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جامعة عين شمس، كلية التربية النوعية، القاهرة، مصر

تحية طيبة وبعد،،،

يسر **معامل التأثير والاستشهادات المرجعية للمجلات العلمية العربية (ارسيف – ARCIF)**، أحد مبادرات قاعدة بيانات **"معرفة"** للإنتاج والمحتوى العلمي، إعلامكم بأنه قد **أطلق التقرير السنوي الثامن للمجلات للعام 2023.**

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

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

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محتويات العدد

* **بحوث علمية محكمة باللغة العربية:**

Effect of Soybean and Wheat germ as a Source of Branched Chain Amino Acids on Chronic Liver Disease of Rats

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Effect of Soybean and Wheat germ as a Source of Branched Chain Amino Acids on Chronic Liver Disease of Rats

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Abstract

The liver is a vital organ that plays a key role in the detoxification of body endogenous and exogenous substances. These substances can cause hepatic injury. Branched chain amino acids (BCAAs), valine (Val), leucine (Leu) and isoleucine (Ile). Protein of soybean and wheat germ characterized much quantity of BCAAs. This study included chemical analysis and amino acids contents in soybean and wheat germ,). Results indicated that rats feds on different formulas which contained soybean 5% and wheat germ 5% and its mixtures were gradually significant decrease in serum Aspartate Amino Transferase (AST) (from 30.20 to 35.80%), Alanine Amino Transferase (ALT) (from 52.50 to 61.06%) and Alkaline phosphatase (ALP) (from 9.90 to 22.03%) compared to positive group

Keywords: Soybean, Wheat Germ, Branched chain Amino Acids, Chronic Liver Disease

ملخص:

العنوان : تأثير فول الصويا وجنين القمح كمصدر للأحماض الأمينية المتشعبة السلسلة على مرض الكبد المزمن فى الفئران

المؤلفون : أسامة السيد مصطفى ، اسحق مراد الحديدى ، هانى جابر المصرى ، اسراء عبد الله أنور سيد

الكبد هو عضو حيوي يلعب دورًا رئيسيًا في إزالة السموم من المواد الداخلية والخارجية للجسم. يمكن أن تسبب هذه المواد إصابة الكبد. الأحماض الأمينية المتشعبة السلسلة (BCAAs) والفالين والليوسين والإيزولوسين ، تتميز بروتين كل من فول الصويا وجنين القمح بكمية كبيرة من الأحماض الأمينية المتشعبة السلسلة (BCAAs). تضمنت هذه الدراسة التحليل الكيميائي ومحتويات الأحماض الأمينية في فول الصويا وجنين القمح K أشارت النتائج إلى أن الفئران التى تغذت على تركيبة مختلفة تحتوي على فول الصويا 5٪ وجنين القمح 5٪ وخليط منهما كان هناك انخفاضا تدريجياً كبيرًا في سيرم كل من(AST من 30.20 إلى 35.80٪) ,(ALT من 52.50 إلى 61.06٪) و (ALP من 9.90 إلى 22.03٪) مقارنة بالمجموعة الإيجابية

الكلمات الدالة : الوسواس القهرى ، العلامة التجارية ، الملابس ، الدور الوسيط.

Introduction

The liver is a vital organ that plays a key role in the detoxification of endogenous and exogenous substances. Variety of pathological factors including viral hepatitis (especially hepatitis B and C), alcohol and drug abuse, metabolic diseases, autoimmune diseases and congenital abnormalities can cause hepatic injury. Also, liver cirrhosis is the final stage of all chronic hepatic diseases. Chronic hepatic disease is quite common in daily clinical practice **(Haytham *et al.*, 2019, p. 4254).**

Branched chain amino acids (BCAAs), valine (Val), leucine (Leu) and isoleucine (Ile), are essential amino acids that cannot be synthesized by animals, but only from bacteria, plants and fungi and must be obtained from diet sources. These amino acids have branched hydrophobic side chains and play a primary role in protein structure. All three BCAAs account for approximately 20–25% of most dietary proteins and constitute about 35% of the essential amino acids in mammals **(Dimou *et al.*, 2022, p. 4022).**

Branched chain amino acids (BCAAs) supplementation has benefit to patients with liver cirrhosis and liver cancer. BCAAs have also been used on critically ill patients with severe burn, sepsis, surgery, and trauma in different studies **(Tamanna and Mahmood, 2014, p. 8).**

In the progression of liver cirrhosis, the depletion of BCCAs, such as leucine, isoleucine, and valine inhibits protein synthesis and protein Turnover **(Yoshizawa *et al.*, 2004, p. 1541).** Moreover, the skeletal muscle catabolizes BCAAs more rapidly than most other amino acids **(Kimball and Jefferson, 2001, p. 39),** and these increase the major complications of liver cirrhosis during disease progression: hepatic encephalopathy, edema, and ascites accompanied by hypoalbuminemia, insulin resistance, hepatocarcinogenesis, and infection caused by an impaired immune function **(Nakamura *et al.*, 2007, p. 1062).**

In patients with advanced cirrhosis, decreased serum concentrations of BCAAs and increased concentrations of aromatic amino acids (AAAs), phenylalanine and tyrosine, are often found, resulting in a decreased ratio of BCAAs to AAAs, called the Fischer ratio. A decreased Fischer ratio is thought to be a cause of hepatic encephalopathy (HE) **(Tajiri & Shimizu, 2013, p. 7620).**

The protein of soybean contains the considerable quantity of lysine (6.2g/16gN), but value of protein is limited by methionine and cystine content (2.9g/16gN). With regard on high protein content, the soybean meal is mainly use in poultry nutrition. In mixtures for poultry content of soybean meal can approximate to 40%. The soybean contain very little of starch (4.66-7%) and quite a lot of hemicellulose and pectins. Protein of soybean products characterized much quantity of lysine, tryptophane, isoleucine, valine and threonine **(Hassan, 2013, p. 12).**

Wheat germs are used in human nutrition, inclusion of any other protein sources rich in leucine and isoleucine (e.g. soybean, fish, milk) are necessary, to achieve balanced dietary amino acid pattern. For example, in soy protein the digestible indispensable amino acid score (DIAAS) reference ratio for leucine is 1.18 and for isoleucine is 1.39, in milk whey protein is 1.72 and 1.97 for leucine and isoleucine, respectively **(Nitrayová *et al.*, 2018, p. 762).**

Therefore, this study aim to the role of BCAAs in soybean and wheat germ on rat's liver injury by CCl_4, and effect of these material on hepatic cells cytotoxicity in (HepG-2).

MATERIALS AND METHODS

Materials:

Dried soybean samples were obtained from Agricultural Research Centre, Giza, Egypt. While, dried wheat germ samples were acquired from El Salam mills, Cairo, Egypt. Branched Chain

Amino Acids (BCAAs) (Leucine, Isoleucine. Valine) were obtained from Sigma Co., Cairo, Egypt. Carbon tetrachloride and paraffin oil were purchased from El-Gomhoryia Company for Chemicals; Cairo, Egypt as a liquid packed in brown bottles. Ninety Six male albino rats (Sprague Dawley strain) weighing an average (190 ± 10g) were obtained from animal house in Food Technology Research Institute, Agricultural Research center, Giza - Egypt. Casein, vitamins, minerals and cellulose were obtained from El-Gomhoryia Company for Chemicals; Cairo, Egypt. Kits used to determine serum of Aspartate Amino Transferase (AST), Alanine Amino Transferase (ALT) and Alkaline phosphatase (ALP) were Purchased from El-Gomhoryia Company for Chemicals; Cairo, Egypt.Hepatic carcinogenic experiment were prepared in the National Oncology Institute, Giza, Egypt.

Methods:

The chemical analyses such as, moisture, ash, crude oil, crude fiber and crude protein were determined in dried soybean and wheat germ according the procedures described in **(A.O.A.C. 2012),** while, total carbohydrates was calculated by difference according to **(Mathew et al., 2014, p. 113).**

Amino acids for soy protein and wheat germ were fractionated by Amino Acid Analyzer according to **Karr-Lilienthal et al. (2005, p. 2146). Yiqiang *et al*. (1999, p. 52) and Carcia *et al*. (1972, p. 158)** for wheat germ and soy protein.

Diet composition and animal groups: Diet composition: Basal diet was prepared according to **(Reeves et al., 1993, p. 1939).**

The vitamin and mineral mixtures had prepared according to **(Campbell, 1963).**

Experimental design: In animal house in Food Technology Research Institute, Agriculture Research Center; albino rats were adapted for one week prior to commencement of

the experiment, housed in well aerated cages under hygienic conditions and water was introduced ad-libitum. After this week, rats were divided into two main groups as follows: **The first main group:** Control negative group (1): consists of 8 rats fed on the basal diet during the experimental period.

The second main group: consists of 92 rats fed on the basal diet and injected with CCl_4 (2ml/Kg body weight), dissolved in paraffin oil (50%, V/V) intraperitoneally twice/week for 2 weeks, to induce chronic liver disease, according to the method of **(Jayasekhar *et al.*, 1997, p. 426).**

The second main group divided into eleven subgroups (eight rats) as follows: **sub group (1):** Positive control group was fed on the basal diet till final experiment. **Sub group (2):** fed on the basal diet contained soybean 5%. **Sub-group (3):** fed on the basal diet contained wheat germ 5%. **Sub-group (4):** fed on the basal diet contained 2.5soy + 2.5wheat germ. **Sub-group (5):** fed on the basal diet contained 8.6 gm leucine. **Sub-group (6):** fed on the basal diet contained 4.3 gm leucine. **Sub-group (7):** fed on the basal diet contained 4.3 gm Isoleucine. **Sub-group (8):** fed on the basal diet contained 2.15 gm Isoleucine. **Sub-group (9):** fed on the basal diet contained 4.3 gm Valine. **Sub-group (10):** fed on the basal diet contained Leucine (8.6gm) + Isoleucine (4.3gm) + Valine (4.3gm). **Sub-group (11):** fed on the basal diet contained Leucine (4.3gm) + Isoleucine (2.15gm) + Valine (4.3gm).

Serum sampling: At the end of the experiment period (4 weeks after induced CCl_4), the rats were fasted overnight then anaesthetized, sacrificed and blood samples were collected from the aorta. The blood samples were centrifuged for 15 minutes at 3000 rpm to separate the serum. The serum was carefully separated into dry clean Wassermann tubes by using a Pasteur pipette and kept frozen till analysis at -20°C.

Determination of Aspartate Amino Transferase (AST) and Alanine Amino Transferase (ALT) were determined according to

the method of **(Reitman and Frankel, 1975, p. 65),** Alkaline phosphatase (ALP) described by **(Belfield and Goldberg, 1971, p. 561).**

Hepatic cytotoxicity (HpG-2) cell line Toxicity: Samples were prepared by dissolving 1:1 Stock solution and stored at -20°C in dimethylsulfoxide (DMSO). Different concentrations of dried soybean and wheat germ extracts were used (Range of concentration used by µg/ml according to **(Skehan and Strong, 1990, p. 1107).**

Statistical analysis: Results were analyzed using SPSS 19.0 Program**.** Means and standard deviations were determined using descriptive statistics. Comparison between means of samples was determined using analysis of one way variance (ANOVA) and multiple range tests. Statistical significance was defined at $P \leq 0.05$ **(Steel *et al.*, 1997, p. 666).**

RESULTS and DISCUSSION

1- Chemical composition of dried soybean and wheat germ (% on dry weight basis):

Table (1): Chemical composition of dried soybean and wheat germ (g/100gm) % on dry weight basis.

Components	Soybean	Wheat germ
Ash	5.51	4.80
Crude oil	22.19	11.92
Crude protein	40.09	35.75
Fiber	25.51	24.25
Total carbohydrates	32.21	47.53

Table (1) explained that the major components in dried soybean and wheat germ. The results in this table showed the content of protein in soybean was higher than in wheat germ (40.09 and 35.75 % respectively).

Also, the content of oil of soybean was higher (22.19%) than oil content in wheat germ (11.92 %).

On the other hand, the results of ash and fiber were nearly in soybean and wheat germ (5.51, 25.51% and 4.80, 24.25% respectively).

2- Amino acids contents for soybean and wheat germ (g/100g protein) on dry weight basis:

Table (2): Amino acids contents for soybean and wheat germ (g/100g protein) on dry weight basis.

Item	Soybean g/100g protein	Wheat germ g/100g protein
Arginine	5.86	7.17
Histidine	2.87	3.54
Isoleucine	5.38	1.90
Leucine	7.50	4.97
Lysine	6.49	5.70
Methionine	1.61	4.16
Phenylalanine	5.16	4.75
Threonine	4.40	5.03
Valine	4.30	3.46
Tryptophane	1.56	0.66
Alanine	4.75	6.54
Aspartic acid	9.15	12.75
Cystine	2.18	0.15
Glutamic Acid	19.08	14.62
Glycine	5.25	7.71
Proline	5.10	5.68
Serine	5.73	7.84
Tyrosine	3.63	3.37

Results in table (2) showed that, the content of branched Chain Amino Acids (BCAAs) in soybean and wheat germ protein were (5.38 and 1.90 g/100g protein) Isoleucine, (7.50 and 4.97 g/100g protein) leucine and (4.30 and 3.46 g/100g protein) Valine, respectively.

These results indicated the content of isoleucine and valine were higher in dried soybean than wheat germ. While, wheat germ protein was higher content of arginine, histidine, methionine, threonine, alanine, aspartic acid, glycine, proline and

serine (7.17, 3.54, 4.16, 5.03, 6.54, 12.75, 7.71, 5.68 and 7.84 g/100g protein) respectively than soybean protein.

The protein quality is characterized not only based on protein and amino acid content but also based on amino acids utilization by body **(Nitrayová et al., 2018, p. 762).**

Table (3): The quality Parameters of soybean and wheat germ protein:

Comparison between Arginine/Lysine ratio and Methionine/Glycine ratio in Soybean and Wheat germ	Arginine/Lysine ratio		
		Soybean	Wheat germ
	Arginine	5.86	7.17
	Lysine	6.49	5.70
	Arginine/Lysine	0.90	1.26
	Methionine/Glycine ratio		
		Soybean	Wheat germ
	Methionine	1.61	4.16
	Glycine	5.25	7.71
	Methionine/Glycine	0.31	0.54
Total Branched Chain Amino Acid, Aromatic Amino Acid and Fischer ratio	Branched Chain Amino Acids (BCAAs)		
		Soybean	Wheat germ
	Leucine	7.50	4.97
	Isoleucine	5.38	1.90
	Valine	4.30	3.46
	Total	17.18	10.33
	Aromatic Amino Acids (AAAs)		
		Soybean	Wheat germ
	Phenylalanine	5.16	4.75
	Tryptophane	1.56	0.66
	Tyrosine	3.63	3.37
	Total	10.35	8.78
	Fischer ratio		
		Soybean	Wheat germ
	Total BCAAs	17.18	10.33
	Total AAAs	10.35	8.78
	T. BCAAs/ T. AAAs	1.66	1.18

Wheat germ was higher in Arginine/lysine and Methionine/Glycine ratios (1.26 and 0.54) than soybean (0.90 and 0.31) respectively, while, the content of branched chain amino acids (BCAAs) and aromatic amino acids (AAAs) were the highest in soybean (17.18 and 10.35 g/100 gm protein), these results reflected to fisher ratio (1.66 in soybean and 1.18 in wheat germ protein).

In patients with advanced cirrhosis, decreased serum concentration of branched chain amino acids (BCAAs) and increased concentrations of aromatic amino acids (AAAs), phenylalanine and tyrosine, are often found, resulting in a decreased ratio of BCAAs to AAAs, called the Fischer ratio. A decreased Fischer ratio is thought to be a cause of hepatic encephalopathy (HE) **(Tajiri and Shimizu, 2013, p. 7620).**

3- Liver function indicators for Chronic Liver Disease Rats:

Table (4): Effect of different diets content of soybean, wheat germ on Aspartate Amino Transferase (AST) levels for chronic liver disease rats **(IU/L).**

Groups \ Experimental periods		Zero time	1 week	2 weeks	3 weeks	4 weeks	Decrement %
Control Group	Negative (-)	119.20h ± 3.56	115.20h ± 4.96	114.20h ± 5.93	112.40h ± 5.94	112.00h ± 6.40	-6.04
	Positive (+)	198.40a ± 4.56	183.20b ± 5.71	175.60c ± 3.84	170.8c ± 3.49	168.80cd ± 4.08	-14.90
Soybean	5%	192.40a ± 4.44	172.20c ± 4.32	155.60e ± 2.073	143.40f ± 2.96	125.20g ± 3.70	-34.90
Wheat germ	5%	201.60a ± 5.22	178.40c ± 3.50	160.40d ± 3.04	153.20e ± 5.11	129.40g ± 3.50	-35.80
Soybean + Wheat germ	2.5% + 2.5%	198.20a ± 3.83	185.40b ± 3.71	166.20d ± 4.20	156.20e ± 2.38	130.00g ± 4.74	-34.40
Leucine	8.6 gm	198.40a ± 2.88	172.60c ± 4.15	166.40d ± 4.09	154.20e ± 4.43	133.40g ± 3.43	-32.70
	4.3 gm	200.40a ± 5.41	192.00a ± 4.74	174.20c ± 3.56	162.60d ± 4.15	135.40g ± 3.64	-32.40
Isoleucine	4.3 gm	196.40a ± 2.40	186.20b ± 3.19	175.60c ± 2.40	152.20e ± 3.56	132.00g ± 3.87	-32.80
	2.15 gm	202.40a ± 4.21	195.60a ± 2.07	183.20b ± 5.71	149.20e ± 4.32	132.60g ± 2.40	-34.40
Valine	4.3 gm	189.40a ± 4.50	181.60b ± 6.06	170.40c ± 6.80	163.20d ± 3.56	138.60fg ± 2.70	-26.80
Leucine+ Isoleucine+ Valine	(8.6gm) + (4.3gm) + (4.3gm) (2:1:1)	195.20a ± 2.38	183.40b ± 5.41	173.20c ± 4.65	160.20d ± 2.77	130.60g ± 3.91	-33.09
Leucine+ Isoleucine+ Valine	(4.3gm) + (2.15gm)+ (4.3gm) (2:1:2)	198.40a ± 3.04	173.20c ± 4.65	162.40d ± 3.84	154.20e ± 3.19	131.40g ± 4.03	-30.20

- Zero time after induced CCl_4.
- All results are expressed as Means ± SD.
-Values in each column & raw which have different letters are significantly different ($p<0.05$).

Results in table (4) showed that, the significant increase in Aspartate Amino Transferase (AST) value in rats fed on basal diet after CCl_4 injection (from 112.00 to 168.8 IU/L).

Also, results indicated that gradually significant decrease in rats fed on different formula which contained soybean 5% and wheat germ 5% and its mixtures were gradually significant decrease in serum AST, ALT and ALP compared to positive group .

Generally, all groups fed on soybean 5% and wheat germ 5% and branched chain amino acids and its mixture were significant decrease in AST level (from 125.20 to 138.60 IU/L) compared to group was fed on basal diet after injection (168.80 mg/dl).

The percentage of decreament were from 26.80 % to 34.90 % at the end of expermint (after 4 weeks).

Table (5): Effect of different diets content of soybean, wheat germ and branched chain amino acids on Alanine Amino Transferase (ALT) levels for Chronic Liver Disease Rats **(IU/L).**

Groups \ Experimental periods		Zero time	1 week	2 weeks	3 weeks	4 weeks	Increment and Decrement %
Control Group	Negative (-)	27.60f ± 3.20	27.40g ± 3.64	29.20g ± 4.65	28.20g ± 3.03	28.00g ± 3.53	-0.40
	Positive (+)	107.20ab ± 5.26	110.20ab ± 7.82	119.00ab ± 8.33	119.20ab ± 5.80	123.60a ± 13.16	+15.20
Soybean	5%	103.40ab ± 3.43	82.40c ± 11.61	65.20e ± 3.96	58.60d ± 3.20	37.60f ± 3.78	-63.60
Wheat germ	5%	105.40ab ± 2.40	83.40c ± 17.64	66.40cd ± 2.88	59.20e ± 3.11	39.60f ± 3.78	-62.40
Soybean + Wheat germ	2.5% + 2.5%	102.40ab ± 2.07	76.40cd ± 10.64	59.60e ± 3.64	50.60e ± 4.03	32.40f ± 3.64	-68.30
Leucine	8.6 gm	113.40ab ± 3.43	104.40ab ± 10.43	73.40cd ± 2.70	65.40cd ± 2.88	43.20ef ± 3.11	-61.90
	4.3 gm	110.40b ± 3.43	108.40ab ± 7.92	75.60cd ± 3.04	63.60cd ± 2.70	42.20ef ± 3.56	-52.70
Isoleucine	4.3 gm	100.40b ± 2.88	93.40b ± 12.93	69.40cd ± 4.037	60.20cd ± 3.19	46.40ef ± 2.40	-53.70
	2.15 gm	116.60ab ± 3.43	108.40ab ± 4.72	72.40cd ± 4.21	58.40e ± 3.36	45.40e ± 3.36	-61.06
Valine	4.3 gm	120.20a ± 2.58	116.60ab ± 7.43	78.20c ± 3.19	68.60cd ± 3.64	52.40e ± 4.21	-56.40

Leucine+ Isoleucine+ Valine	(8.6gm) + (4.3gm) + (4.3gm) (2:1:1)	99.60b ± 3.64	93.40b ± 12.93	80.40c ± 3.04	76.20cd ± 5.06	44.60ef ± 3.36	-55.20
Leucine+ Isoleucine+ Valine	(4.3gm)+ (2.15gm) + (4.3gm) (2:1:2)	102.40ab ± 4.15	91.40b ± 5.36	82.40c ± 3.04	73.20cd ± 4.91	48.60ef ± 3.20	-52.50

- Zero time after induced CCl_4.
- All results are expressed as Means ± SD.
-Values in each column & raw which have different letters are significantly different ($p<0.05$).

Serum Alanine Amino Transferase (ALT) level was gradually increase in rats fed on basal diet after CCl_4 injection (from 107.2 to 123.6 IU/L) compared to rats group were fed on basal diet through the experiment period (from 27.6 to 28.00 IU/L).

Results indicated that rats fed on mixture of soybean 2.5% and wheat germ 2.5% was more effect than rats fed on soybean 5% or wheat germ 5%, the percentage of decrement was (68.30, 63.60 and 62.40 %, respectively. While, rats fed on leucine 8.6 and isoleucine 2.15 were more effect than other branched chain amino acids and its mixtures.

Also, non-significant decrease results between rats fed on groups 12 which contained Leucine 4.3gm, Isoleucine 2.15gm and Valine 4.3gm), group 7 (leucine 4.3gm) and group 8 (Isoleucine 4.3gm), these results were 52.50, 52.70 and 53.70%, respectively, but its decreased significant compared to positive group. The same observation, in rats fed on group10 (valine 4.3gm) and group11 (Leucine 8.6gm, Isoleucine 4.3gm and Valine 4.3gm), the percentage of decrement were 55.20 and 56.40% were not significant.

Generally soybean 5% and wheat germ 5% and its mixtures improved the serum ALT parameter from 52.5% to 63.6% compared to the decrement of ALT in positive control group (+15.2%) compared to branched chain amino acids and its mixtures.

Table (6): Effect of different diets content of soybean, wheat germ and branched chain amino acids on Alkaline phosphatase (ALP) levels for chronic liver disease rats **(IU/L).**

Groups \ Experimental periods		Zero time	1 week	2 weeks	3 weeks	4 weeks	Decrement %
Control Group	Negative (-)	125.40g ± 4.50	125.60g ± 5.72	124.40g± 5.54	125.80g ± 4.96	124.78g ± 5.98	-0.62
	Positive (+)	192.00a ± 8.63	188.20a ± 8.81	188.00a ± 7.61	187.00a ± 4.52	186.20a ± 4.14	-3.02
Soybean	5%	190.00a ± 4.94	183.00a ± 4.94	175.00b ± 4.35	160.00d ± 4.0	145.20f ± 3.27	-23.50
Wheat germ	5%	187.00a ± 3.39	173.00c ± 4.47	165.00cd ± 5.00	157.00d ± 3.16	145.80e ± 5.49	-22.03
Soybean + Wheat germ	2.5% + 2.5%	190.00a ± 6.20	180.00a ± 4.00	175.00b ± 3.39	168.00cd ± 5.91	155.80e ± 6.30	-17.90
Leucine	8.6 gm	186.00a ± 2.91	181.00a ± 5.43	175.00b ± 2.91	170.00c ± 3.87	161.40d ± 5.31	-13.20
	4.3 gm	189.00a ± 5.47	183.00a ± 5.83	177.00ab ± 3.87	170.00c ± 4.00	165.20cd ± 2.58	-12.50
Isoleucine	4.3 gm	186.00a ± 2.91	179.00ab ± 3.53	171.00bc ± 3.53	166.00cd ± 2.91	160.60d ± 3.64	-13.60
	2.15 gm	191.00a ± 2.91	181.00a ± 3.39	176.00b ± 3.16	171.00c ± 4.94	166.80cd ± 2.38	-12.60
Valine	4.3 gm	189.00a ± 3.87	186.00a ± 4.94	182.00a ± 4.47	175.00c ± 3.80	170.20c ± 7.19	-9.90
Leucine+ Isoleucine+ Valine	(8.6gm) + (4.3gm) + (4.3gm) (2:1:1)	188.00a ± 3.87	182.00a ± 4.74	170.00b ± 3.87	162.00d ± 4.52	149.80e ± 3.96	-20.20
Leucine+ Isoleucine+ Valine	(4.3gm)+ (2.15gm) + (4.3gm) (2:1:2)	187.00a ± 2.91	177.00ab ± 3.80	169.00c ± 3.80	162.00d ± 3.87	153.20e ± 3.56	-18.07

- Zero time after induced CCl4.
- All results are expressed as Means ± SD.
-Values in each column & raw which have different letters are significantly different ($p<0.05$).

Results indicated that significant decrement in Alkaline phosphatase (ALP) level after induced CCl_4 was ranged from 190 to 186 IU/L compared to negative control group rats (125.4 IU/L) at start of experiments. After that the ALP level was gradually decrease significant till the end of experiments. The highest significant decrease in ALP level of rats fed on all diets (ranged from 9.90 to 23.50 %) compared to rats induced CCl_4 fed on basal diet (3.02%).

Results in table (6) showed the improvement in ALP parameter was observed which ranged from 23.50 to 9.90 %. It's noticed that the lowest significant decrement in rats groups fed

on Valine 4.3gm (9.90 %), while the best rats groups fed on soybean 5%, wheat germ 5%, and group 11 (Leucine 8.6gm, Isoleucine 4.3gm and Valine 4.3gm) (145.20, 145.80 and 149.80 mg/dl) respectively compared to the significant decrement of positive control group (3.02%).

4- 4- Effect of soybean in hepatic cytotoxicity (HEPG-2):

Table (7): Effect of soybean in hepatic cytotoxicity.

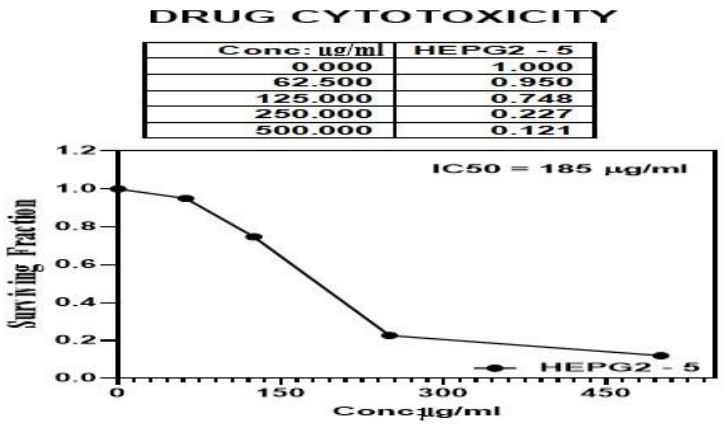

Fig 1. The effect of soybean in hepatic cytotoxicity.

5- Effect of wheat germ in hepatic cytotoxicity (HEPG-2):

Table (8): Effect of wheat germ in hepatic cytotoxicity.

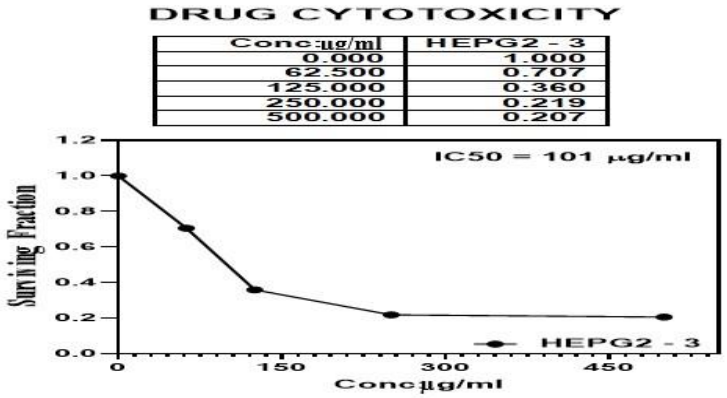

Fig 2. The effect of wheat germ in hepatic cytotoxicity.

Results in table (7) showed IC50 on hepatic cytotoxicity in case of soybean was185 µg/ml. While, IC50 in table (8) was101 µg/ml in case of wheat germ.

This result was indicated that soybean and wheat germ had an effect on cancer (HepG-2 cells). While, wheat germ was more hepatic effect than soybean in (HepG-2 cells).

Branched chain amino acids (BCAAs) supplementation has benefit to patients with liver cirrhosis and liver cancer. BCAAs have also been used on critically ill patients with severe burn, sepsis, surgery, and trauma in different studies **(Tamanna and Mahmood, 2014, p. 8).**

CONCLUSION

Conclousfly, soybean and wheat germ supplementation has benefit to liver chronic and cancer patient, due to the content of branched chain amino acids (BCAAs) (leucine, isoleucine and valine).

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