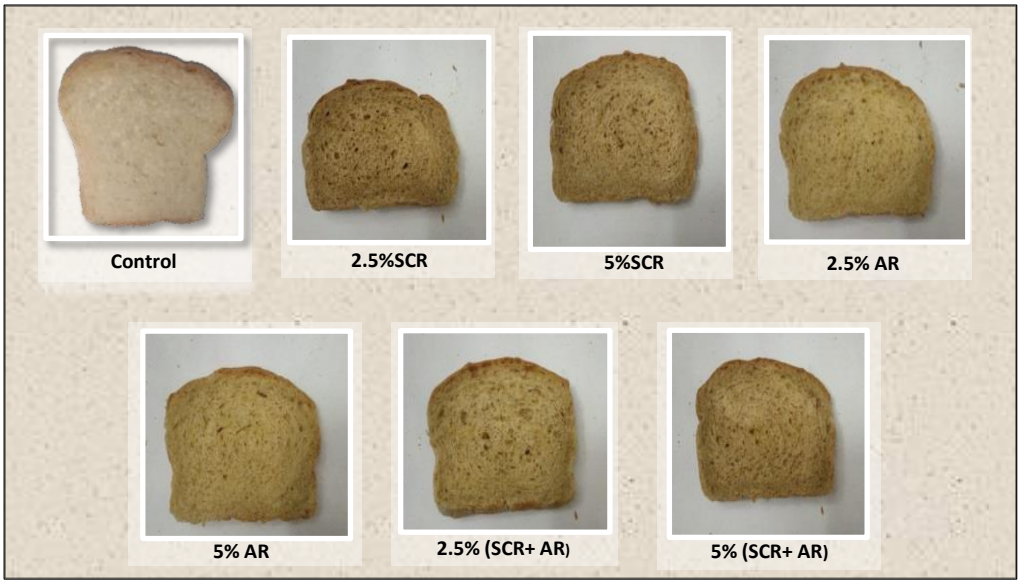

Fig (3): Pan bread fortified with *Saussurea costus* and Ashwagandha roots.

Conclusion

In conclusion, the present study has shown that *Saussurea costus* and Ashwagandha roots serve as an important source of many bioactive compounds; they improve liver enzyme activity and lipid profile. Besides, it maintains the integrity of the kidney and liver functions and lowers oxidative stress in the body. Overall, improvements were increased with the increase of the tested plants' concentration. So, people with liver disorders may benefit from incorporating *S. costus* and ashwagandha root into their diet during treatment. The study recommended using *S. costus* and ashwagandha root powder as additives in pharmaceutical industries and different food applications.

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التأثيرات المحتملة لجذور القسط الهندي والأشواجاندا على اضطرابات الكبد في الفئران

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تعتبر أمراض الكبد مشكلة صحية عالمية بسبب الدور الذي يلعبه الكبد في إزالة السموم والمركبات التي تولد الشقوق الحرة. وبالتالي، يُمكن تقليل سمية الكبد من خلال التدعيم بعدة عوامل مثل مضادات الأكسدة. تُعد جذورنبات القسط الهندي والأشواجاندا من أكثر النباتات الطبية احتواءً على نسبة عالية من مضادات الأكسدة. لذلك، تم تصميم الدراسة الحالية للتحقيق في التأثير المحتمل لجذور القسط الهندي و الأشواجاندا على الفئران المصابة بأمراض الكبد. تم تقسيم أربعون فأرًا من فئران الألبينو أوزانهم (١٥٠ ± ١٠ جرام) إلى مجموعتين رئيسيتين: تم تغذية المجموعة الرئيسية الأولى (٥ فئران) على نظام غذائي أساسي كمجموعة ضابطة سلبية. تم حقن المجموعة الرئيسية الثانية برابع كلوريد الكربون لإحداث تلف حاد في الكبد، ، ثم قُسِّمت إلى سبع مجموعات فرعية (٥ فئران لكل مجموعة). تم تغذية المجموعة الفرعية الأولى على نظام غذائي أساسي كمجموعة ضابطة إيجابية. المجموعتان الفرعيتان الثانية والثالثة تم تغذيتهم على نظام غذائي أساسي يحتوي على ٢,٥٪ و٥٪ من مسحوق جذور القسط الهندي على التوالي. المجموعتان الفرعيتان الرابعة والخامسة تم تغذيتهم على نظام غذائي أساسي يحتوي على ٢,٥٪ و٥٪ من مسحوق جذور الاشواجاندا على التوالي. بينما المجموعة الفرعية السادسة تم تغذيتهم على نظام غذائي أساسي يحتوي على ٢,٥٪ من مخلوط جذور القسط الهندي والاشواجاندا. المجموعة الفرعية السابعة تم تغذيتهم على نظام غذائي أساسي يحتوي على ٥٪ من مخلوط جذور القسط الهندي و الاشواجاندا. وأشارت النتائج الى تحسنات كبيرة في المعايير الغذائية والكيميائية الحيوية في مجموعات الفئران التي كانت تعاني من أمراض الكبد عند علاجها بمستويات مختلفة من جذور القسط الهندي و الاشواجاندا ومزيجهما أدى إلى تحسين النسبة المئوية للزيادة في وزن الجسم ، وزن الأعضاء ، وظائف الكلى، دهون الدم، وظائف الكبد و مضادات الأكسدة الانزيمية وذلك عند مقارنتها بمجموعة التحكم الإيجابية. حيث كان التحسن الأكثر وضوحاً في المجموعتين المعالجتين بمخلوط من جذور القسط الهندي و الاشواجاندا وكان أفضلها هو النسبة العالية للمخلوط ٥٪. كما أكد الفحص النسيجي للكبد تحسنًا تدريجيًا في جميع المجموعات المعالجة. أظهرت نتائج التقييم الحسي أن جميع عينات الخبز حصلت على أكثر من ٧٥٪ في القبول العام. في الختام، بالإضافة إلى فوائدهما الصحية العديدة ، يتمتع كلٌّ من نبات القسط الهندي وجذور الأشواجاندا بخصائص وقائية محتملة للكبد، ويمكن استخدامهما في المشروبات العادية وغيرها من التطبيقات الغذائية لعلاج مشاكل الكبد.

الكلمات المفتاحية: جذور القسط الهندي، جذورالأشواجاندا، وظائف الكبد، دهون الدم، وظائف الكلى، مضادات الأكسدة الانزيمية.