

# Study the Protective Role of Arabic Gum Extract on Some Physiological and Histological Criteria for Liver of Male Rabbit Treated with Atrazine

Noor Rahi AL-Shimmari<sup>1</sup>, Rasha Abdulameer Jawad<sup>1</sup>

<sup>1</sup>Department of Biology, College of Education for pure Sciences, Karbala University, Iraq

## Abstract

The study involved fifteen health adult male rabbit white (*Lepus arcticus*), weight ranged (1480-1550) kg. and age between 6 to 8 month, divided to three groups the first group treated 10 ml of atrazine orally for five rabbits daily for two weeks and second group is treated with aqueous extract of Arabic gum (AG) (*Acacia Senegal*) 30% and atrazine 10 ml between one day and another for two weeks and third group was the control group given 10 a normal saline. In the search the levels of hepatic enzyme were presented (AST) Aspartate transaminase, (ALT) Alanine transaminase and (ALP) Alkaline phosphate, also the rate of diameters of liver sinuses, the diameters of central veins and hepatocellular diameter, and found through this study:

- ❖ The presence of a significant increase ( $P \leq 0.05$ ) in the levels of hepatic enzyme (AST), (ALT) and (ALP) and rate of diameters each of liver sinuses, central veins, and hepatic cells in atrazine group compared with the control group.
- ❖ The presence of significant decrease ( $P \leq 0.05$ ) in levels of hepatic enzyme (AST) and (ALT) in the group treated with aqueous extract of AG in 30% concentration and 10 ml of atrazine compared with control group, and did not occur significant differences ( $P \geq 0.05$ ) in the level of (ALP) and in rate of diameters each of liver sinuses, central veins, and hepatic cells in aqueous extract of *Acacia Senegal* and atrazine group compared with the control group

**Keyword:** Atrazine, *Acacia Senegal*, AST, ALT, ALP, Hepatocytes.

## Introduction

Medicinal plants containing active substances used for therapeutic purposes or in the pharmaceutical industry and that the natural products of these plants are an effective source to discover vital effectiveness like anticancer, antioxidant<sup>(1)</sup>, antibacterial and antiparasitic<sup>(2)</sup>. Where these plants are a food source on the one hand and medication against various diseases on the other due to the contents of its parts of chemical compounds of great interest and importance because of therapeutic activity of humans<sup>(3)</sup>. where used as catalysts for growth<sup>(4)</sup>. the orientation for medicinal plant treatment is justified and taking chemically manufactured drugs has been shown to have serious side effects that may appear over time and cumulatively<sup>(5)</sup>.

AG is a substance collected from the secretion of the stems and branched of *Acacia Senegal* tree, it consists mainly of large molecules carbohydrates, proteins, minerals and amino acid<sup>(6)</sup>. AG has a high molecular weight as polysaccharide it contains galactose, rhamnose, glucuronic acid and arabinose also calcium, potassium and sodium salts. It also contains 1.5%-2.6% protein, 0.22%-0.39% nitrogen and amino acid mainly like hydroxyproline, serine, proline, and aspartic acid, it has high solubility in water but does not dissolve in alcohol<sup>(7)</sup>. And has many uses in the industrial section such as textiles and cosmetics, and pharmaceutical and nutritional uses where in the pharmaceutical field as a pharmaceutical compound because of its antioxidant activity and positive effect in treatment of urinary tract, cardiovascular and digestive system also improves the liver, kidney & heart tissue<sup>(7)</sup>.

Atrazine effects on the body's various organs, including the liver, which is the main organ for detoxification of the body. Research has shown a decrease in the accumulation of hepatic glycogen and the presence of early symptoms of liver cytotoxicity, this is due to the toxic effects of atrazine on the liver that inhibit the activity of major enzymes such as hexokinase, glycogen synthase and glucokinase<sup>(8)</sup>, this explains the low body weight has been observed to lead to decrease in glycogen and increase fat in liver<sup>(9)</sup>. In addition, bile duct hypertrophy and renal tube necrosis have been observed<sup>(10)</sup>. It has also been found to lead to changes in insulin resistance and disturbance in fat digestion<sup>(11)</sup>. Atrazine also attacks fats, proteins and DNA molecules, causing metabolic changes in severe cases, it can lead to cell death and lead to oxidative stress and emergence and development of many diseases such as atherosclerosis, cancer, psoriasis, Alzheimer's, high blood pressure, heart disease, liver, kidney and brain<sup>(12)</sup>.

### Materials and Method

AG samples were collected from Babylon governorate in October 2018, Gum Arabic powder was moistened with water by 1:5 where 50 of gum Arabic was mixed with 250 ml of distilled water<sup>(13)</sup>, a horizontal shaker GFL type, 3015 modules for 30 minutes and the sample was left static and then filtered using filter paper three times, after that centrifuge was used 3000 rpm for 15 minutes. the extract was concentrated using a rotary evaporator and dried 45 in an oven.

#### Experimental design.

The experiment was designed to investigate the effect of aqueous extract of AG and atrazine on liver tissue and its effect on some functional blood parameters in local male rabbits. then divided into three groups, including 5 rabbits are:

- 1- Atrazine group 10 ml for two weeks daily.
- 2- Aqueous extract of AG and atrazine group from day to day at dose of 10 ml and at concentration of 30% of AG and 10 ml of atrazine and for two weeks daily.

#### Anatomy of animals and blood collection

The animals were then numbed with chloroform and anatomy by opening the abdominal cavity and draw blood through heart puncture to get the most amount of blood. Blood samples were placed directly into sterile anticoagulant –free test tubes its capacity 10 ml, it was

left for 15-20 minutes at laboratory temperature and then transferred to a centrifuge 3000 rpm for 15 minutes for the purpose of obtaining the serum stored in the refrigerator at temperature  $-4\text{ }^{\circ}\text{C}$ <sup>(15)</sup> for the purpose of physiological testing that including AST and ALT<sup>(16)</sup> ALP<sup>(17)</sup>. The liver was removed after fatty substances were removed fixed with formalin concentration 10% so as to preserve the cellular structure and the natural structure and the natural state of the tissue, after two days 48 hours I was extracted from formalin and washed with tap water for a (3-6) hours, then a series of operation took place to prepare tissue slides colored Eosin and Hematoxylin then the prepared tissue slides were examined using a compound microscope and under a 10X magnification force, and measured the rate of diameters of liver sinuses, the diameters of central veins and hepatocellular diameter.

### Results and Discussion

**Physiological study:** The results of the physiological study are shown in Table 1 for atrazine group 10 ml in rabbits' serum there is a significant increase ( $P\leq 0.05$ ) in the average level of ALT, AST and ALP enzymes for two weeks compared with the control group. The results of this study are consistent with which reached Jested and his group<sup>(18)</sup> in his study with doses rats 300mg/kg from atrazine for one month noting an increase in the levels enzymes mentioned above, consistent with the results of this study of Hussein and his group<sup>(19)</sup> which he made on rats by taking them 400gm/kg from atrazine for two weeks, the cause of the increase in rate of enzymes levels in the blood serum indicates the extent of damage to the liver and heart tissues the result of treatment with atrazine leading to a change in hepatic metabolic functions through damage to hepatocytes or the increase may due to kidney damage due to heart damage which leads to the occurrence of chronic renal insufficiency due to necrosis, degeneration or damage to renal cells leading to enzymes leaking into the blood serum<sup>(20)</sup> Or due to increased oxidative stress resulting from the high proportion of oxidant from the liver because atrazine is an oxidizing agent capable of attacking the cell membrane and antioxidant molecules which cause damage and breakdown in DNA, protein and fat<sup>(21)</sup>. consistent with this result what he pointed out Mohammad and his group<sup>(22)</sup>. to born of free radicals (ROS) Reactive Oxygen Species leads to the breakdown of mitochondria containing the enzymes listed, because oxidative stress increases the process of lipid peroxidation leading to cirrhosis.

The results of the physiological study are shown in Table 1 for atrazine group 10 ml that treatment with aqueous extract of AG at concentration in rabbits to presence of significant decrease ( $P \leq 0.05$ ) in AST and ALT, there is no significant difference ( $P \geq 0.05$ ) in rate of ALP for two weeks compared with the control group. Consistent with the results of our study<sup>(23)</sup>, noting when dosing mice with 10% from AG for three month leads to decrease in the concentration of enzymes mentioned above, the results of the atrazine group with the results of this group treatment with AG to increase in average level of liver cell enzymes that lead to oxidative stress of the kidney and liver that results born of free radicals

capable of attacking the cell membrane and antioxidant molecules which cause damage and breakdown in DNA, protein and fat<sup>(22)</sup>. Also the study of Ayaz and his group<sup>(24)</sup> referred to the protective role of aqueous extract go AG when give 0.5 g/kg daily for two weeks to mice treated with toxic substance Trichloroactate where they notice a decrease in the levels of hepatic enzymes mentioned above perhaps due reason to improvement in the liver functions due to increased enzymatic antioxidants such as Catalase and Glutathione peroxidase, and non-enzymatic such as vitamin A, C, and because the plant contains phenolic compounds which working on decrease free radical formation<sup>(25)</sup>.

**Table (1): effects of aqueous extract of AG in concentration 30% on levels rate of some enzymes in serum of rabbits that treated with atrazine.**

parameters groups	AST U/L	ALT U/L	ALP U/L
Control group Normal saline (10)ml	18.80± 0.84	20.60± 1.52	23.40± 3.78
Atrazine 10 ml	27.38± 3.91	29.40± 1.52	44.60± 6.50
Atrazine 10 ml and gum Arabic 30%	16.30± 0.84	18.80± 0.84	23.46± 2.21
LSD	2.74	1.51	4.89

**Histological study:** Figure 1 represents the normal histological structure of the control group for two weeks for a rabbit liver, the liver consists of a central vein and hepatic cords surrounding cubic cells distributed between hepatic cord and hepatic sinuses. The results have shown the formal and histological measurements in Table 2 and Figure 2 to section of liver tissue for atrazine group treated with AG 30% to absence of significant differences ( $P \geq 0.05$ ) in diameters rate each of hepatic sinuses, central vein and hepatic cells compared with the control group, as for the histological composition for atrazine group treated with AG 30% note a slight improvement in liver tissue represented by lack of central vein expansion compared with group treated with atrazine alone, absence lack in sinuses and less cellular infiltration.

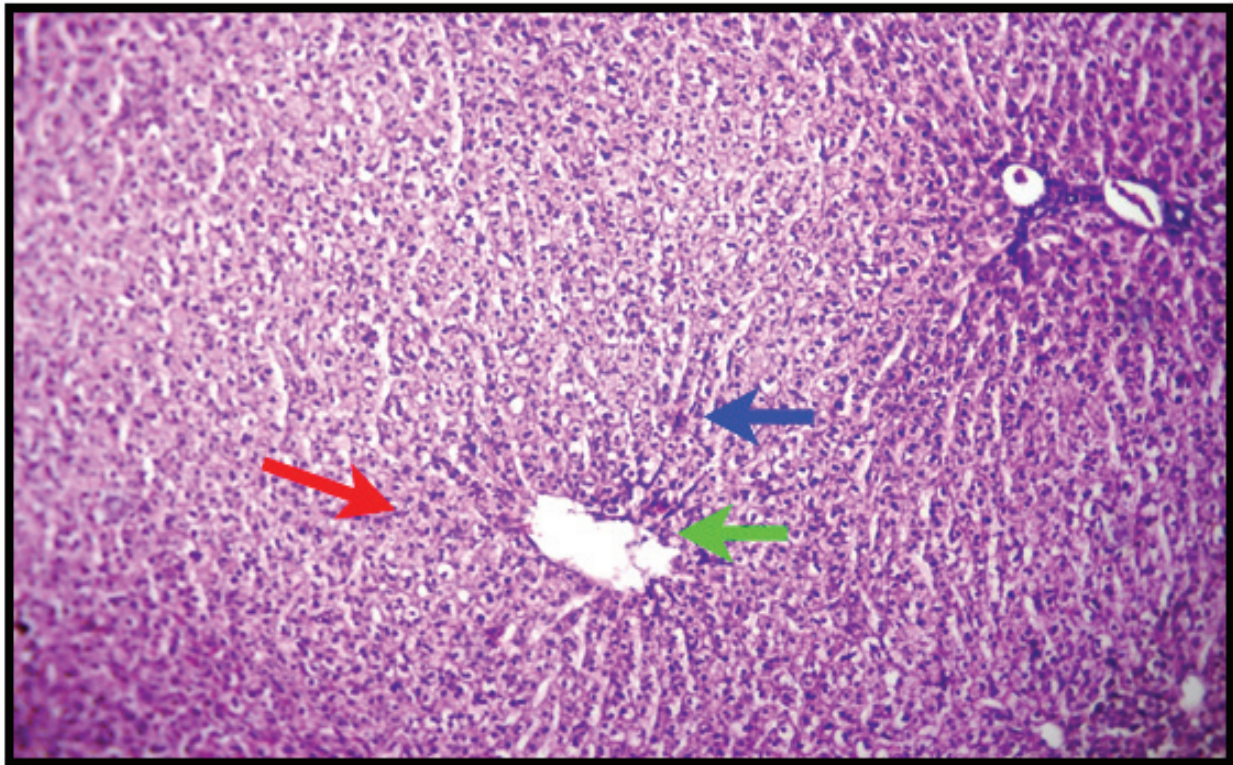
The study of<sup>(26)</sup> pointed to that oral dosage with alcoholic extract of AG plant 400,800mg/kg

daily for one week for mice treated with toxic carbon tetrachloride prevent necrosis, fatty infiltration and liver damage resulting from this toxic substance CCL4 that the histological structure of the hepatic cords and tissue normal appears addition to low in hepatic enzymes. This confirms the effectiveness of AG as a medicinal plant used in folk medicine because it contains many effective chemical compounds such as carbohydrates rhamnose, galactose and arabinose, acids such as glucuronic and mineral salts such as calcium, potassium, magnesium and sodium, addition to medically effective compounds such as phenols, flavonoids and alkaloids<sup>(27)</sup>. Also the study of Ram and his group pointed to a role of alcoholic extract of gum Arabic plant when dosing rabbit 500mg/kg daily for forty five days leads to remain the liver, kidney, heart and aorta tissues similar to normal, addition to low in hepatic enzymes and liver lipids that pointed to the possibility of the plant in the treatment of cases of

fats and atherosclerosis<sup>(28)</sup> .

**Table (2): Measurement of liver sinusoids diameters, central veins diameters and hepatocyte diameters measured in micrometers for male rabbits after dosing with aqueous extract of AG and atrazine for two weeks.**

hepatocytes groups	Diameters of liver sinususes $\mu\text{M}$	Diameters of central veins $\mu\text{M}$	Diameters of hepatic cells $\mu\text{M}$
Control group Normal saline (10ml)	41.2 $\pm$ 9.1	72.7 $\pm$ 8.4	17.3 $\pm$ 2.0
Atrazine 10 ml	80.7 $\pm$ 2.3	*98.7 $\pm$ 209	*23.2 $\pm$ 3.1
Atrazine 10 ml and AG 30%	46.9 $\pm$ 9.1	80.2 $\pm$ 9.0	19.9 $\pm$ 3.1
LSD	13.0	12.2	2.9



**Figure 1** Section of liver tissue in control group for two weeks notes presence of the central vein (→) regularity in the hepatic cords (→) with presence of sinusoids (→) (10X H&E)

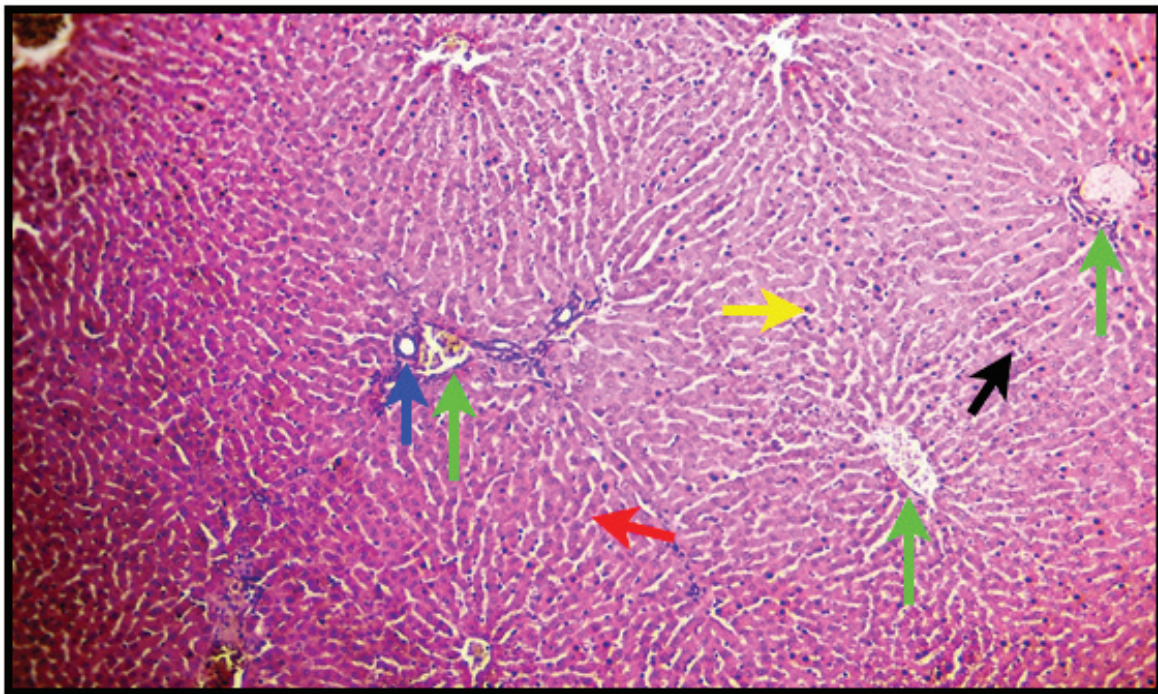


Figure 2 Section of liver tissue in atrazine group treated with 10 ml for two weeks notes presence of congestion and dilatation of central vein (→) and portal vein (→) dilatation in sinusoids (→) cellular infiltration (→) severe irregularity in hepatic cords (→) with water degeneration (→). (10X H&E)

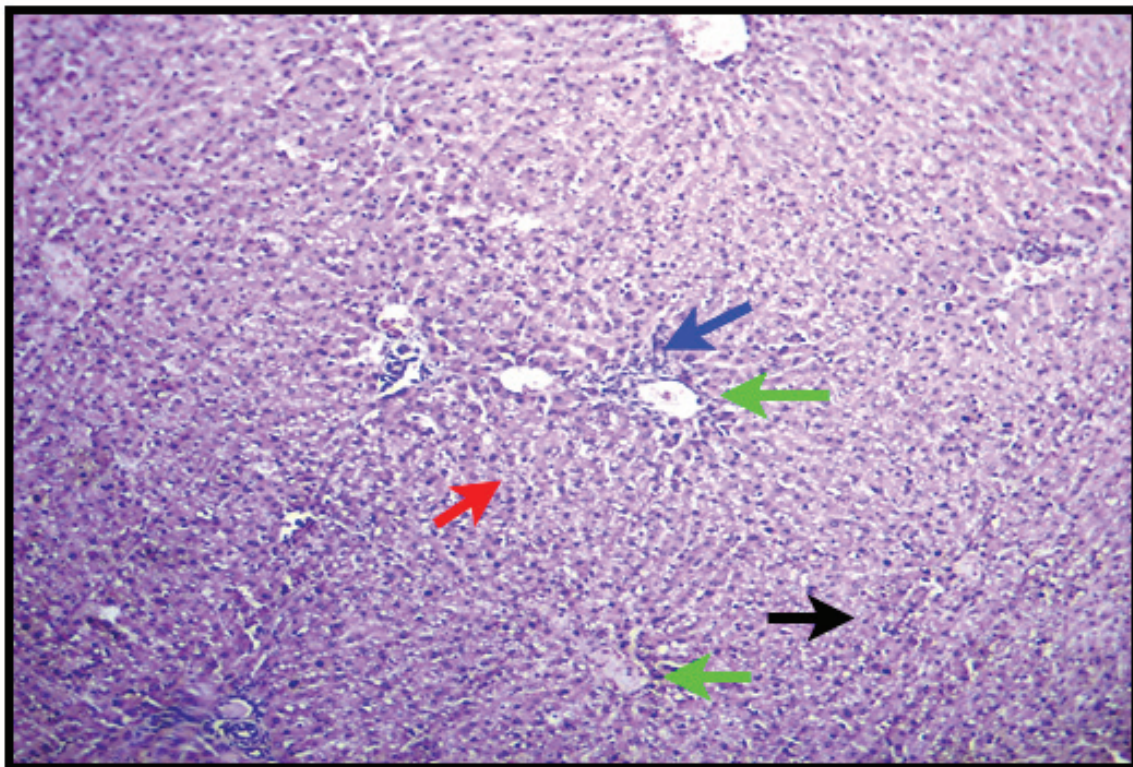


Figure 3 Section of liver tissue in atrazine group treated with 10 ml for two weeks with AG with 30% concentration notes presence of congestion and dilatation of central vein less than previous treatment (→) less cellular infiltration (→) presence water degeneration (→) dilatation in sinusoids (→). (10X H&E)

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

**Conflict of Interest:** The authors declare that they have no conflict of interest.

**Funding:** Self-funding

## References

- (1) Adebayo, A.H; Abolaji , A.O.; Opata , T.K. and Adegborno , I.K. Effects of ethanolic leaf extract of chrysophyllum albidum Go. on biochemical and hematological parameters of albino wister rats. African Journal of Biotechnology. (2010). 9(14) : 2145-2150.
- (2) Saeid , S. and Tariq, P. Antibacterial activities of emblica officinalis and (Corianderum sativium L.) against gram negative urinary pathogens. Pak.J. pharm Sci Jan: (2007). 20(1): 32-35.
- (3) Zuhair, N.; Huda, A. and Faris, K. Iraqi Medical Herbs Guide. Ministry of Health. (1996).
- (4) Cabuk, M.; Alcicek, A.; Bozkurt, M. and Imre, N. Antimicrobial properties of essential oils isolated from aromatic plants and using possibility as alternative feed additives II. National Animal Nutrition Congress.18-20 September, Konya, Turkey: (2003). 184-187.
- (5) Saibara , T.; Toda , k.; Wakatsuki , A. and Ogawa. Protective effect of 3-methyl-1-pyrenyl-2-pyrazolin-5-one, free radical scavenger, on acute toxicity of paracetamol in mice. Toxicol let; (2003). 134: 51-54.
- (6) Marian, A.; Maria, L. and Claudio. Products and Applications of Biopolymer, Edited by Dr. Johan Verbeek: (2012). 1-26.
- (7) Ali, B.H.; Ziada, A .and Blunden, G. Biological effects of gum Arabic: a review of some recent research. Food and Chem. Toxicol, (2009). 47: 1-8.
- (8) Gluzczak,L.; Miron, D.S.; Moraes,B.S.; Simoes, R.R.; Schetinger, M.R.; Morsch, V.M.; Loro,V.R. Acute effect of glyphosate herbicide on metabolic and enzymatic parameters of silver catfish (Rhamdia quelen). Comparative Biochemistry and Physiology. (2007). 146:519-524.
- (9) Curic, S.; Gojmerac, T. and Zuric, M. Morphological changes in the organs of gilts induced with low-dose atrazine. Vet. Archiv., (1999). 69: 135-148.
- (10) Hussain, R.; Mahmood, F.; Khan, M. and Muhammad, F. Pathological and genotoxic effects of atrazine in male Japanese quail (Coturnix japonica). Ecotoxicology, (2011). 20: 1-8.
- (11) Lim, S.; Ahn, Y.; Song, C.; Chung, H.; Jang, C.; Par, S.; Lee, K. and Pak, K. Chronic Exposure to the Herbicide Atrazine Causes Mitochondrial Dysfunction and Insulin Resistance. PLOS ONE, (2009). 4(4):1-11.
- (12) Bhatti, J.; Sidhu, I. and Bhatti, G. Ameliorative action of melatonin on oxidative damage induced by atrazine toxicity in rat erythrocytes. Mol. Cell. Biochem. (2011). 353:139-149.
- (13) Harborne, J. Phytochemical methods. A guide to modern techniques of plants analysis. 2nd.ed. Chapman & Hall, London, New York. (1984).
- (15) Pinon – Lataillade, G.; Thoreux- Manaly, A.; Coffigny , H. ; Masse , R. and Soufir , J.C. Reproductive toxicity of chronic laed exposure in male and femal mice. Hum. Experiment. Toxicol., (1995). 11: 872-878
- (16) Bergmeyer, H.U. and Rej , M.H. International Fedration of Clinical Chemistry (IFCC) scientific committee, Analytical section. J. Clin. Chem. Clin. Biochem., (1985). 24: 481- 495.
- (17) Belfield, A., & Golderg, G. M. Revised assay for serum phenyl phosphatase activity using 4-amino-antipyrine-Enzyme., (1971). 12, 561–573.
- (18) Jestadi, D.B.; phanien dra, A.; Babji, U.; Srinu,T.; Shanmuganathan, B. and Periyasamy, I. Effect of short-term exposure of atrazine on the liver and kidney of normal and diabetic rats. Journal of Toxicology., (2014). 10(11): 7.
- (19) Hussain, R.; Mahmood, A.; Khan, A.; Javed, M, T.; Rehan, S. and Mehdi, T. Cellular and biochemical effects induced by atrazine on blood of male Japanese quail (Coturnix Japonica) Pesticide Biochemistry Physiology (2012). 103(1) :38-42.
- (20) Fowler, P. A; Bellingham, M. and Sinclair, K.D. Impact of endocrine- disrupting compounds (EDC5) on female reproductive molecular study. Ecotoxicology and environmental safety., (2012). 78: 170-177.
- (21) Xing,H.; Li, Z.; Wang, X.;Gao, S.;Xu, S. and Wang, X. Oxidative stress response and histopathological changes due to atrazine and chlorpyrifos exposure in common carp. Biochem. Physiol., (2012). 103(1): 74-80.

- (22) Mohammad, R.S.; Attabi, A.L. and Diwan, A.L. protective role of clomiphene citrate from the biochemical effects of atrazine exposure in adult male rats. *Basrah Journal of Veterinary Research.*, (2012). 11 (2): 82-92.
- (23) Babiker, Abbas.T.; Elimam, M. and Mohammad.A. Effect of Gum Arabic on liver function and antioxidant enzymes of Sprague- Dawley rats. *Journal of pharmacy and biological Sciences.*, (2017). 12 (2): 29-33.
- (24) Ayaz,N.O.; Ramadan, K.H.S.;Farid. H.E.A. and ALnahdi,H.S. Protective role and antioxidant activity of AG against trichloroacetate – induced toxicity in liver of male rats, *Indian Journal of Animal Research.*, (2017). 51(2): 303-309.
- (25) Verbeken, Dewettinck exudate gums: occurrence, production and application . *Applied Microbiology and Bio technology .*, (2003). 63: 10-21.
- (26) Singh, J.; Bias, C.S.; Hoda, M. S .and Pal, R. Hepaty protective activity of *Acacia Senegal* pod against carbon tetrachloride – induced hepato toxicity in rats. *International Journal of Pharmaceutical Science Review and Research.*, (2014). 26 (1): 165-168.
- (27) Hooda, M.S.; Rishi, P.; Bhandari, A. and singh, J. Antioxidant potential and free radical scavenging activity by pod extract of *Acacia Senegal*. *Int.J. Phar chem and biological sciences.*, (2013). 2(4):500-506.
- (28) Ram, H.; Jatwa, R. and Purohit. A. Antiatherosclerosis and cardioprotective potential of *Acacia Senegal* seeds in diet – induced atherosclerosis in rabbits. *Biochemistry Research International.*, (2014). ; 2014: 6.