Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 9[12] November 2020 :142-148

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD
Global Impact Factor 0.876
Universal Impact Factor 0.9804
NAAS Rating 4.95

ORIGINAL ARTICLE **OPEN ACCESS**

Therapeutic Application of Novel Multi Herbal Formulation (AKSS16LIV01) against the inductive influence of Carbon Tetrachloride (CCl_4) upon Tissue and serum protein in Experimental animals

Soumendra Darbar[1,2], Srimoyee Saha[3], Kausikisankar Pramanik[2] and Atiskumar Chattopadhyay[1]*
[1]Faculty of Science, Jadavpur University, Raja S C Mallick Road, Kolkata-700032, West Bengal, India
[2]Department of Chemistry, Jadavpur University, Raja S C Mallick Road, Kolkata-700032, West Bengal, India
[3]Department of Physics, Jadavpur University, Raja S C Mallick Road, Kolkata-700032, West Bengal, India
E-mail:atischatterjee@gmail.com

ABSTRACT
Serum proteins are an important indicator of the nutritional status. Disturbance in normal protein levels indicates vital organs dysfunctions which may be fatal for life. The main aim of the study was to determine the serum and tissue protein levels against CCl_4 intoxication and its mitigation by newly developed novel herbal medicine (AKSS16-LIV-01). Healthy adult swiss albino mice were assigned to four groups of six mice each according to their weights. Group-I serve as control, Group-II received Multi herbal formulation(AKSS16-LIV01)400 mg/kg/day, Group-III received carbon tetrachloride (CCl_4) 1 ml/kg-bw and Group-IV received CCl_4(1 ml/kg-bw) along withAKSS16-LIV01 (400 mg/kg).Administration of carbon tetrachloride (CCl_4) showed decline body weight, food consumption and water intake in mice whereas treatment with Multi herbal formulation(AKSS16-LIV01)normalized the same as compared with untreated animals. Treatment with CCl_4 (Group-III) decline the total protein, albumin and globulin levels in serum, liver and kidney compared with control. Pre-treatment with AKSS16-LIV01 significantly ($p<0.001$) increased thetotal protein, albumin and globulin levels in serum, liver and kidney compared CCl_4 intoxicated animals. On the other hand higher level of albumin/globulin ratio clearly indicate that liver and kidney might be affected by CCl_4 treatment. Multi herbal formulation (AKSS16-LIV01) protect the liver and kidney by maintaining the albumin/globulin ratio. So, in conclusion it may be predicted that phyto constituents and antioxidant enrich this novel formulation maintain normal protein pattern and protects the body from various dysfunctions.
Keywords:*Multi herbal formulation;serum protein; albumin; globulin; Swiss albino mice*

Received 21.09.2020 Revised 09.10.2020 Accepted 20.11.2020

INTRODUCTION

The body needs *protein* to function and survive. Alteration of serum total protein leads to various complications and sometimes damage vital organs[1]. The major components of the serum protein are albumin and globulin, represents the nutritional status of the body which maintain the colloidal osmotic pressure in blood[2-4]. These two proteins also maintain body's immune function, prevent infection[5]. Scientific literature revelled that albumin-to-globulinratio (AGR) is a prognostic factor of various diseases and medical complications[6,7]. Clinical study indicate that decline albumin levels showed poor nutrition status, sometimes very fatal to survive [8,9].

Carbon tetrachloride (CCl_4) is a major industrial pollutant associated with production of free radicals which creates various organ dysfunction like liver and kidney[10]. It is established that metabolic activation ofCCl_4 by cytochrome P450 produced trichloromethyl radical ($\cdot CCl_3$)and peroxytrichloromethylradical ($\cdot OOCCl_3$) which initiates lipid peroxidation, responsible for membrane disruption leads to liver and kidney injury[11]. Long-time exposure of CCl_4 alter the normal protein level in the body which creates various type of organ dysfunctions[12]. Animal study showed that

administration of CCl_4 decrease normal food and water intake, produce nutrition deficiency syndrome[13].

Long term safe and symptomatic medication without side effects is one of the main approach of alternative system of medicine comprising herbal products[14]. The plant based formulation is enrich with various essential phytochemicals and enormous antioxidants, serrates to prevent diseases[15,16]. With view this concept, we developed a novel, low cost herbal formulation composed of six Indian medicinal plants and three Indian spices. Our previous study upon animals showed that this formulation does not produce any toxic effects upon animals and safe for therapeutic medication[17,18]. Here we try to apply this traditional medicine for maintaining the essential protein levels caused by CCl_4.

MATERIAL AND METHODS

Chemicals

Carbon tetrachloride (CCl_4) and TRIS buffer were obtained from Merck, India. PBS pH 7.4 was procured from Sigma-Aldrich. Biochemical determination kits i.e. total protein, albumin and globulin were procured from Thermo Scientific, USA. All others reagents used in this study are laboratory grade.

Preparation of plant extract

All the medicinal plant and spice ingredients were collected from registered local herbal suppliers and authenticated by pharmacognosist. Plants parts were cleaned and dry with normal temperature. The dried plant parts were used for preparation of multi herbal formulation as per standard validated protocol[19]. The plants and plant parts used in preparation of the extract are listed in Table 1.

Animals

Twenty four young, healthy swiss albino mice weighing 25g ± 5g have been randomly included for the study. The animals have been housed in healthy atmospheric conditions (12 h light and dark cycles, at 25±2 ºC and 50-60% humidity), normal feeding, drinking, and medical care based on the CPCSEA guidelines. Mice were kept under observation for one week before the onset of the experiment for acclimatization and to exclude any unsercurrent infection. The experimental procedures were approved by the Institutional Animal Ethics Committee (IAEC) (Approval No. 261/JU/s/IAEC/Pharma/2018).

Experimental procedure

The mice were randomly assigned to four major groups of six mice each according to their body weights such that each group was made up of mice within the close range of body weight. The groups are as follows: Group-I serve as control, Group-II received Multi herbal formulation (AKSS16-LIV01) 400 mg/kg/day, Group-III received carbon tetrachloride (CCl_4) 1 ml/kg-bw and Group-IV received CCl_4 along with AKSS16-LIV01 (400 mg/kg).

Body weight, food consumption and water intake

Body weights were measured on weekly basis from the initial day to the final day of experiment to calculate body weight alteration. Feed intake was determined by measuring feed residue on weekly basis since the beginning of the experiment. Feed conversion was obtained by dividing total feed intake by body weight gain. Water intake was determined by subtracts the remaining of water found in the drinking bottle from the initial water given to the animals.

Blood Collection and serum preparation

At the end of the respective fasting period, blood was collected from each mouse by retro orbital venous puncture. 200 µL of blood sample were collected into micro-centrifuge tubes with and without EDTA (2%). Collected bloods were placed in slanting position at room temperature for 2 hrs. Then, they were centrifuged at 3500 g for 10 min. after centrifugation clear light yellow colour serum was separated and used for further analysis.

Preparation of tissue homogenate

A small portion of the liver and kidney tissues was homogenized in ice-cold 0.9% w/v saline using a homogenizer to obtain 20% homogenate. Aliquots of the liver homogenate were stored at 4^0C prior to biochemical analysis.

Determination of serum, liver and kidney protein

Serum and tissue homogenate were used for the determination of total protein, albumin and globulin. Total protein, albumin and globulin were determined according to the standard biochemical protocol with slight modification using colorimetric kit obtained from Thermo Scientific, USA.

Statistical analysis

Data are presented as mean ±SE. Statistical analysis of the data was carried out using two way analysis of variance (ANOVA) followed by Tukey's test for post hoc analysis. Statistical significance was acceptable to a level of $p< 0.05$.

RESULTS

Effect of multi herbal formulation (AKSS16-LIV01) on Body weight, Food Consumption and Water Intake

Gross body weights and relative changes, food consumption and water intake was presented in table 2. Administration of carbon tetrachloride (CCl_4) (1 ml/kg-bw) significantly reduced ($p<0.001$) the body weight, food intake and water intake capacity as compared with control animals. Treatment with multi herbal formulation (AKSS16-LIV01) 400mg/kg/day normalized the body weight, daily food intake and water intake capacity as compared with control animals. Administration of AKSS16-LIV01 did not show any abnormal changes as compared with control animals.

Effect of multi herbal formulation (AKSS16-LIV01) on serum, liver and kidney total protein

Figure 1 shows the mean serum, liver and kidney total protein (TP) levels in control and experimental groups of mice. Data indicate that CCl_4 intoxicated mice had significantly lower mean serum liver and kidney total protein compared with the control ($p<0.001$). Pre-treatment with multi herbal formulation (AKSS16-LIV01) at a dose of 400 mg/kg/day significantly increased the decline total protein levels when compared with CCl_4 treated mice. 28days treatment with newly developed multi herbal formulation (AKSS16-LIV01) at a dose of 400 mg/kg/day alone did not shows any significant differences in serum, liver and kidney protein levels when compared with control group.

Effect of multi herbal formulation (AKSS16-LIV01) on serum, liver and kidney albumin

Figure 2 shows the mean serum, liver and kidney albumin levels in control and experimental groups of mice. Data indicate that CCl_4 intoxicated mice had significantly lower mean serum liver and kidney albumin compared with the control ($p<0.001$). Pre-treatment with multi herbal formulation (AKSS16-LIV01) at a dose of 400 mg/kg/day significantly increased the decline albumin levels when compared with CCl_4 treated mice. 28days treatment with newly developed multi herbal formulation (AKSS16-LIV01) at a dose of 400 mg/kg/day alone did not shows any significant differences in serum, liver and kidneyalbumin levels when compared with control group.

Effect of multi herbal formulation (AKSS16-LIV01) on serum, liver and kidney globulin

Figure 3 shows the mean serum, liver and kidney globulin levels in control and experimental groups of mice. Data indicate that CCl_4 intoxicated mice had significantly lower mean serum liver and kidney globulin compared with the control ($p<0.001$). Pre-treatment with multi herbal formulation (AKSS16-LIV01) at a dose of 400 mg/kg/day significantly increased the decline globulin levels when compared with CCl_4 treated mice. 28days treatment with newly developed multi herbal formulation (AKSS16-LIV01) at a dose of 400 mg/kg/day alone did not shows any significant differences in serum, liver and kidney globulin levels when compared with control group.

Effect of multi herbal formulation (AKSS16-LIV01) on serum, liver and kidney albumin/globulin (AGR) ratio

Table 3 shows the mean serum, liver and kidney albumin/globulin ratio in control and experimental groups of mice. Data indicate that CCl_4 intoxicated mice had significantly higher mean serum liver and kidney albumin/globulin ratio (AGR) compared with the control ($p<0.001$). Pre-treatment with multi herbal formulation (AKSS16-LIV01) at a dose of 400 mg/kg/day normalized the decline albumin/globulin ratio(AGR) levels when compared with CCl_4 treated mice. 28days treatment with newly developed multi herbal formulation (AKSS16-LIV01) at a dose of 400 mg/kg/day alone did not shows significant differences in serum, liver and kidney albumin/globulin ratio (AGR) levels when compared with control group.

Table 1 Details ofingredient(s) present in the newly developed multi herbal formulation

Sl. No.	Botanical Name	Common Name	Family	Quantity used in extract
1.	*Tinosporacordifolia*	Guduchi	Menispermaceae	20 mg
2.	*Terminaliachebula*	Haritaki	Combretaceae	20 mg
3.	*Azadirachtaindica*	Neem	Meliaceae	50 mg
4.	*Andrographispaniculata*	Kalmegh	Acanthaceae	50 mg
5.	*Aloe barbadensis miller*	Aloe vera	Liliaceae	50 mg
6.	*Curcuma longa*	Curcuma, Haldi	Zingiberales	20 mg
7.	*Trigonellafoenum-graecum*	Methi	Fabaceae	10 mg
8.	*Piper nigrum*	Black pepper	Piperaceae	10 mg
9.	*Elettariacardamomum*		Zingiberaceae	10 mg

* Amount required for preparation of 5 ml extract.

Table-2: Effect of multi herbal formulation (AKSS16-LIV01) on body weight, food consumption and water intake

Parameters	Mice			
	Group-I	Group-II	Group-III	Group-IV
Body weight (g) Initial	26.35±1.91	26.51±2.35	26.71±4.2	26.68±5.1
Body weight (g) Final	37.84±2.03	36.94±1.69	21.81±2.41#	36.97±1.67*
Body weight (g) gain or loss	11.49±0.06	10.43±0.04	4.90±0.006	10.29±0.03
Food consumption (g)	4.52±0.05	4.37±0.07	2.94±0.06#	5.11±0.04*
Water intake (ml)	4.01±0.04	4.25±0.04	3.01±0.02#	4.31±0.06*

All data were expressed as means± SE (n=6/group). Data comparison was performed using two way ANOVA followed by Tukey's Multiple Comparison Test. #Significantly different from the control group at p<0.001 and *Significantly different from (CCl_4) group values at p<0.001

Table 3: Effect of AKSS16-LIV01 on serum, liver and kidney albumin/globulin (AGR) in CCl_4 induced toxicity

Groups	Albumin/Globulin ratio		
	Serum	Liver	Kidney
Control	1.32±0.12	1.45±0.14	1.48±0.11
AKSS16-LIV01	1.31±0.11	1.37±0.16	1.41±0.12
CCl_4	1.76±0.16	1.58±0.14	1.57±0.15
CCl_4 + AKSS16-LIV01	1.26±0.13	1.43±0.19	1.50±0.18

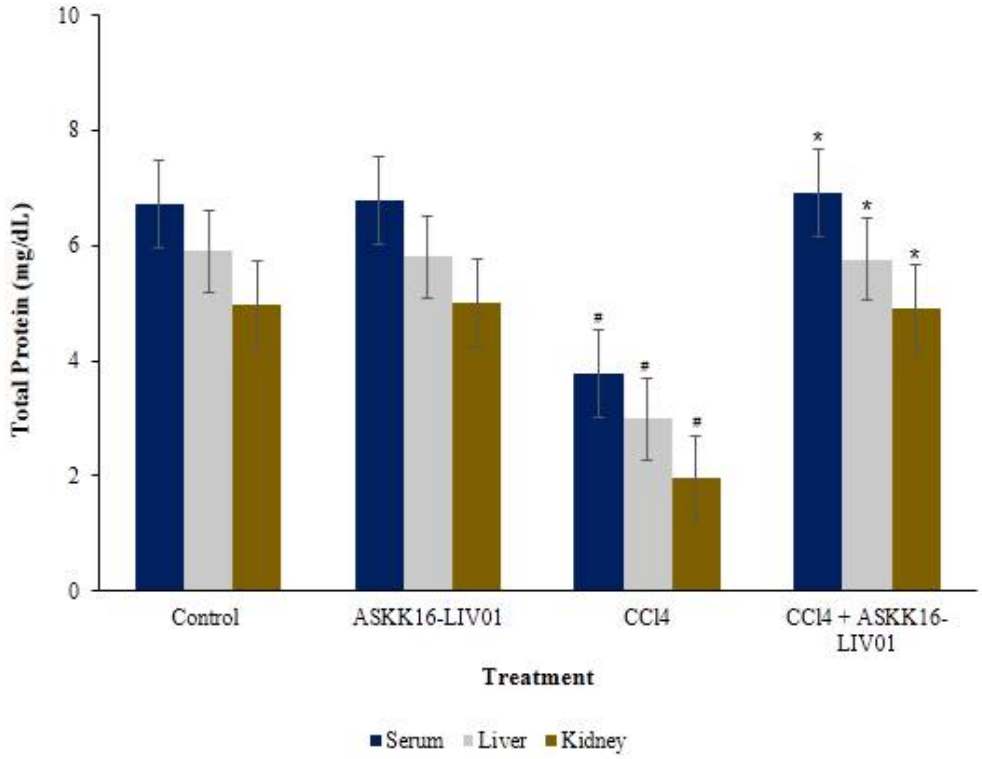

Figure 1: Effect of multi herbal formulation (AKSS16-LIV01) on Total protein levels in mice. All data were expressed as means± SE (n=6/group). #significantly different from the control group at p<0.001 and *significantly different from (CCl_4) group values at p<0.001. Data comparison was performed using one way ANOVA followed by Tukey's Multiple Comparison Test.

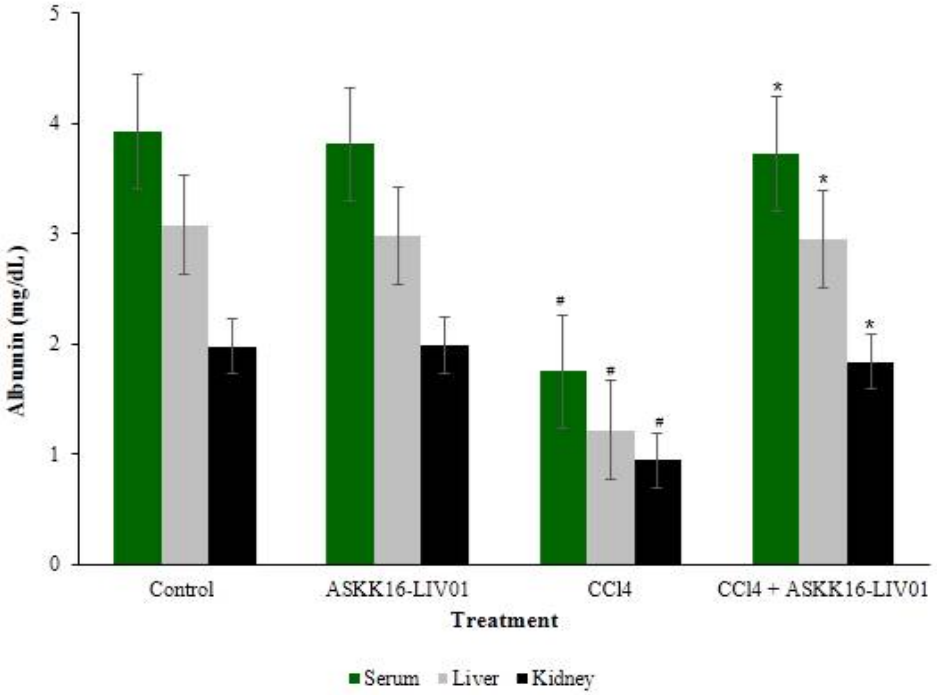

Figure 2: Effect of multi herbal formulation (AKSS16-LIV01) on albumin levels in mice. All data were expressed as means± SE (n=6/group). #significantly different from the control group at p<0.001 and *significantly different from (CCl_4) group values at p<0.001. Data comparison was performed using one way ANOVA followed by Tukey's Multiple Comparison Test.

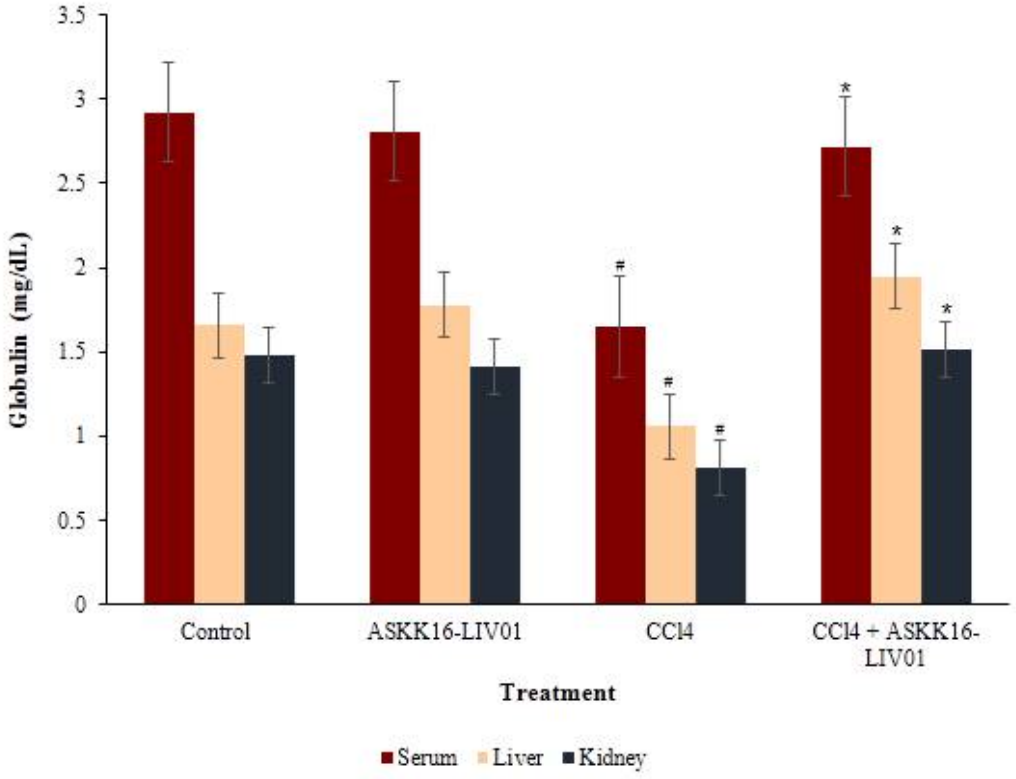

Figure 3: Effect of multi herbal formulation (AKSS16-LIV01) on globulin levels in mice. All data were expressed as means± SE (n=6/group). #significantly different from the control group at p<0.001 and *significantly different from (CCl_4) group values at p<0.001. Data comparison was performed using one way ANOVA followed by Tukey's Multiple Comparison Test.

DISCUSSION

Various secondary metabolites of the medicinal plants are mainly responsible for therapeutic effects[21]. Poly herbal drug are very useful for treatment of various diseases due to the synergistic effects of different plants [22]. Phenolic compounds and flavonoids present in the aromatic plants are mainly responsible for pharmacological functions and prevent oxidative stress [23]. Our study showed that administration of carbon tetra chloride (CCl_4) inhibit normal body growth, food consumption and water intake. Co administration of our developed formulation retained the body weight, food consumption and water intake.

Protein is responsible for normal body growth and development. Abnormal protein level inhibit the body growth which may be occur when subject exposed with environmental toxin [24,25]. Total serum protein is an indicator in liver and kidney damage [26]. In the present study we observed that carbon tetra chloride (CCl_4) significantly decreased the serum, liver and kidney protein levels. Co administration with AKSS16-LIV01 maintained the normal serum, liver and kidney protein levels.

Albumin play a crucial role to maintain physiological activities of human body [27,28]. It is one of the liver biomarker as it generates from the liver cells. Low level of albumin is responsible for poor nutrition [29-32]. In this study we observed that chronic administration of CCl_4 decline normal albumin levels in serum, liver and kidney which was recovered when animals pre-treated with novel multi herbal formulation (AKSS16-LIV01). The result clearly indicate that AKSS16-LIV01 capable to maintain the normal albumin level against the environmental toxicant like CCl_4. On the other hand scientific study revealed that serum globulin is involved in chronic inflammation. Recent study showed that carbon tetra chloride (CCl_4) alter the serum, liver and kidney globulin and disrupt normal homeostasis. Our study also confirm that application of CCl_4 decreased normal globulin levels in serum, liver and kidney. Treatment with the developed formulation (AKSS16-LIV01) normalized the globulin level in experimental animals. Albumin/globulin ratio also confirm the protein alteration.

CONCLUSION

Chronic administration of carbon tetrachloride (CCl_4) suppressed the normal body growth and reduced normal food and water intake capacity in mice. This environmental toxin reduced the total protein, albumin and globulin levels both in serum and tissues. Our developed novel multi herbal formulation might be able to maintain the normal essential protein values and prevent the CCl_4 induced deleterious effects in mice. Thus, we believe that the developed formulation composed of medicinal herbs and medicinal spices might be a therapeutic medicine in future.

ACKNOWLEDGEMENT

The authors are thankful to Prof. S K Pal, Senior Professor Department of Chemical, Biological & Macromolecular Sciences S N Bose National Centre for Basic Sciences JD Block, Sector IIISalt Lake City for his guidance and valuable suggestion during this investigation.

CONFLICT OF INTEREST

All authors report no conflicts of interest regarding this manuscript.

REFERENCES

1. Jiaxi He, Hui Pan, Wenhua Liang, Dakai Xiao, Xuewei Chen, MinzhangGuo, Jianxing He[2017]. Prognostic Effect of Albumin-to-Globulin Ratio in Patients with solid tumors: A Systematic Review and Meta-analysis. *Journal of Cancer*;8(19): 4002-4010.
2. Ataseven B, du Bois A, Reinthaller A, Traut A, Heitz F, Aust S, et al.[2015]. Pre-operative serum albumin is associated with post-operative complication rate and overall survival in patients with epithelial ovarian cancer undergoing cytoreductive surgery. *GynecolOncol.;*138(3):560-565.
3. Chiang J.M, Chang C.J, Jiang SF, Yeh C.Y, You J.F, Hsieh P.S, et al. [2017]. Pre-operative serum albumin level substantially predicts post-operative morbidity and mortality among patients with colorectal cancer who undergo elective colectomy. *Eur J Cancer Care (Engl);*26(2).
4. Ishizuka M, Nagata H, Takagi K, Iwasaki Y, Shibuya N, Kubota K[2016]. Clinical Significance of the C-Reactive Protein to Albumin Ratio for Survival After Surgery for Colorectal Cancer. *Ann SurgOncol;*23(3):900-907.
5. Jiang H, Li H, Li A, Tang E, Xu D, Chen Y, et al. [2016]. Preoperative combined hemoglobin, albumin, lymphocyte and platelet levels predict survival in patients with locally advanced colorectal cancer. *Oncotarget;*7(44):72076-72083.
6. Zhou T, Zhan J, Hong S, Hu Z, Fang W, Qin T, et al. [2015]. Ratio of C-Reactive Protein/Albumin is An Inflammatory Prognostic Score for Predicting Overall Survival of Patients with Small-cell Lung Cancer. *Sci Rep.;*5:10481.
7. Gabay C, Kushner I[1999]. Acute-phase proteins and other systemic responses to inflammation. *N Engl J Med.;*340(6):448-454.

8. Ritchie K, Touchon J[2000]. Mild cognitive impairment: conceptual basis and current nosological status. *Lancet;*355(9199):225–228.
9. DeCarli C[2003]. Mild cognitive impairment: prevalence, prognosis, aetiology, and treatment. *Lancet Neurol.;*2(1):15–21.
10. He S.X, Luo J.Y, Wang Y.P, Wang Y.L, Fu H, Xu J.L[2006]. Effects of extract from Ginkgo biloba on carbon tetrachloride-induced liver injury in rats. *World J Gastroenterol;* 12: 3924-3928.
11. Adewole S.O, Salako A.A, Doherty O.W, Naicker T[2007]. Effect of melatonin on carbon tetrachloride_induced kidney injury in Wistar rats. *Afr J Biomed Res;* 10: 153-164.
12. Khan M.R, Rizvi W, Khan G.N, Khan R.A, Shaheen S. Carbon tetrachloride induced nephrotoxicity in rat: protective role of Digeramuricata (L.) Mart. *J Ethnopharmacol;* 122: 91-99.
13. Sreelatha S, Padma P.R[2009].Umadevi M. Protective effects of Coriandrumsativum extracts on CCl4-induced hepatotoxicity in rats. *Food ChemToxicol;* 48: 702-708.
14. Rajesh M.G, Latha M.S[2004]. Protective activity of *Glycyrrhizaglabra*Linn. on carbon tetrachloride-induced peroxidative damage. *Indian J. Pharmacol;*36: 284-287.
15. Sane R.T, Kuber V.V, Chalissery M.S, Menon S[1995]. Hepatoprotection by *Phyllanthus amarus* and *Phyllanthus debili* in CCl4 induced liver dysfunction. *CurrSci*; 68: 1243-1246.
16. Rawat A.K.S, Mehratra S, Tripathi S.C, Shome U[1997]. Hepatoprotective activity of *Boerhaavia diffusa* L. root- a popular Indian ethnomedicine. *J. Ethanopharmacol*; 56: 61-66.
17. Darbar S, Saha S, Pramanik K.S and Chattopadhyay A.K[2018]. Preliminary Acute Oral Toxicity Study of a Newly Developed Herbal Formulation. *World Journal of Pharmaceutical Research*; 7(5): 924-930.
18. Darbar S, Saha S, Pramanik K.S and Chattopadhyay A.K [2019]. Preliminary Assessment of Acute and 28-Day Repeated Dose Oral Toxicity of a Newly Developed Herbal Mixture on Experimental Animal. *Indian Journal of Pharmaceutical Education and Research;* 53(4) (Suppl): 669-676.
19. Adhikari A, Darbar S, Chatterjee T, Das M, Polley N, Bhattacharyya M, Pal S.K[2018]. Spectroscopic Studies On Dual Role Of Natural Flavonoids In Detoxification Of Lead Poisoning: Bench-To-Bedside Preclinical Trial. *Acs Omega;3*(11): 15975-15987.
20. Saini R, Mishra1 A.K, Mishra S.K, Tiwari K.N[2019]. Evaluation of Polyphenolic Content and Anti-oxidant Potential of Poly-herbal Formulation.*International Journal on Emerging Technologies;* 10(2): 243-248.
21. Mahdi-Pour B. Jothy, S.L Latha, L.Y Chen, Y, Sasidharan S[2012]. Antioxidant activity of methanol extracts of different parts of Lantana camara. *Asian Pacific Journal of Tropical Biomedicine;*2: 960-965.
22. Ekor M[2014]. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. *Frontiers in Pharmacology;*4: 1-10.
23. Kumar A, Kulkarni S.K[2006]. Effect of BR-16A (Mentat), a polyherbal formulation on drug-induced catalepsy in mice. *Indian Journal of Experimental Biology;*44:45-48.
24. Gupta D, Lis C.G[2010]. Pretreatment serum albumin as a predictor of cancer survival: a systematic review of the epidemiological literature. *Nutr J.;*9:69.
25. Onate-Ocana L.F, Aiello-Crocifoglio V, Gallardo-Rincon D, Herrera-Goepfert R, Brom-Valladares R, Carrillo JF, et al.[2007]. Serum albumin as a significant prognostic factor for patients with gastric carcinoma. *Ann SurgOncol.;*14(2):381-389.
26. Sejima T, Iwamoto H, Masago T, Morizane S, Yao A, Isoyama T, et al.[2013]. Low pre-operative levels of serum albumin predict lymph node metastases and ultimately correlate with a biochemical recurrence of prostate cancer in radical prostatectomy patients. *Cent European J Urol.;*66(2):126-132.
27. Suh B, Park S, Shin D.W, Yun J.M, Keam B, Yang H.K, Ahn E, Lee H, Park J.H, Cho B[2014]. Low albumin-to-globulin ratio associated with cancer incidence and mortality in generally healthy adults. *Ann Oncol;*25(11):2260–2266.
28. Queiroz M.J.R.P, Ferreira I.C.F.R, Calhelha R.C, Estevinho L.M[2007]. Synthesis and antioxidant activity evaluation of new 7- aryl or 7-heteroarylamino-2,3-dimethylbenzo[b]thiophenes obtained byBuchwald_Hartwig C-N cross-coupling. *Bioorg Med ChemLett;* 18: 1788-1794.
29. Rice-Evans C.A, Miller N.J, Paganga G[1996]. Structure_antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biol Med;* 20: 933-938.
30. He X, Guo S, Chen D, Yang G, Chen X, Zhang Y, et al.[2017]. Preoperative Albumin to Globulin Ratio (AGR) as Prognostic Factor in Renal Cell Carcinoma. *J Cancer;*8(2):258-265.
31. Toiyama Y, Yasuda H, Ohi M, Yoshiyama S, Araki T, Tanaka K, et al. [2017]. Clinical impact of preoperative albumin to globulin ratio in gastric cancer patients with curative intent. *Am J Surg.;*213(1):120-126.
32. Xu WZ, Li F, Xu ZK, Chen X, Sun B, Cao JW, et al.[2017]. Preoperative albumin-to-globulin ratio and prognostic nutrition index predict prognosis for glioblastoma. *Onco Targets Ther.;*10:725-733.

CITATION OF THIS ARTICLE

B C Semwal, A Mishra, S Singh. *In Vivo* Anti-Arthritic Effect of Ethanol Extract of *Plunchea Lanceolata* on Complete Freund's Adjuvant (CFA)-Induced Arthritis in Rats . Bull. Env. Pharmacol. Life Sci., Vol 9[12] November 2020 :142-148