WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 11, Issue 16, 2007-2018. Research Article ISSN 2277– 7105

PHYTOCHEMICAL SCREENING, BIOCHEMICAL PARAMETERS AND HISTOPATHOLOGICAL CHANGES IN LIVER OF ALBINO RATS ORALLY DOSED WITH *CHROZOPHORA PLICATA* ETHANOLIC EXTRACT

Manal A. Ibrahim*[1], Safia A. Ahmed[1] and Nadia N. Abd-AL-Gader[2]

[1]Department of Botany, Faculty of Science and Technology, Omdurman Islamic University, Sudan.

[2]Department of Biochemistry, Faculty of Nursing Sciences, Omdurman Islamic University, Sudan.

Article Received on
19 October 2022,
Revised on 09 Nov. 2022,
Accepted on 29 Nov. 2022
DOI: 10.20959/wjpr202216-26406

***Corresponding Author**
Manal A. Ibrahim
Department of Botany, Faculty of Science and Technology, Omdurman Islamic University, Sudan.

ABSTRACT

Chrozophora plicata (Family Euphorbiaceae) traditionally used in folkloric medicine to cure some diseases. The leaves were extracted and tested for their secondary metabolites and toxic effects. *C plicata* ethanolic extracts represented high presence of saponins and tannins, moderate presence of steroids, trace presence of alkaloids and triterpens were observed. Also negative result of Cumarins and flavonoids were detected. Most rats which were dosed with *C plicata* extracts exhibited significant decrease of ALT, AST, ALP, and total protein, globulin and albumin, while significant increase represented in bilirubin. The histopathological change varied between congested portal veins, necrotic, kuppfer cells proliferation and cytoplasmic eosinophilia.

KEYWORDS: Phytochemical screening, Biochemical Parameters, Histopathological Changes, *Chrozophora plicata*.

INTRODUCTION

Plant supplied shelter, oxygen, food and medicine needed by animals with the evolution and need of humans, they recognize and categorize plants materials as their importance and necessities of life. The plants separated, identified and grouped according to their therapeutic value which developed traditional medicine system, (Bajpai et al; 2015). Human societies

have been in close contact with their environments since the beginning of their formation and they used the ingredients of an environment to obtain food and medicine. Awareness and application of plants to prepare food and medicine have been realized through trial and error, and gradually human became able to meet his needs from his surroundings (Jamshidi et al; 2018).

Medicinal plants include a various types of species used in herbalism and some of these plants have a medicinal activities, and they consider as a rich resources of ingredients which can be used in drug development and synthesis. Also they play a critical role in the development of human cultures around the whole world (Rasool Hassan BA 2012).

The traditional system of treatment, differing in concept and protocol, exemplifies well-developed systems such as allopathic, homeopathic, Ayurvedic, and Chinese systems of treatment. Most of the civilized nations have developed their own Materia Medica, compiling details about various plants used for therapeutic purposes (Khan, 2014).

Chrozophora plicata (Family euphorbiaceae) is a prostrate, branched annual or perennial herb to under shrub up to 60 cm high. Leaf blades are ovate (fig. 1) (Tabussum, 2017). It occurs throughout tropical Africa to Northern ripe, South Africa, Egypt, Syria, Palestine, and North-Western India to the Mediterranean, It grows in warmer climate and temperate regions.

In Sudan, the pounded stems or whole plants are used for wounds and improve healing. In Ethiopia an infusion of the seeds and leaves is taken as a laxative. The plant is also used medicinally in Saudi Arabia, Pakistan and India, against jaundice and to purify blood. Literature survey revealed the presence of diterpenoids, triterpenoids, flavonoids, and glucosides in different species of the genus *Chrozophora*, while hydrocarbons, cholesterol, stigmasterol, b-sitosterol, b-amyrin, squalene, octacosanol, hexacosanol and tetracosanol have previously been reported from C. plicata. (Tabussum, et al 2013).

Toxicology studies are the interaction between chemicals and biological systems to determine the potential of chemicals to produce adverse effects in living organisms. Also it investigates the nature, incidence, mechanisms of production, factors influencing their development, and reversibility of such adverse effects (Dekant and Vamvakas, 2000).

0ne major and overriding criterion in the selection of herbal medicines for use in health services is safety. Plants extracts should not only be efficacious but safe for consumption.

Therefore, closely associated with screening of plants extracts for their activities against microorganism or disease conditions is the need to know their toxic potentials (Bulus et al; 2011).

Some unpleasant side effects which may be related to over doses or other factors occur in various plants despite the benefits of them. This may lead to acute toxicity and death but when these problems are carefully addressed, will help to harness the therapeutic potentials of medicinal and aromatic plants for further drug development in the future. (Okigbo et al; 2009).

The major criterion in the selection of herbal medicines for use in health services is safety, and plants extracts should bet safe for consumption. Therefore, the study aimed to evaluate the toxicity and screened the major classes of secondary metabolites affecting in the level of toxicity.

Fig: 1 *Chrozophora plicata.*

Plant Materials and extraction

The plant *chrozophora plicata* obtained from White Nile State and identified at the Institute of medicinal and aromatic plants _ Sudan. Extraction was carried out according to method described by (Sukhdev et al; 2008). The leaves was coarsely powdered using mortar and pestle. Coarsely sample was extracted by soaking in 80% ethanol for about five days then filtrated and evaporated to dryness under reduced pressure using rotary evaporator apparatus.

Phytochemical screening

Phytochemical screening for the active constituents was carried out on ethanolic extract using the methods described by Martinez & Valencia (2003), Sofowora (1993) and Harborne (1984) with few modifications.

Experimental design

Twenty four white male albino rats were obtained from the Institute of medicinal and aromatic _ Sudan, the animals were divided to tow groups. Group 1 the control contain 6 rats which orally dosed with distilled water, group 2 contain 18 rats divided into 3 sub-groups which orally dosed with extract of *Chrozophora plicata* (low 100mg _ medium 500mg_ high 1000mg) for 14 days.

Blood Sampling and Processing

At the end of the experiment (14 days) the rats were decapitated and blood samples were obtained from each rat, the samples were collected in plain tube to obtain serum; blood was left for one hour to clot and the tube was centrifuged at 3000 rpm for 15 minutes and the harvested serum was used for biochemical analysis. The rats were quickly dissected and the whole liver was excised, preserved in a formalin solution. Tissues sectioning were made, stained and histopathological examination was done.

Biochemical Parameters

Serum samples were analyzed for the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and for the concentrations of total protein, albumin, total bilirubin, alkaline phosphate and urea by the Roche Diagnostic Hitachi 902 Analyzer. Assay kits (Bio Systems S. A.Costa Brava, 30, 08030 Barcelona Spain) were employed for the analyses, the parameters determined according to the procedure described in the kits.

Statistical analysis

The collected data were analyzed and expressed as means ± standard deviation SD of six replicates and were subjected to one way analysis of variance (ANOVA) followed by Duncan multiple range test to determine significant differences in all the parameters. Values considered statistically significant at $p < 0.01$ (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

The plant *C plicata* was tested for secondary metabolites by using procedures described by Harborne (1984). The results indicated high presence of saponins and tannins, moderate presence for steroids, trace presence for alkaloids and triterpens, and absence of cumarins and flavonoids (Table 1). The result was disagreed with what was founded by Alsiede; et al (2015).

Table (1): phytochemical screening of the plants.

Compounds	Saponin	Cumarins	Alkaloids	flavonoids	Tannins	Triterpenes	Steroids
Presence	+++	-	+	-	+++	+	++

Keys: + + + = High presence. + + = Moderate presence. + = Weak presence. - = Absent

Biochemical Finding

Activities of certain enzymes detectable in the serum, commonly called the liver enzymes, their uses are broad ranging from screening for liver disease, to monitoring side effects of medications, and to determining responses to treatment for a given liver disease. AST and ALT catalyze the transfer of the α-amino group from alanine and aspartic acid to α-ketoglutaric acid (Lee; 2012). Blood tests are routinely prescribed in healthcare systems to analyze the biochemistry to determine a diseased or a healthy state. (Bandesh 2019).

Administration of 250, 500 and 1000 mg of *(Chrozophora plicata)* extract resulted in a significantly lower mean of ALT (2.63, 6.00 and 4.65 U//L, resp.) in rats than control (18.91U/L), with a reduction estimated by about 86.1%, 68.3% and 75.4%, respectively (Table2). Also it was observed that ALT level was insignificantly lower in the low dose (250 ml) as compared to medium (500 ml) and high (1000 ml) doses (Table 2).

In addition to AST was significantly reduced by administration of *C plicata* extract, the parameter was significantly reduced in the three doses (2.98, 2.65 and 3.70 U/L.) than in control (85.82 U/L) by about 96.5%, 96.9% and 95.7%, respectively. Also it could be observed that this parameter was insignificantly higher in high dose than in low and medium doses by about 24.2% and 39.6%, respectively. A chronology between the use of the suspected substance and compatible liver damage is required, in addition to the exclusion of alternative causes (Nunes; 2020). AST and ALT are considered two of the most important enzymes that indicate liver injury. Generally increased AST and ALT levels are associated with liver cell damage. During the destruction of liver cells a peak in AST and ALT elevation

occurs, but as the process progresses, enzyme levels may decrease even to the normal level.(kiss 2017). However, AST and ALT were considered useful markers for liver damage (Yakubu, 2007). Mohammed et al.(2010) observed that decreased levels of ALT and AST in rats receiving ethanol and butanol fraction of *Buchholzia coricea* to which streptozotocin was added. Also The level of ALP was significantly reduced in low, medium and high dose (1.57, 1.77 and 1.92 U/L.) as compared to control (88.87 U/L) by about 98.2%, 98.0% and 97.8%, respectively, whereas the differences between *(C plicata)* treatment means themselves were statistically insignificant.

Protein level in rats treated with low dose of *(Chrozophora plicata)* extract was significantly reduced (5.25g/l) as compared to control (6.76g/l) as well as that treated with high dose (6.50g/l), with a reduction estimated by about 22.3% and 9.2% respectively, whereas under medium dose it showed no significant differences than control as well as with low and high doses. Serum or plasma protein is a very complex mixture of proteins and their determination is clinically valuable and reflects the internal status of an individual. Proteins are essential for growth and replacement of worn-out tissues. The rate of cell divisions is determined chiefly by the rate of protein synthesis (Baxter et al., 1987). These results may indicate liver damage and reduction in hepatic function due to the plant extract treatment (Yakubu et al., 2007). Reduction in total protein levels was reported by Atere and Ajoa (2009) in rats dosed with crude alkaloidal fraction from *Gnestis ferruginea* D. C. roots.

Administration of high dose of *Chrozophora* extract significantly increased the level of rats albumin (2.6g/L) as compared to control. Statistically, there were no significant differences between control and both low and medium doses, although the latter two treatments had low level of albumin than the former one by about 12.1% and 7.8%. The decreased levels of total protein and albumin may be due to reduction in protein intake from the intestine (Rolls, 2000) which is considered as an indication of diminished synthetic function of the liver damage.

The level of globulin was significantly reduced in low (3.37g/L), medium(5.87g/L) and high (3.92g/L) doses than the control (4.52g/L) by about 25.4%, 14.45 and 13.3%. No significant differences were observed between doses themselves for this parameter, however it was slightly higher in high dose, followed by medium and finally low dose. Liver damage reduced albumin synthesis and overall decreased plasma protein is observed (Dhanotiya, 2004). Also the result illustrated that the high and medium doses reported a significantly higher level of bilirubin (2.17 and 2.0 mg/dl) than control (0.065 mg/dl) and low dose (1.65 mg/dls), with an

increasing estimated by about 3238.5% 31.5% for high dose and 2976.9% and 21.2% for medium dose. Moreover, the level of this parameter was significantly higher in low dose than in control by about 2438.5%. Liver injury was defined as a 2-fold increase in alanine aminotransferase (ALT) or conjugated bilirubin levels above the upper limit of normality (ULN), or a combined increase in aspartate aminotransferase (AST), alkaline phosphatase (AP) and total bilirubin levels, one of them 2 times above ULN(Lucena et al; 2016). The only study from available previous literature was clinical effects on Nubian goats and desert sheep. The poisoning signs were observed in species (salivation, dyspnea, bloat diarrhea) (Galal et al S1988). The high toxicity of extracts may be attributed to high level of tannin and saponin which considered in some classes of it as hepatotoxic(Jess, 1995, Wiesman & chapagain 2005).

Table (2): Effect of administration of different doses of *Chrozophora plicata* on biochemical parameters.

Parameters	Control	250	500	1000	P-value	Sig. level
ALT (U/L)	18.91±5.65^{a}	2.63±0.84^{b}	6.00±0.48^{b}	4.65±1.53^{b}	0.000	**
AST (U/L)	85.82±23.08^{a}	2.98±0.62^{b}	2.65±0.56^{b}	3.70±1.63^{b}	0.000	**
ALP (U/L)	88.87±31.64^{a}	1.57±0.27^{b}	1.77±0.23^{b}	1.92±0.17^{b}	0.000	**
Protein (g/L)	6.76±1.25^{a}	5.25±0.57^{b}	6.00±0.48^{ab}	6.50±0.69^{a}	0.020	**
Albumin (g/L)	2.31±0.13^{b}	2.03±0.20^{b}	2.13±0.20^{b}	2.60±0.41^{a}	0.008	**
Globulin (g/L)	4.52±1.06^{a}	3.37±0.45^{b}	3.87±0.36^{ab}	3.92±0.40^{ab}	0.042	*
Bilirubin (mg/dl)	0.065±0.047^{c}	1.65±0.27^{b}	2.00±0.29^{a}	2.17±0.40^{a}	0.000	**

Means within rows which having similar letters are not significantly different:

*Significant at 0.05 level of probability.

**: Significant at 0.01 level of probability.

Histopathological Examination of Liver

The liver is the first organ to encounter ingested nutrients, drugs and environmental toxicants that enter the hepatic portal vein from the digestive system, it can be detrimentally altered by injury caused by acute or chronic exposure to toxicants. (Shyamal et al 2010).

Rats treated with 250mg/kg body weight *C.plicata* ethanolic extract exhibited big alteration histological features, the sections showed congested portal veins(Fig. 3), necrotic hepatocyte cell and lymphocytes cells were appeared too when compared with the control (Fig. 2). On the other hand rats which were treated with 500mg/kg body weight showed dilated central vein, loss of nuclei and sinusoidal dilatation (Fig 4). Also Necrotic cells, kuppfer cells proliferation and few lymphocytes cells were appeared. Moreover hepatocyte dissociation

and cytoplasmic eosinophilia (degenerative change) were seen too. Furthermore, rats treated with 1000mg/kg showed dilated portal veins, necrotic cells, kuppfer cells proliferation, lymphocytes cells, cytoplasmic eosinophilia, and hepatocyte dissociation with loss of nuclie (Fig.5,6 and 7). The only result which was found from previous literature was hepatoprotective function of extract preserving the hepatic architecture of the liver tissue to near normal (Kumar and Rao, 2017).

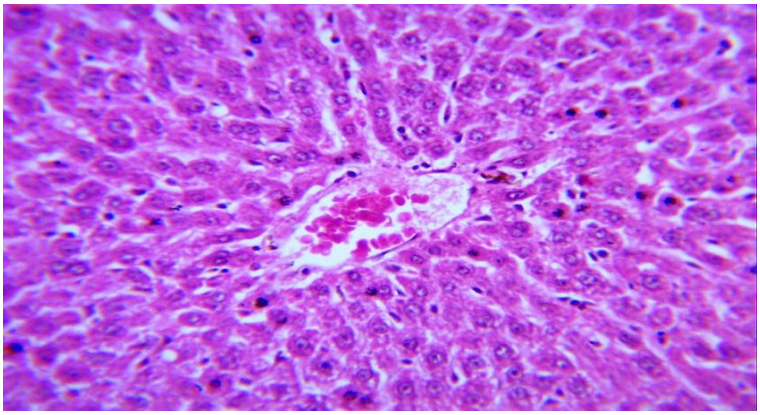

Fig.2. Liver rat treated with distal water (control). showing normal structure. H&E x250.

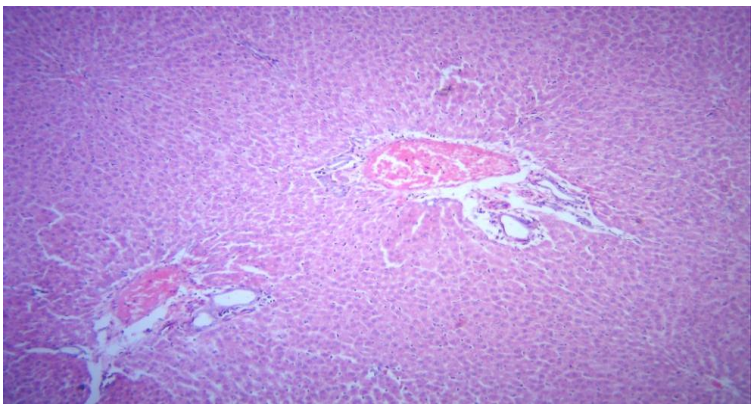

Fig.(3): Liver rat treated with *C. plicata* extract (250 mg/kg) showing Congested portal veins. H&E x 100.

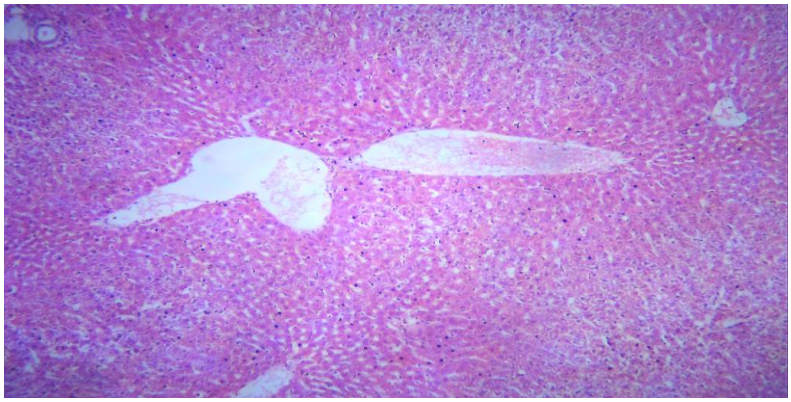

Fig. 4. Liver rat treated with *C. plicata* extract (500 mg/kg) showing dilated veins containing fibrin and dilated sinusoids. H&E x 100.

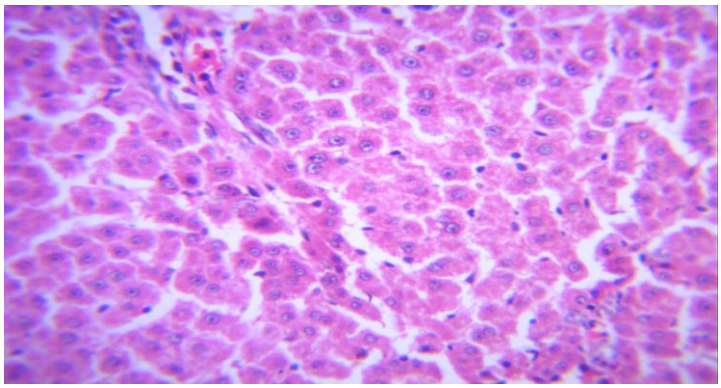

Fig. 5. Liver rat treated with *C. plicata* extract (1000 mg/kg) showing dissociated hepatocytes H&E x 250.

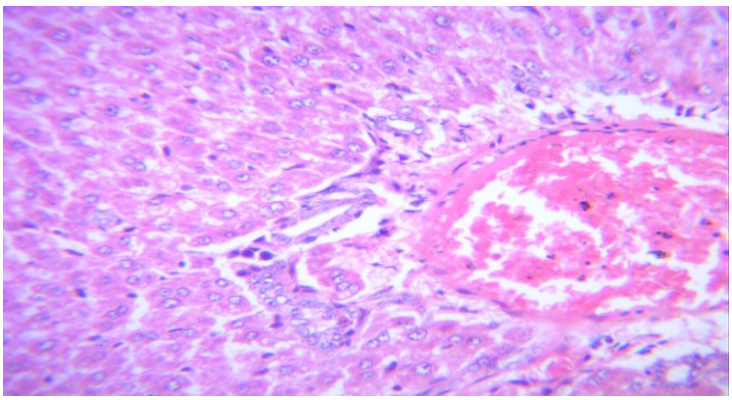

Fig. 6 Liver rat treated with *C. plicata* extract (1000 mg/kg) Portal area showing dilated vein and many small bile ducts. H&E x250.

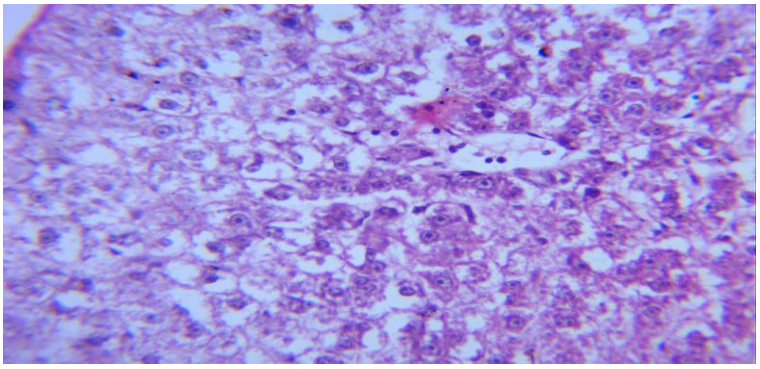

Fig. 7.Liver rat treated with *C. plicata* extract (1000 mg/kg) showing marked hepatocyte vacuolations and loss of nuclei. H&E x 250.

CONCLUSION

These alterations in some of the parameters and histological changes which reported in this study may indicate that the leave extracts of C. plicata possess high toxicity affecting liver function. Therefore, leave crude extracts may be not completely safe as oral remedies.

ACKNOWLEDGEMENTS

The authors are grateful to the management and staff of the Department of Histopathology, Faculty of Veterinary, University of Khartoum, Pharmacology laboratory, Medicinal and Aromatic Plants Research Institute (MAPRI) for technical supports and service of animal facilities.

REFERENCES

1. Atere, T., & Ajao, A. (2009) Toxicological implications of crude alkaloidal fraction from Gnestis ferruginea D. C. root on liver function indices of male Wistar rats. International Journal of Biomedical and Health Sciences, 5(3): 1189-1195.
2. Alsiede, M. M. S. A., Abddrahman, M. A., & Saeed, A. E. (2015). Total phenolic content, flavonoid concentration and antioxidant activity of *Chrozophora plicata* leaves and seeds extracts. International Journal, 3(8): 986-993.
3. Bajpai, A. K., & Agarwal, D. K. K. (2015). Uses and Importance of Medicinal Plants As An Alternative Medicine A Historical Review. Journal of Environmental Science, Toxicology and Food Technology, 1(1): 21-23.
4. Bandesh, K., Jha, P., Giri, A. K., Marwaha, R. K., INDICO, Scaria, V., ... & Bharadwaj, D. (2019). Normative range of blood biochemical parameters in urban Indian school-going adolescents. PloS one, 14(3): e0213255. Baxter, G., & Stanners, C. (1987) The

Effect of Protein Degradation on Cellular Growth Characteristics. Journal of Cellular Physiology, 96: 139-14.

5. Bulus, T., Atawodi, S. E., & Mamman, M. (2011). Acute toxicity effect of the aqueous extract of Terminalia avicennioides on white albino rats. Science World Journal, 6(2): 1-4.
6. Dekant, W., & Vamvakas, S. (2000). Toxicology, 1. Fundamentals. Ullmann's Encyclopedia of Industrial Chemistry, 56.
7. Dhanotiya, R. (2004) Textbook of Veterinary Biochemistry 2nd edition. India: Jaypee Brothers Medical Publishers Ltd, 271-444.
8. Galal, M., & Adam, S. E. (1988). Experimental *Chrozophora plicata* poisoning in goats and sheep. Veterinary and human toxicology, 30(5): 447-452.
9. Gomez KA, Gomez AA, (1984) Statistical procedures for agricultural research 2nd Ed. John Wiley and Sons, Inc., New York.
10. Harborne, J. B. (1984). Phytochemical methods. 2nd edition. Chapman and Hall.
11. Jamshidi-Kia, F., Lorigooini, Z., & Amini-Khoei, H. (2018). Medicinal plants: Past history and future perspective. Journal of herbmed pharmacology, 7(1).
12. Khan, H. (2014). Medicinal plants in light of history: recognized therapeutic modality. Journal of evidence-based complementary & alternative medicine, 19(3): 216-219.
13. Kiss, T., Szabó, A., Oszlánczi, G., Lukács, A., Tímár, Z., Tiszlavicz, L. and Csupor, D., (2017). Repeated-dose toxicity of common ragweed on rats. *Plos one*, *12*(5): 167-188.
14. Kumar, K., S. and Rao, S., A.(2017). Evaluation of hepatoprotective and invitro cytotoxic activity of leaves of *Chrozofora plicata Linn.* Res J. Pharmacology and Pharmacodynamics, 9(1): 91-96.
15. Lee, T.H., Kim, W.R. and Poterucha, J.J., (2012). Evaluation of elevated liver enzymes. *Clinics in Liver Disease*, *16*(2): 183-198.
16. Martinez A, Valencia G: Marcha fitoquimica. (2003). In Manual de prácticas de Farmacognosia y Fitoquímica: 1999. 1st edition. Medellin: Universidad deAntioquia; Phytochemical screening methods 59-65. Mohammed et al. (2010). Hypoglycemic activity of Buccholzia coricea (Capparaceae) seeds in streptozotocin induced diabetic rats and mice. Experimental and Toxicologic Pathology, 10: 1016.
17. Nunes, V. and Mendez-Sanchez, N., (2020). Impact of Herbal and Dietary Supplements Causing Drug-Induced Liver Injury in Latin America. *Clinical Liver Disease*, *16*(3): 83.

18. Okigbo, R. N., & Mmeka, E. C. (2009). An appraisal of phytomedicine in Africa. Okigbo plants used in Moroccan traditional medicine. Current Applied Science and Technology, 6(2): 83-94.
19. Rasool Hassan BA (2012) Medicinal Plants (Importance and Uses). Pharmaceut Anal Acta, 3: e139.
20. Shyamal, S., Latha, P. G., Suja, S. R., Shine, V. J., Anuja, G. I., Sini, S., ... & Rajasekharan, S. (2010). Hepatoprotective effect of three herbal extracts on aflatoxin B1-intoxicated rat liver. Singapore medical journal, 51(4): 326.
21. Sofowora, A. (1993). Medicinal Plants and Traditional Medicines in Africa. Chichester John, Willey & Sons New York, 256.
22. Sukhdev. S. H; Suman. P. S. K; Gennaro. L and Dev. D. R (2008). Extraction technologies for medicinal and aromatic plants. United Nation Industrial Development Organization and the International Center for Science and High Technology, 116.
23. Tabussum, a., (2017). Phypochemical investigation on chorozophora plicata and heliotropium crispum (doctoral dissertation).
24. Tabussum, A., Riaz, N., Saleem, M., Ashraf, M., Ahmad, M., Alam, U., Jabeen, B., Malik, A. and Jabbar, A., (2013). a-Glucosidase inhibitory constituents from *Chrozophora plicata*. *Phytochemistry Letters*, *6:* 614-619.
25. Jess D. Reed, (1995) Nutritional toxicology of tannins and related polyphenols in forage legumes. Journal of Animal Science, 73(5): 1516–1528.
26. Wiesman & chapagain (2005). Larvicidal effects of aqueous extracts of *Balanites aegyptiaca* (desert date) against the larvae of *Culex pipiens* mosquitoes. Afr J Biotechnol, 4: 1351-4.
27. Yakubu et al. (2007) Effect of repeated administration of sildenafil citrate on selected enzyme activities of liver and kidney of male albino rats. Nig. J. Pure Appl. Sci., 18: 1395-1400.
28. Rolls, B. (2000) The role of energy density in the over consumption of fat. J. Nutr., 130: 268.