Available free online at www.medjbio.com

Mediterranean Journal of Biosciences

Mediterranean Journal of Biosciences **2016**, 1(3), 114-119

Hepatoprotective activity of ethanolic and ethyl acetate extracts of *Sterculia Setige* against carbon tetrachloride induced hepatotoxicity in albino rats

Tarig Hussein A. Bilal [1,*], Idris, O.F [2], Khalid, H.E [3] and Samia H Abdelrahman [4]

[1] Sharg Alneel College, School of Medical Laboratories. Khartoum North Sudan.
[2] Department of Biochemistry, Faculty of Science and technology, Alneelain University, Khartoum, Sudan.
[3] Faculty of Pharmacy, University of Khartoum. Khartoum, Sudan
[4] Department of Biochemistry, Veterinary Research Institute, Khartoum Sudan.

Abstract: The ethanolic and ethyl acetate extracts of *Sterculia Setigera* stem bark that belongs to the family of Sterculiaceae was studied for its hepatoprotective activity in albino rats with liver damage induced by carbon tetrachloride (CCL4). These plants are used in traditional medicine for the treatment of jaundice and other diseases. No systematic studies on their hepatoprotective activity have been reported before.
The hepatotoxcity was produced by the administration of CCL4 at a dose of 0.2 ml/kg for 10 days. The treatment was carried out by oral administration of ethanolic and petroleum ether extracts of *Sterculia Setigera* stem bark at a dose of 200 and 400 mg/kg body weight. The hepatoprotective activity was also supported by histopathological studies of liver tissue. The extracts exhibited moderate protective effect by lowering the serum levels of Alanine amino transferase (ALT), Aspartate amino transferase (AST), total protein, Albumin (ALB), Bilirubin concentration and alkaline phosphatase (ALP) to significant extent. In addition, the concurrent administration of the plant extracts with CCL4 for 10 days masked the liver changes induced by the hepatotoxic compound in rats and compared with hepatoprotective effect of the standard drug Silymarin.

Key words: Sterculia Setigera; stem bark; hepatoprotective activity; carbon tetrachloride.

Introduction

Plants are the most important source for traditional medicines and may contribute to the development of new medicines for various human health problems. Medicinal plants have played an essential role in the development of human culture, religions and ceremonies. 80% of the world's inhabitants rely mainly on traditional medicines for their primary health care [1].

Drugs are sometimes associated with side effects, whereas phytochemical (plant extract) have been found to have fewer side effects, better patient tolerance, relatively less expenses and a long history of use and renewability in nature .

Liver is one of the largest organs in human body and the chief site for intense metabolism and excretion. The major functions of liver are carbohydrate, protein and fat metabolism, detoxification, secretion of bile and storage of vitamins. Thus, maintaining a healthy liver is a crucial factor for an overall health and well-being. However, liver is in continuous environmental exposition to toxins and poor drug habits, alcohol and prescribed or over-the-counter drug which can eventually lead to various liver damage [2,3].

The manifestations of drug-induced hepatotoxicity are highly variable, ranging from asymptomatic elevation of liver enzymes such as ALT (alanine aminotransferase) and AST (aspartate amino-transferase) to fulminate hepatic failure and taken together, these two conditions can cause severe hepatotoxicity. There are not much drugs available for the treatment of liver disorders [4,5]. Therefore, many folk remedies from plant origin are tested for their potential and hepatoprotective activity in experimental animal model [6,7].

Carbon tetrachloride (CCl4) is widely used for the study of hepatoprotective effects of drugs and plant extracts. CCl4 can induce liver damage through the formation of reactive free radicals that can bind covalently to cellular macromolecules forming nucleic acid, protein and lipid adducts; and hypomethylated ribosomal RNA, resulting in inhibition of protein synthesis. CCl4 can also induce centrilobular steatosis, inflammation, apoptosis and

Corresponding author: Tarig Hussein Abdelrahman
E mail address: Ranin20102010@hotmail.com
DOI: http://dx.doi.org/

necrosis. If the damage continues, the liver will progress to fibrosis and cirrhosis [8].
One of the most known drugs for its hepatoprotective activity is Silymarin; a unique flavonoid complex derived from the milk thistle plant (Figure 1). It was traditionally used by nursing mothers to increase milk but, it is best known for its use as a liver protectant and decongestant. Moreover, Silymarin was used to cleanse the liver and spleen and to treat jaundice and gallstones.

The plant is also cultivated as a food, providing leaves for salad, seeds for a coffee-like drink, and flowers, which were eaten as artichokes are today.
Since then, hundreds of studies have been done on silymarin, and it has been approved as a supportive treatment for inflammatory liver conditions such as cirrhosis, hepatitis, and fatty infiltration caused by alcohol and other toxins [9].

Figure 1. Milk thistle forehead (Wikipedia)

Sudan with its uniquely variable climatic conditions possesses a huge wealth of flora, cultivated or wild. These later found their way to folk medicine and are used especially by natives in rural areas. Many phytochemical plants are used in the treatment of much liver disorder like jaundice, liver cirrhosis and fatty liver, but in most cases their effectiveness has never been evaluated nor receive any comprehensive scientific evaluation.

Sterculia Setigera of the family of Sterculiaceae is savanna tree wide spread in tropical Africa, sometimes up to 40 ft high and 5 ft girth (Figure 2). Pale purplish bark with thin scales peels off to expose yellowish patches; slash crimson, exuding a gummy sap. The anti-bacterial, anti-fungal and anti-viral activities of dried bark, dried fruit and the root of *S. setigera* have been already reported [10].

Figure 2. *Sterculia Setigera*, the tree on the left and the bark on the right. (The virtual botanic garden).

The present study was conducted to evaluate the hepatoprotective activity of (ethanolic and petroleum ether) extract against CCL4-induced toxicity in rats. The plant extracts of *Sterculia Setigera* stem bark is commonly known and ethnobotanically investigated in the central region of Burkina Faso where it is also used frequently and widely in traditional medicine to treat various diseases. In Sudan at the Kordofan region, the plant was used to treat various kinds of diseases such as fever, pain, and is believed to possess antibacterial, anti-viral and hepatoprotective activities. Hepatotoxicity is indicated by elevated liver enzymes including Alanine amino transferase (ALT), Aspartate amino transferase (AST) and Alkaline phosphatase (ALP) as Bilirubin [11,12].

Material and Method

Animals

Thirty, male and female Wister white (albino) rats weighting 100-150g were obtained from the Veterinary Research Institute, Soba, Khartoum, where they were housed in cages and maintained in a room under standard environmental condition, controlled temperature (22±2c), relative humidity (60%) with free access to water and food.

Plant Materials

Sterclia setigera bark were collected in October 2012 from Alnohod city. The plant was used in this area to treat hepatitis C. The plant was authenticated by the botanists in medicinal and aromatic plants research institute. The tree is often found on hills, rocky, poor and little deep soil. The plant is greyish, roots are small. Fruits are follicles containing big seeds. The stem bark of the plant was collected and then dried. The powder was weighed and then prepared for extraction.

Preparation of extract:

Extraction was carried out according to method described by Harborne [9]. 300g of plant sample was successively extracted by soaking in, ethyl acetate and 80% ethanol for about seventy two hours in each solvent with daily filtration and evaporation. Solvents were evaporated under reduced pressure to dryness using rotary evaporator apparatus. The yield percentages were calculated as followed:
Weight of extract / weight of sample * 100

Experimental Section

The rats were divided randomly into five groups of six rats each. The hepatoprotective activity of the plant extracts was tested using CCl4 model. Group A (normal control) received neither the plant extract nor CCl4 for 72 hours that is, they receive only food and water ; Group B (induction control) was given a single intraperitonial dose of 0.2 mg/kg BW CCl_4. Group C was given CCL4 together with the standard Silymarin (Prime Healthcare) at a dose rate of 100mg/kg BW. Group D was given CCl_4 together with 200 mg/kg BW of the plant extract. Group E was given CCl_4 together with 400 mg/kg BW of the plant extract. Clinical signs were recorded. Blood samples were obtained from the ocular vein before the start of the experimental dosing and there after fortnightly for serum analysis. Sera were analyzed for the activities of AST, ALT, ALP and for the concentrations of metabolic indicators, total protein, urea, albumin. After 10 days the rats were dissected and liver tissues were fixed in 10% neutral buffered formalin and processed for histopathology.

Biochemical Analysis

Serum AST, ALT and ALP activities were measured by a commercial kit (Randox Laboratories Ltd, U.K.) [10,13]. Serum albumin and total bilirubin was measured by a colorimetric method using a commercial kit (Randox Laboratories Ltd., U.K.).

Statistical analysis:

The values were expressed as mean ± SD. Statistical analysis and comparison between the groups was performed by one way analysis of variance (ANOVA) using SPSS version 10.0.

Results:

The effect of *Steculia Setigera* ethyl acetate and ethanolic extract on serum constituents are shown in Tables 1 and 2 . CCl_4 administration produced significant elevations of serum level of certain serum marker enzymes, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) level and bilirubin concentration in rats compared to the normal control and standard drugs group (P<0.05-0.01). Pretreatment of rats with 200 and 400 mg/kg animal body weight of *Sterculia setigerd* stems ethyl acetate and ethanolic extract significantly increased liver condition (P<0.05-0.01) when compared with group B that was given only CCL_4 .

Table 1. Effect of *Sterculia Setigera* stems ethyl acetate extract administered simultaneously with CCL_4 on Serum Constituents on rats.

Groups	*Total Protein (Mean±S.E.)*			*Alb (Mean±S.E.)*		
	Day 0	*Day 5*	*Day 10*	*Day 0*	*Day 5*	*Day 10*
A	7.40±0.00	*7.50±0.00*	*7.30±0.00*	*5.00±0.00*	*4.60±0.00*	*4.90±0.00*
B	7.30±0.00	*9.70±0.00*	*10.15±0.00*	*4.80±0.00*	*9.10±0.00*	*10.20±0.00°*
C	7.20±0.00	*10.20±0.00*	*10.90±0.00*	*4.10±0.00*	*7.00±0.00*	*8.50±0.00*
D	8±0.7.70	*7±0.10.05*	*°°7±0.11*	*7±0.5.20*	*6±0.7.55*	*°0.64±658.*
E	8±0.7.70	*7±0.10.05*	*°°7±0.70.*	*7±0.5.20*	*6±0.7.55*	**0.64±65.9.*

Groups	***ALT GPT (Mean±S.E.)***			***AST GoT (Mean±S.E.)***		
	Day 0	*Day 5*	*Day 10*	*Day 0*	*Day 5*	*Day 10*
A	11.20±0.00	*11.50±0.00*	*12.00±0.00*	*12.00±0.00*	*12.10±0.00*	*12.20±0.00*
B	11.10±0.00	*14.00±0.00*	*17.00±0.00°°*	*11.50±0.00*	*13.20±0.00*	*15.20±0.00°*
C	9.70±0.00	*14.00±0.00*	*16.10±0.00**	*10.20±0.00*	*13.20±0.00*	*15.30±0.00*
D	1.06±7.95	*1.06± 14.75*	*°0.2815.79±*	*1.41± 10.00*	*6±0.14.45*	*°°6±0.65.15.*
E	1.06±7.95	*1.06± 14.75*	**0.28±06.51*	*1.41± 10.00*	*6±0.14.45*	**6±0.65.6.1*

Groups	**Total BiL (Mean±S.E.)**			**Direct Bil (Mean±S.E.)**		
	Day 0	Day 5	Day 10	Day 0	Day 5	Day 10
A	1.20±0.00	1.10±0.00	0.96±0.00	0.25±0.00	0.24±0.00	0.25±0.00
B	1.20±0.00	2.80±0.00°	3.90±0.00°	0.26±0.00	1.80±0.00°	2.20±0.00°°
C	1.30±0.00	2.90±0.00*	3.40±0.00	0.35±0.00	1.16±0.00	1.91±0.00
D	07±0.1.15	*57±0.3.40	°35±0.95.4.	0.06±0.25	21±0.1.25	°°02±0.21.2.
E	07±0.1.15	*57±0.3.40	°35±0.95.4.	0.06±0.25	21±0.1.25	°022.98±0.

Groups	**Indirect BiL (Mean±S.E.)**			**ALP (Mean±S.E.)**		
	Day 0	Day 5	Day 10	Day 0	Day 5	Day 10
A	0.95±0.00	0.86±0.00	0.71±0.00	99.00±0.00	99.10±0.00	96.00±0.00
B	0.94±0.00°	1.00±0.00°	1.70±0.00	95.00±0.00	112.00±0.00	130.00±0.00°°
C	*0.95±0.00	1.74±0.00	*2.74±0.00	98.00±0.00	115.00±0.00*	129.10±0.00°
D	01±0.0.91	* 35±0.51.2	0.37±1.75	7.07±85.00	0.71±112.50	°3.54±50.131.
E	01±0.0.91	35±0.2.35	0.3797±.1	7.07±85.00	0.71±112.50	* 3.5±50.132.

The result of histopathological changes is presented in Figures 3 and 4 showing steatosis (fatty changes in hepatocytes) and perivenular fibrosis in group B. These changes were not observed when the extracts of the *Sterculia Setigerd* were administered in Groups D and E or in presence of Silymarin in Group C.

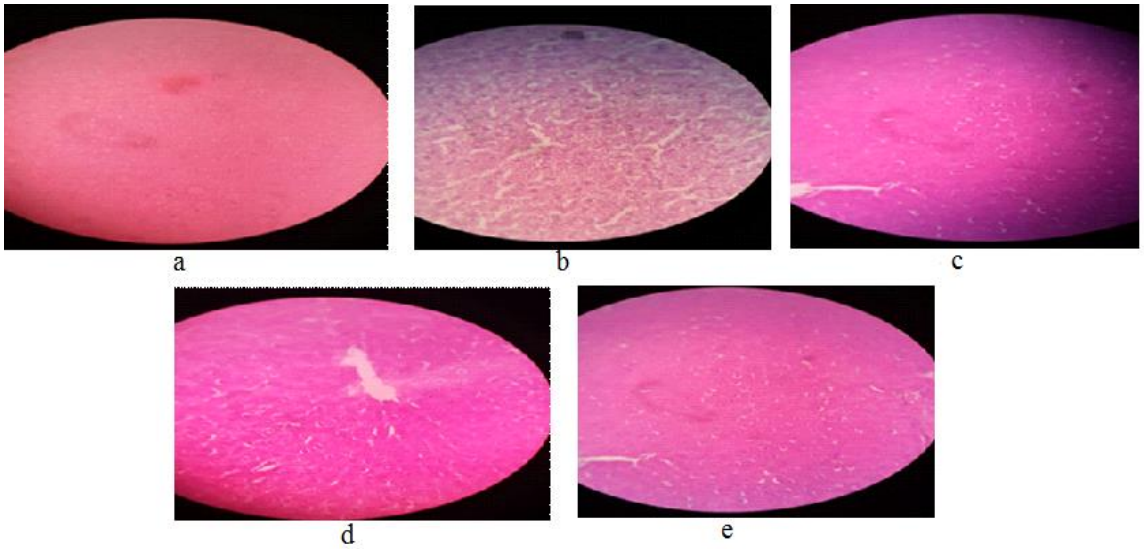


Figure 3. Histopathological changes in rats livers given ethyl acetate extract of *Sterculia Setigera* stem simultaneously with CCL4 with a: Group A, b: Group B, c: Group C, D: Group D and e : Group E

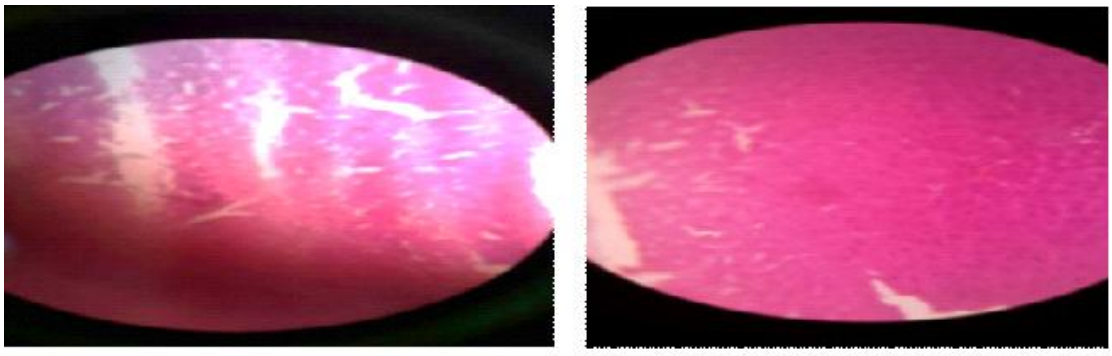
Table 2. Effect of *Sterculia Setigera* stems ethanolic extract administered simultaneously with CCL_4 on serum constituents in rats.

Groups	ALT GPT (Mean±S.E.)			AST GoT (Mean±S.E.)		
	Day 0	Day 5	Day 10	Day 0	Day 5	Day 10
A	11.20±0.00	11.50±0.00	12.00±0.00	12.00±0.00	12.10±0.00	12.20±0.00
B	11.10±0.00	14.00±0.00°	17.00±0.00$^{\circ\circ}$	11.50±0.00	13.20±0.00	15.20±0.00$^{\circ\circ}$
C	9.70±0.00	14.00±0.00	10.10±0.00*	10.20±0.00	13.20±0.00	11.30±0.00
D	9.30±1.13	14.60±0.85	11.25±0.35°	10.50±1.84	14.40±0.71	11.45±0.49*
E	9.30±1.13	14.60±0.85	11.25±0.35**	10.50±1.84	14.40±0.71	11.45±0.49$^{\circ\circ}$

Groups	Total BiL (Mean±S.E.)			Direct Bil (Mean±S.E.)		
	Day 0	Day 5	Day 10	Day 0	Day 5	Day 10
A	1.20±0.00	1.10±0.00	0.96±0.00	0.25±0.00	0.24±0.00	0.25±0.00
B	1.20±0.00	2.80±0.00°	3.90±0.00°	0.26±0.00	1.80±0.00°	2.20±0.00$^{\circ\circ}$
C	1.30±0.00	2.90±0.00*	3.40±0.00	0.35±0.00	1.16±0.00	1.91±0.00
D	07±0.1.15	*57±0.3.40	$^{\circ}$35±0.95.4.	0.06±0.25	21±0.1.25	$^{\circ\circ}$02±0.21.2.
E	07±0.1.15	*57±0.3.40	$^{\circ}$35±0.95.4.	0.06±0.25	21±0.1.25	$^{\circ}$022.98±0.

Groups	Total Protein (Mean±S.E.)			ALP (Mean±S.E.)		
	Day 0	Day 5	Day 10	Day 0	Day 5	Day 10
A	7.40±0.00	7.50±0.00	7.30±0.00	5.00±0.00	4.60±0.00	4.90±0.00
B	7.30±0.00	9.70±0.00	10.15±0.00°	4.80±0.00	9.10±0.00	10.20±0.00$^{\circ\circ}$
C	7.20±0.00	10.20±0.00	6.90±0.00*	4.10±0.00	7.00±0.00**	4.50±0.00
D	8.45±0.49	1.99±0.26	8.45±0.49°	4.65±0.78	9.5±0.21$^{\circ\circ}$	5.25±.042*
E	8.45±0.49	1.99 ±0.26	$_{*}$ 8.45±0.49*	4.65±0.78	9.05±0.21	5.25±.042*

Group	Indirect BiL (Mean±S.E.)			ALP (Mean±S.E.)		
	Day 0	Day 5	Day 10	Day 0	Day 5	Day 10
A	0.95±0.00	0.86±0.00	0.71±0.00	99.00±0.00	99.10±0.00	96.00±0.00
B	0.94±0.00°	1.00±0.00°	1.70±0.00	95.00±0.00	112.00±0.00	130.00±0.00$^{\circ\circ}$
C	*0.95±0.00	1.74±0.00	*2.74±0.00	98.00±0.00	115.00±0.00*	129.10±0.00°
D	01±0.0.91	* 35±0.51.2	0.37±1.75	7.07±85.00	0.71±112.50	$^{\circ}$3.54±50.131.
E	01±0.0.91	35±0.2.35	0.3797±.1	7.07±85.00	0.71±112.50	*.3±50.132.



a b

Figure 4. Histopathological changes in rats livers given ethanolic extract of Sterculia stem simultaneously with CCL4. Perivenular fibrosis were observed in section (a) and appeared normal when rats were treated with *Sterculia Setigera* in scetion (b).

Discussion

Hepatotoxicity is the most widespread liver pathology world-wide. Hepatitis, viral infection, food additives, alcohol, toxic industrial chemicals, and air and water pollutants are the major risk factors of liver toxicity.

The effect of *Steculia. setigera* ethyl acetate and ethanolic extract on serum constituent are shown in Tables 1and 2 and Figures 2 and 3. CCl4 administration produced significant elevations of serum level of certain serum marker enzymes, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP)

level and bilirubin concentration in rats compared to the normal control and standard drugs group (P<0.05-0.01). Following administration of the ethyl acetate and ethanolic extracts of *Sterculia setigera* stems at dose rates of 200 and 400 mg/kg B.W the level of (ALT), (AST) and (ALP) decreased significantly (P<0.05-0.01) when compared to the group B that was given only CCL4. At the end of the experimental period, it was clear that the enzymes (ALT), (AST) and (ALP) returned to the normal levels when 400 mg/kg BW of *Steculia Setigera* was used compared which was more efficient than 200 mg/kg of the extract.

Histological changes showed stenosis (fatty changes in hepatocytes) and perivenular fibrosis. These changes were not observed when the extracts of the *Sterculia Setigera* were administered.

Our result agrees with that reported in[10]. It is important to evaluate other plant extracts that can be used to improve treatment of hepatic failure caused by severe oxidative stress and necrosis [4].

Conclusion

Both ethanolic and ethyl acetate extracts of *Sterculia Setigera* plant have protective effect against CCL4-induced hepatotoxicity in rats . Our results using the model of CCL_4 induced hepatotoxicity in rats caused a significant elevation of ALT, AST, ALP, Total protein as well as bilirubin concentration. However, sever necrotic hepatic lesions induced by CCL_4 were reduced by ethanolic and ethyl acetate extract of plant stems which indicates the protective activity of *Sterculia Setigera* stem bark (200-400 mg/kg). A more efficient result was given with the highest dose of 400mg/kg body weight showing that the protective effect is dose dependent.

References

1. Chaterrjee, T, K “Medicinal Plants with hepatoprotective Properties”. Herbal option Books and Applied AUied (p) L td, Calcutta. 2000, 143.
2. Jude, E.O and Paul, A.N. Anti-inflammatory, analgesic and antipyretic activities of ethanolic root extract of *croton zambesicus. Phytochemistry.* 2007- **51**: 171-174
3. Karan, M. ,Vasisht,K., Handa,S.S. Anhepato toxic activity of swertia chirata on carhon tetrachlon iduced hepatotoxicity in rats, phytotherapy Research. 1999: 13, 24-30.
4. Owoabi, O.J., E.K.I. Omogbai and O. Obasuyi, Antifungal and antibacterial activities of the ethanolic and aqueous extract of Kigella Africana (Bignoniaceae) stem bark. Afr. J. Biotechnology.2007: 6: 1677-1680.
5. Reitman, S. and Frankel, S. Amer. J. Clin. Path.1957: 28-56.
   Schmidt, E. and Schmidt, F. W. Enzyme, Biol. Clin.1963- 3:1.
6. Rubinstein,D. “Epirephnre release and liven glycogen level after canbon tetrachloride administration”. American Journal of physiology.1962: 203, pp.1033-1037.
7. Shanma,A., chakraborti K.K, and Handa, S,S. “Anti-hepatotoxic activity of some Indian herbal formulations as compared to silymarin” Fitoterapia .1991: 62, pp.229-235.
8. Shana R. Dalton a,b , Serene M.L. Lee. Carbon tetrachloride-induced liver damage in asialoglycoprotein receptor-deficient mice. biochemical pharmacology. 2009: 77,1283–1290
9. Wagner, H., et al. "The Chemistry of Silymarin (Silybin), the Active Principle of the Fruits of Silybum marianum." Arzneim-Forsch Drug Res. 1968; 18:688-96.
10. Sharma Bhawna and Sharma Upendra Kumar Hepatoprotective activity of some indigenous plants. International Journal of PharmTech Research. 2009: Vol.1, No.4, pp 1330- 1334.
11. Rajib Ahsan, Km Monirul Islam, A. Musaddik and E. Haque. Hepatoprotective Activity of Methanol Extract of Some Medicinal Plants Against Carbon Tetrachloride Induced Hepatotoxicity in Albino Rats- Global Journal of Pharmacology. 2009:3 (3);116-122.
12. Isubramoninm, A, and Push pangadan, P. “Development of ply to medicines for liver diseases”. Indian of pharmacol. 1999) 31, pp.166-175.
13. Kamsiah Jaarin , U. Nor-Aini , M.A. Siti-Aishah and Srijit Das. Palm Oil Fat Diet Consumption and its Effects on Serum Liver Enzymes and Microscopic Changes in Experimental Rats. Pakistan Journal of Nutrition. 2015:14 (9): 575-580.