INDO AMERICAN JOURNAL OF PHARMACEUTICAL RESEARCH

STUDY OF GROWTH PROMOTION AND HEALTH BENEFITS OF AYU-809 GENERAL NUTRITIONAL SUPPLEMENT IN RATS

Priyanka Shrestha[1], Biplav Syngkli[1], Sri Vidya[2], Shanaz Tejani-butt[3], Shivalinge Gowda KP.[*]

[1]*Department of Pharmacology, PES College of Pharmacy, Bengaluru, Karnataka, India.*
[2]*Ayurwin Pharma Pvt. Ltd, Bengaluru, Karnataka.*
[3]*University of the Sciences in Philadelphia, Philadelphia, PA, USA.*

ARTICLE INFO

Article history
Received 14/01/2017
Available online
30/04/2017

Keywords
Ayu-809 A General Supplement,
Carbon Tetrachloride,
Body Circumference,
Silymarin,
Superoxide Dismutase,
And Lipid Peroxidation.

ABSTRACT

The growth promotion and health benefits of AYU-809 –a general nutritional supplement was investigated using carbon tetrachloride (CCl_4 in olive oil 0.5ml/kg bw ip) intoxicated Sprague Dawley rats. The AYU-809 in milk was administered orally at a dose of 4.80 g/kg bw po for 28 days. The CCl_4 was administered to all the groups except group I (normal control) once in three days. Silymarin (100 mg/kg po) was used as a standard hepatoprotective drug. Body weight, feed efficiency and body circumference were measured every 5th day during the study. On 29th day, animals were anaesthetized and blood was collected by retro orbital. Hemoglobin content and RBC count, serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) and alkaline phosphatase (ALP) were determined. Liver homogenate was prepared and lipid peroxidation (LPO) and Superoxide Dismutase (SOD) levels were measured and histopathology of liver tissue also examined. Our findings suggest that AYU-809, a general nutritional supplement formulated by Nutraceuticals division M/s Ayurwin Pharma Pvt Ltd, Bengaluru, possesses growth promoting, hepato protecting and antioxidant properties. The data provide useful insight into the possibility of using AYU-809 to promote the growth in children associated with growth related disorders.

Corresponding author
Priyanka Shrestha
Department of Pharmacology,
PES College of Pharmacy,
Bengaluru, Karnataka, India.
shivalinge65@gmail.com

*Please cite this article in press as **Priyanka Shrestha** et al. Study of Growth Promotion and Health Benefits of AYU-809 General Nutritional Supplement in Rats. Indo American Journal of Pharmaceutical Research.2017:7(04).*

Copy right © 2017 This is an Open Access article distributed under the terms of the Indo American journal of Pharmaceutical Research, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

INTRODUCTION

Normal growth in children is promoted by human growth hormone (hGH). Normal hGH levels in new born is 5-40ng/ml (in children 0-20 ng/ml). Many factors stimulate the release of hGH. For example, regular exercise, sleep (8 h/d), melatonin (0.5 to 5 mg) before bedtime, (ref-Life Extension Magazine March 2009), GABA (1.5 to 3 g), or 2 g of glutamate before bedtime have all been found to increase hGH levels by up to 200 % [ref]. Consuming high protein and low carbohydrate snack before bedtime can also increase hGH levels in the blood. The combination of L-arginine and L-lysine together before bed or exercise increases growth hormone production by up to 700 % (ref -Life Extension Magazine March 2009).

Several health problems in children and adults may lead to growth failure, including nutritional disorders, gut malabsorption syndrome, various diseases of the heart, lungs, and kidneys, bone disorders such as skeletal dysplasia, intrauterine growth retardation, Turner syndrome, thyroid hormone deficiency and growth hormone deficiency. Some conditions that cause growth failure may be inherited or caused by other health problems [1].

Research over the past couple of decades has provided evidence for the biological activity of dietary factors that influence specific molecular systems and pathways related to body functions. There are many types of natural growth promoters. Which include predominantly organic acids, probiotics, prebiotics, synbiotics, phytogenics, feed enzymes and immune stimulants [2]. In addition, nutritional supplements such as vitamins, minerals, herbs, meal supplements, sports nutrition products, natural food supplements, and other related products can also be used to boost the nutritional content of the diet.

Thus, an ideal natural growth promoting agent should promote body growth by build ing muscle mass, promoting new cells and tissue growth. It should also strengthen the nervous system, maintain cholesterol level, help insomnia, enhance memory, and provide the necessary amino acids required for the synthesis of hGH in the anterior pituitary gland.

The aim of the present study is to investigate the growth promoting effects of AYU-809, a general nutritional supplement, in rats. This study will measure various physical, enzymatic and serum parameters to determine the growth promoting, organ protective and health benefits of AYU-809.

MATERIALS AND METHODS

Chemicals and reagents

Ethylenediaminetetraacetic acid (EDTA), Sodium carbonate, Nitroblue tetrazolium (NBT), Hydroxylamine, Phenylazinemethosulphate (PMS), carbon tetrachloride (CCl_4) Drugs and chemicals were procured from VASA scientific suppliers, Bengaluru, biochemical kits for SGOT, SGPT, ALP were obtained from Anjan distributors Bengaluru and Sample-AYU-809 was supplied by Nutraceuticals division, Ayurwin Pharma Pvt Ltd, Bengaluru.

Experimental Animals

Thirty Sprague Dawley male rats (120-150 g) were ordered from M/s Aditya Biosys Pvt ltd, Bengaluru. The animals were exposed to 12 h/d and 12 h night cycle with a temperature between 25 ^{o}C to 27 ^{o}C. The animals were housed in spacious, hygienic, polypropylene cages with paddy husk as bedding during the experimental period. The animals were fed with water and rat pellet feed (M/s Aditya Biosys Pvt Ltd, Bengaluru.) *ad libitum*. All experimental procedures and protocols used in this study were reviewed by Institutional Animal Ethics Committee (PESCP/IAEC/32/2015) and were in accordance with the guidelines of the CPCSEA (PES College of Pharmacy, CPCSEA registration No: 600/PO/Ere/S/02/CPCSEA) New Delhi.

The human dose for AYU-809 was reported as 30 g in 200 ml of milk. Based on this, the dose for rat was calculated.

Experimental design-

Study of growth promotion and health benefits of AYU-809 general nutritional supplement in rats. Animals are acclimatized (for about 10 d) to laboratory conditions before the study. Rats (120-150 g) were divided into 5 groups (n = 6) and studied for 28 d as per Table 1.

Table 1: Experimental design.

Group (n = 6) Treatment (dose & route)		Treatment schedule
I Normal control	Vehicle- milk	D 1 to 28
II CCl_4 control	CCl_4 (0.5ml/kg bw) i.p. olive oil (1:1)	D 2,5,9,12,16,19,23,26
III AYU-809 in milk	4.80 g/kg bw p.o.	D 1 to 28
IV AYU-809 + CCl_4	4.80 g/kg bw p.o.	D 1 to 28
	(0.5 ml/kg CCl_4)ip	D 2,5,9,12,16,19,23,26
V Silymarin + CCl_4	100mg/ kg p.o.	D 1 to 28
	(0.5 ml/kg 50 % CCl_4) ip	D 2,5,9,12,16,19,23,26

Body weight, feed efficiency and body circumference were measured every 5th day during the study.

Body weight was measured using digital weighing machine, feed efficiency was measured using the following formula, Feed efficiency = [Body weight (g) /food consumed (g)] x 100 and body circumference was measured with the help of thread and expressed in cm.

Collection of blood by retro orbital at the end of study

On 29th day, animals were anaesthetized and blood was collected by retro orbital[14]. A portion of the collected blood was used for the estimation of blood parameters. The remaining blood was processed to serum. Blood was used to measure hemoglobin content and RBC count. Serum was used to measure serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) and alkaline phosphatase (ALP) using Erba biochemical kits procedure.

Tissue parameters- Liver Weight:

On 29th day, rats were sacrificed as per the CPCSEA prescribed method and liver was excised, blotted on a filter paper and the relative organ weight was calculated.

Liver tissue homogenate:

Liver homogenate was prepared and lipid peroxidation (LPO) and Superoxide Dismutase (SOD) levels were measured according to (Noori *et al*) [15].

Histopathology:

Isolated liver tissues from all groups were immediately blotted using filter paper and then weighed with an analytical balance. Thereafter, the tissues were suspended in 10 % v/v formalin for fixation preparatory to histological processing.

Statistical Analysis

The values were expressed as Mean ± SEM from 6 animals /group. The results were subjected to Statistical analysis by using one-way ANOVA followed by Bonferroni's method of statistics to calculate the significance. $P<0.05$ was considered as significant.

RESULTS

Body weight-

Over a period of 28 days**,** the percentage weight gained in control rats in group I was 9.2±2.9. In contrast, rats treated with CCl_4 (group II) showed a significant decrease in mean percentage weight of 3.4±10.3 within the same time frame. Interestingly, rats in group III that were treated with AYU 809 showed a significant increase in percentage weight of 21.8±3.9. In group IV rats (AYU-809+CCl_4), the mean increase in percentage weight was 8.5±4.7, and rats in group V (silymarin + CCl_4) showed a percentage change in body weight of 12.10±3.6. Thus, data indicate that AYU-809 significantly increased the percentage change in the body weight compared to the control as well as CCl_4 treated during the 28 d study period.

Table 2: Effects of AYU 809 on body weight of rats (% change).

Animal group n=6	Treatment	% change in body weight
I	Normal control	9.2 ±2.9
II	CCl_4 control	3.44±10.3*
III	AYU 809 control	21.8±3.9*
IV	CCl_4 +AYU 809	8.5±4.7
V	CCl_4 + silymarin	12.1±3.6

Body circumference-

Control rats in Group I showed an increase in percentage change in body circumference of 3.2±3.2 whereas group II group rats (CCl_4), show a significant decrease in percent body circumference of 0.83+ 3.5 over the course of 28 days study. In contrast, rats in group III that were treated with AYU 809 were closer in body circumference to control group I (4.95±2.3). Interestingly, group IV rats (AYU+CCl_4) shows an increase in body circumference of 3.2±3.2 and group V rats (silymarin+CCl_4) show a mean increase in body circumference of 3.8±2.2 when compared with group II CCl_4 rats (body circumference of 0.833±3.536). Thus it appears that AYU-809 protects against the effects of CCl_4.

Table 3: Effects of AYU 809 on body circumference (cm) of rats (% change).

Animal group n=6	Treatment	% change in body circumference
1.	Normal control	3.2 ±3.2
2.	CCl_4 control	0.83±3.5*
3.	AYU 809 control	4.95±2.3 *
4.	CCl_4 +AYU 809	3.2±3.2
5.	CCl_4 + silymarin	3.8±2.2

Feed efficiency-

Rats in group III administered with AYU-809 show a significant difference compared to control rats (group I) as well rats treated with CCl_4. Rats from group IV and V show a significant increase in the percentage of feed efficiency, when compared to group II rats suggesting a growth promoting effect of AYU 809.

Table 4: Effects of AYU 809 on feed efficiency of rats.

Animal group n=6	Treatment	% change in feed intake
1.	Normal control	12.7
2.	CCl_4 control	5.95*
3.	AYU 809 control	17.3*
4.	CCl_4 +AYU 809	9.5
5.	CCl_4 + silymarin	9.6

Hemoglobin content-

Compared to control rats (Group 1, 9.778±5.892 g/dL) and rats treated with CCl4 (Group II, 2.189±2.334 g/dL), rats treated with AYU -809 (Group III) show a significant increase (11.97±2.4 g/dL) in hemoglobin content.AYU-809.

Table 5: Effects of AYU 809 on Hemoglobin content of rats.

Animal group n=6	Treatment	Hemoglobin count g/Dl (g%) Mean± SEM
1.	Normal control	9.8 ±5.9
2.	CCl_4 control	2.2±2.3*
3.	AYU 809 control	11.97±2.4
4.	CCl_4 +AYU 809	6.5±6.96
5.	CCl_4 + silymarin	8.7±1.7

RBC count-

Rats treated with AYU -809 (group III) show significant increase in RBC count (19.94±2.4million cells/ mm3 of blood) when compared to group I normal rats (14.6 ±13.55 million cells/ mm3 of blood). The group IV rats treated with (AYU-809+CCl_4) have shown significant increase (15.23±22.57 million cells/ mm3 of blood) when compared with group II rats treated with CCl_4 (5.376±10.18 million cells/ mm3 of blood). Similarly, Group V rats (silymarin+CCl_4) showed an increase in RBC count (8.480±3.664 million cells/ mm3 of blood) when compared with group II rats. These results support the growth promoting effects of AYU-809.

Table 6: Effects of AYU 809 on RBC count in rats.

Animal group	Treatment	RBC count million cells/ mm3 Mean± SEM
1.	Normal control	14.6±13.6
2.	CCl_4 control	5.4±10.2*
3.	AYU 809 control	19.9±2.4*
4.	CCl_4 +AYU 809	15.2±22.6
5.	CCl_4 + silymarin	8.5±3.7*

Serum parameters-

The serum ALP level in group I normal control rats was found to be 108±10.47 U/L. Similarly group II CCl_4 control rats were found to be 268.1±9.478 U/L. The rats treated with AYU-809+CCl_4 (group IV) have also shown significant decrease in the serum ALP level (187±7.236 U/L) when compared to CCl_4 control rats. The insignificant activity of serum ALP, SGOT and SGPT in AYU 809 treated rats (group II) when compared to the normal rats suggests that AYU-809 is safer to vital organs like liver.

Table 7: Effects of AYU 809 on Serum parameters: ALP, SGPT & SGOT.

Animal group	Treatment	SGOT IU/L Mean± SEM	SGPT IU/L Mean± SEM	ALP U/L Mean± SEM
I	Normal control	7.5±0.69	35.7±3.7	108.0±10.47
II	CCl_4 control	89.7±14.5[a]***	103.2±10.4 [a]***	268.1±9.5 [a]***
III	AYU 809 control	12.1±0.7	53.8±1.45	131.2±4.4
IV	CCl_4 +AYU 809	30.2±2.5 [b]***	77.49±2.5 [b*]	187.0±7.2 [b]***
V	CCl_4 + silymarin	13.2±1.1 [b]***	64.4±7.5 [b***]	175.5±2.6 [b]***

Tissue parameters: **Effect of AYU-809 on rat liver tissue**

LPO and SOD-

The SOD levels in normal control rats were found to be (3.78±0.05 units/mg protein) and in rats treated with CCl_4 there was a significant decrease in tissue SOD level (0.73±0.09 units/mg protein). The group IV rats treated with AYU-809+ CCl_4 (1.26±0.029 units/mg protein) and group V rats treated with silymarin+CCl_4 (1.76±0.014 units/mg protein) show a significant increase in SOD level when compared with group II rats. This result indicates the hepatoprotective effects of AYU-809 in rats.

The LPO level in group I rats (normal control) was found to be (31.86±0.64 µmol MDA/mg protein) whereas the LPO level in group II rats (CCl_4 treated) was found to be (78.9±0.74 µmol MDA/mg protein), which is significantly higher when compared with group I. Group IV rats (AYU-809+CCl_4) showed decrease in LPO level and was found to be (68.3±0.27 µmol MDA/mg protein). Similarly group V rats (silymarin+CCl_4) with LPO level of (41.30±0.38 µmol MDA/mg protein) have showed significant decrease in LPO level when compared with group II rats. Thus this result indicates that AYU-809 possesses good hepatoprotective activity in rats.

Table 10: Table showing effect of AYU-809 on rat liver tissue LPO and SOD.

Animal group	Treatment	LPO µmol MDA/mg protein Mean± SEM	SOD units/mg protein Mean± SEM
I	Normal control	31.86±0.6433	3.778±0.05218
II	CCl_4 control	78.90±0.7440 [a]***	0.7335±0.09351[a]***
III	AYU 809 control	39.95±0.8971	3.073±0.04595
IV	CCl_4 +AYU 809	68.29±0.2686 [b]***	1.258±0.02982[b]***
V	CCl_4 + silymarin	41.30±0.3829 [b]***	1.764±0.01394 [b]***

Histopathological study of liver tissue:

The normal microscopic architecture of the liver is composed of hexagonal lobules and acini. Hexagonal lobules are centered on the central vein and have a portal triad containing branches of the portal vein, hepatic artery and bile duct (Figure 1).

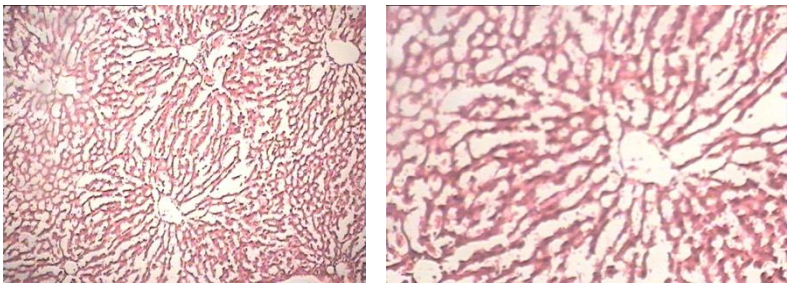

Fig. 1: Group I rats (normal control).

The CCl_4 administered rats (group II) liver sections revealed severe congestion of the central vein, disorganization of hepatic cords, moderate congestion of the hepatic artery, sinusoids and portal vein with dilatation of the portal vein. In addition some hepatocytes were dissociated from hepatic cords, indicating liver injury (figure 2).

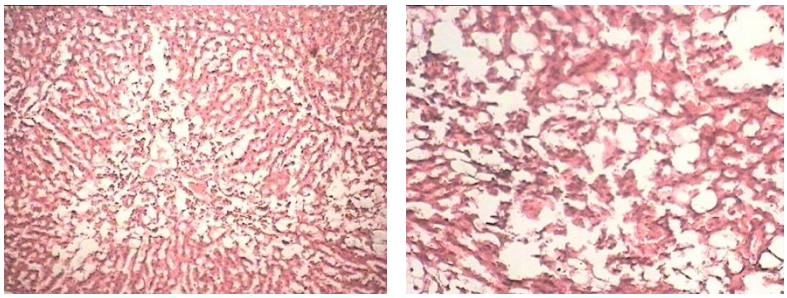

Fig. 2: Group II rats (CCl_4) treated.

Histological observations of group III rats treated with AYU-809 showed normal microscopic architecture of liver. Hexagonal lobules were centered on the central vein and have a portal triad containing branches of the portal vein, hepatic artery and bile duct. This observation indicates safety of AYU-809 on liver tissue (figure 3).

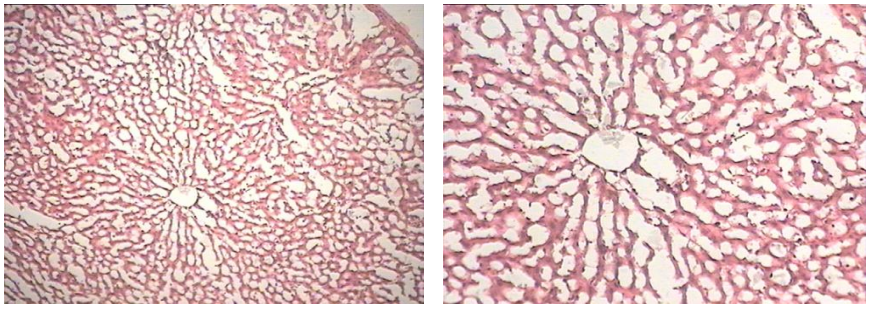

Fig. 3: Group III (AYU-809).

Group IV rat (CCl_4 + AYU 809) sections revealed slight congestion of the central vein, disorganization of hepatic cords, minor congestion of the hepatic artery, sinusoids and portal vein with mild dilatation of the portal vein. Since some hepatocytes were not dissociated from hepatic cords, it suggests a protective effect of AYU-809 from CCl_4 induced injury (figure 4).

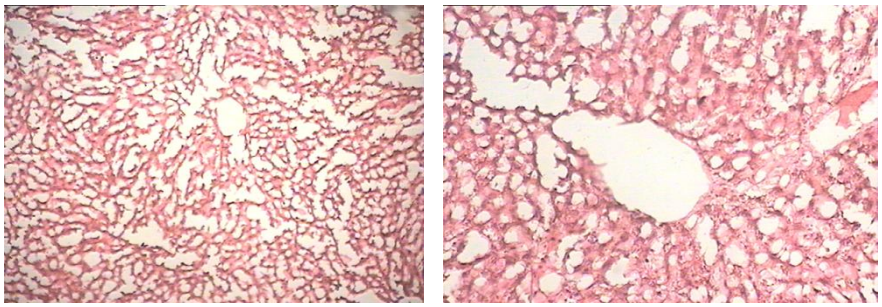

Fig. 4: Group IV(AYU-809+ CCl_4).

The CCl_4 + silymarin administered rat (group V) liver sections revealed slight congestion of the central vein, little disorganization of hepatic cords, slight congestion of the hepatic artery, sinusoids and portal vein with mild dilatation of the portal vein. In addition some hepatocytes were not dissociated from hepatic cords, indicating protective effect of Silymarin from the CCl_4 induced liver injury (figure 5).

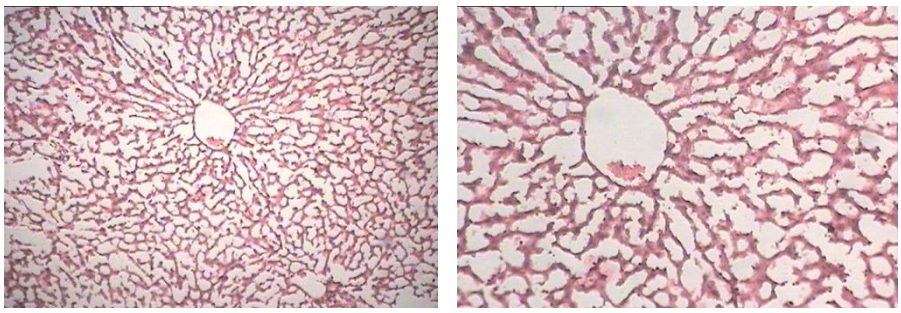

Fig. 5: Group V(Silymarin+CCl_4).

DISCUSSION

Phytochemical investigations-

A preliminary phytochemical investigation of Ayu-809 indicated that AYU 809 consisted of carbohydrate, amino acid, alkaloids, flavonoids, saponins, glycosides and tannins.

Body weight, body circumference and feed efficiency-

Good nutrition is an important factor in helping a child achieves normal growth and development as well as protection from illness. Body weight is one of the most reliable measures of health and nutrition. The present study indicates that AYU-809 significantly increased body weight, body circumference, and feed efficiency when compared rats treated with CCl_4 alone group. In the present study, AYU-809 promoted weight gain during the 28 d study period.

Haemoglobin level-

Blood hemoglobin level was positively correlated with relative body weight and serum IGF-1 and IGFBP-3 (growth factors) levels. Treatment with growth hormone (GH) accelerates growth and elevates the concentrations of hemoglobin and serum IGF-1 and IGFBP-3 levels (ref) CCl_4 treated rats show a decrease in Hemoglobin **(**Hb) content when compared to normal rats. This may be due to a growth inhibitory effect of CCl_4 as growth factors get affected due to liver injury. The rats treated with AYU-809 show an increase in blood Hb content when compared to normal rats. Group IV and V rats show the same effect when the results are compared to group II rats (toxic control). These observations suggest a growth promoting effect of AYU-809. Given that the GH-IGF (growth hormone-insulin derived growth factor) axis is involved in the physiological elevation of Hb levels (ref), it is possible that AYU-809 is working to promote growth by similar mechanisms.

RBC count-

Blood RBC count was positively correlated with body weight and serum IGF-1 and IGFBP-3 (growth factors) levels. Treatment with growth hormone (GH) accelerates the growth and elevates RBC count and serum IGF-1 and IGFBP-3 levels (ref). Group III rats (AYU-809) show a significant increase in RBC count compared to group I normal rats. The group IV (AYU-809+CCl_4) and V (silymarin+CCl_4) rats show significant increases in RBC count when compared with group II disease control rats. These results also support the growth promoting effects of AYU-809.

Serum SGOT, SGPT and ALP-

AST (SGOT) occurs in all human tissues and is present in large amounts in liver, renal, cardiac and skeletal muscle tissue. Increased levels are associated with liver diseases or damage, myocardial infarction, muscular dystrophy and cholecystitis. Increase in ALT (SGPT) levels is greater in hepatocellular disease compared to AST. In viral hepatitis associated with necrosis the elevation of enzyme may be of 20 to 50 fold, in alcoholic hepatitis there is moderate increase in the enzyme but in viral hepatitis ALT levels will be increased before initiation of jaundice and persists slightly even after disappearance of jaundice. he rats treated with AYU-809+CCl_4 (group IV) have also shown significant decrease in the serum

Elevations in serum ALP activity commonly originates from the liver and bone. Consequently, serum ALP measurements are of particular interest in the investigation of hepatobiliary disease and bone disease associated with increased osteoblastic activity. ALP level when compared to CCl_4 control rats. The results of this study show insignificant activity of serum ALP, SGOT and SGPT in AYU 809 treated rats (group II) when compared to the normal rats, suggesting that AYU-809 is safer to vital organs such as the liver.

Tissue LPO and SOD-

Intrauterine fetal growth restriction (IUGR), the main cause of premature delivery and fetal mortality, has been suggested to involve oxidative stress. The elevated values of indices of oxidative stress in the blood serum of pregnant women with increased levels of malondialdehyde and 4-hydroxyalkenals decreased total antioxidant capacity of the serum, with respect to healthy pregnancy.

This study shows that group II rats have showed significant increase in LPO activity when compared to group I normal rats. This could be due to induction of lipid peroxidation caused by CCl_4 in group II rats. The group III rats (AYU-809) show insignificant change in the LPO level suggesting that AYU-809 is safer for liver tissue. Significant decrease in the LPO level in group IV rats (CCl_4 and AYU-809) and group V rats (AYU-809and Silymarin) may be due to hepatoprotective activity of AYU-809 and Silymarin.

Histopathological observations:

The histopathology of normal rat liver reveals normal microscopic architecture composed of hexagonal lobules and acini. Hexagonal lobules are centered on the central vein and have a portal triad containing branches of the portal vein, hepatic artery and bile duct. The histopathological observations of normal rats confirm the normal heath of the liver tissue. The CCl_4 administered rats (group II) liver sections revealed severe congestion of the central vein, disorganization of hepatic cords, moderate congestion of the hepatic artery, sinusoids and portal vein with dilatation of the portal vein. In addition some hepatocytes were dissociated from hepatic cords, indicating liver injury. These observations confirm the hepatotoxicity effects caused by the administration of CCl_4. The exposure of CCl_4 leads to the formation of its metabolite and this metabolite forms covalent bond with the cellular components of the liver leading to its toxicity. The cellular components may be lipids and proteins. Binding occurs preferentially to triglycerides and phospholipids. The liver injury is also due to lipid peroxidation potency of CCl_4.

The histological sections of rats (III group) (treated with AYU-809 alone) show normal microscopic architecture of the liver. Hexagonal lobules are centered on the central vein and have a portal triad containing branches of the portal vein, hepatic artery and bile duct. These observations suggest that AYU-809 is safe for liver tissue.

Histology slides of liver tissue in group IV rats (CCl_4 + AYU- 809) show slight congestion of the central vein, some disorganization of hepatic cords, slight congestion of the hepatic artery, sinusoids and portal vein with dilatation of the portal vein. In addition, some hepatocytes were not dissociated from hepatic cords, indicating protective effect of AYU-809 from the CCl_4 induced liver injury. This protection of AYU-809 may be due to inhibitory effect on CCl_4 binding with lipids and proteins. The protection may also be due to the blockage of lipid peroxidation caused by CCl_4.

The histology examination of liver tissue of group V rats (CCl_4 + Silymarin), shows slight congestion of the central vein, some disorganization of hepatic cords, slight congestion of the hepatic artery, sinusoids and portal vein with mild dilatation of the portal vein. In addition some hepatocytes were not dissociated from hepatic cords, indicating protective effect of Silymarin from the CCl_4 induced liver injury. This protection of silymarin may be due to inhibition effect on CCl_4 binding with lipids and proteins. The protection may also due to the blockage of lipid peroxidation caused by CCl_4. The growth retardation is also due to oxidative stress due to the release of oxygen free radicals. SOD levels decreased in CCl_4 treated rats. The increase in the levels of tissue (liver) SOD is an index of hepatoprotective and health benefit of AYU-809.

CONCLUSION

Our findings suggest that AYU-809, a general nutritional supplement formulated by Nutraceuticals division M/s Ayurwin Pharma Pvt Ltd, Bengaluru, possesses growth promoting, hepato protecting and antioxidant properties. The data provide useful insight into the possibility of using AYU-809 to promote the growth in children associated with growth related disorders. Future studies aimed at understanding the mechanisms by which AYU-809 exerts its growth promoting and other health benefits is certainly warranted.

REFERENCES

1. Jiongran Chen, Yanfei Yang, Sepideh Abbasi, Daryoush Hajinezhad, Saija Kontulainen and Ali Honaramooz. The effects of Elk Velvet Antler dietary supplementation on physical growth and bone development in growing rats. Evid Based Complement Alternat Med.2015; 2015, 1-10.
2. Goodman, Gilman. The Pharmacological Basis of Therapeutics. 10th ed. McGraw- Hill; 2001.
3. Rang HP, Dale MM, Ritter JM, Moore PK. Pharmacology. 5th ed. London: Churchill Livingstone; 2003.
4. Available from [online, nutritional supplement, dated 27 feb. 2016] URL: http://medical-dictionary.thefreedictionary.com/Nutritional+Supplements
5. Hajian S. Positive effect of antioxidants on immune system. Immunopathologia Persa 2015;1(1).
6. Zabriskie JB. Essential clinical immunology. New York: Cambridge University Press; 2009.
7. El-Missiry MA. Biochemistry, Genetics and Molecular Biology. Croatia: Intec; 2012.
8. Mates JM, Gomez CP, Decastro IN. Antioxidant Enzymes and Human Diseases. Clinical Biochemistry. 1999;32 (8) :595–603.
9. Eidil A, Mortazavi P, Tehrani1 ME, Rohani AH, Safi S. Hepatoprotective effects of pantothenic acid on carbon tetrachloride-induced toxicity in rats. EXCLI Journal 2012;11:748-59.
10. Sujaa SR, Lathaa PG, Pushpangadan P, Rajasekharana S. Evaluation of hepatoprotective effects of *Helminthostachys zeylanica* (L.) Hook against carbon tetrachloride-induced liver damage in Wistar rats. Journal of Ethnopharmacology. 2004; 61–6.
11. Nidhiya ISR, Pai KSR, Mallikarjun Rao C. Growth promoting potential of *Ficus bengalensis* root extracts in immature female rats. 2009. 47(4): 268-73.
12. Vipul Gujrati, Nilesh Patel, Venkat N Rao, Nandakumar K, Md.Shalam, Shanta Kumar SM. Hepatoprotective activity of alcoholic and aqueous extracts of leaves of *Tylophora indica* (Linn.) in rats. 2007, 39(1), 43-7.
13. Jawaid T, Shakya AK, Siddiqui HH. Effect of ethanolic extract of *Bambusa arundinacea* on scopolamine induced amnesia in rats. Excli Journal 2012;11:748-59.
14. Herck H Van, Baumans V, Brandt C. J. W. M, Boere H A G, Hesp A. P. M. H. Lith A Van, Schurink M *et al.* Blood sampling from the retro-orbital plexus, the saphenous vein and the tail vein in rats: comparative effects on selected behavioral and blood variables. Laboratory animals. 2001; 35, 131-9.
15. Noori S, Nasir K and Mahboob T. Effects of cocoa powder on oxidant/ antioxidant status in liver, heart and kidney tissues of rats. The Journal of Animal & Plant Sciences.2009; 9(4), 174-8.