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PROTECTIVE EFFECT OF *EUCALYPTUS GLOBULUS* EXTRACT ON GLUFOSINATE AMMONIUM –INDUCED HEPATOTOXICITY IN WISTAR RATS.

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ABSTRACT: The present study aims to investigate the hepatoprotective effect of *Eucalyptus globulus* extract against pesticide liver damage in comparison to silymarin, a classical antioxidant liver medicine. Liver damage was induced by oral administration of toxicant i.e. Glufosinate ammonium. The extent of damage was studied by assessing biochemical parameters and histopathological evaluations. The aqueous extracts of *Eucalyptus globulus* were administered respectively to the animals pretreated with pesticide and its effects on biochemical parameters were compared with standard drug silymarin (100mg/kg b.wt). *Eucalyptus globules* showed significant reduction of serum enzymes AST, ALT, ALP & Bilirubin (Aspartate Transminase, Alanine Transminase, Alkaline Phosphatase & Total bilirubin) when compared to control counterparts. The hepatoprotective effect of *Eucalyptus globules* was comparable with the standard drug silymarin and it was confirmed by histopathological findings. Moreover, these effects presented in a dose-dependent manner.The present study showed that aqueous extract of *Eucalyptus globulus* at the dosage level of 500 mg/kgb.wt may play a protective role against pesticideinduced hepatotoxicity.

Keywords: Glufosinate-ammonium (GA), silymarin, Liver toxicity, *Eucalyptus globulus*

Abbreviations: Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline Phosphatase (ALP)

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INTRODUCTION

Human health risks vary with the type of the pesticides and also with the extent of vulnerability.

The ruralpopulation is exposed to ahigher concentration of pesticides due to their application in fields. Most pesticides cause harm to theenvironment and animals by entering the food chain. There are reports that, our vegetables, dairyproducts, meat products and even mother's milk have pesticide residues (Kumar Set.al, 2006). According to the World Health Organization, 3 million cases of pesticide poisoning occur every year, resulting in more than 250,000 deaths.Despitethis alarming figure, there is currently no global system to track and stem poisoning or diseases associated with pesticide use(Koirala et.al, 2007).

One such organophosphate which has spurred interest is Glufosinate ammonium.Glufosinate ammonium structurally resembles glutamate, a typical excitatory amino acid in thecentral nervous system. It is recognised that excess release of glutamate results in the death ofnerve cells in the brain (Jewell and Buffin, 2001). Glufosinate can cause marked inhibition of the enzyme, glutamine synthetase in liver which interferes with ammonia detoxification (EFSA, 2005).

Plant products have been the basis for many medicinal therapies. Eucalyptus plant belongs to the Myrtaceae (Myrtle) family, is a herbal drug which is being extensively used in the Indian traditional system of medicine for diabetes & liver components. Commonly known as Safeda in Hindi, distributed in Gujarat, Himachal Pradesh, south west Bengal, Shivalik ranges in Haryana and Chhattisgarh. Eucalyptus has an extensive record of curative uses with a variety of important beneficial properties (Arti et.al, 2012).The plant is considered as an indigenous source of medicine exhibiting phytochemical constituents which contain flavonoids, alkaloids, tannins and propanoids, which are present in the leaves of the plant. Numerous studies have shown that *Eucalyptus globulus* exhibit various properties like anti-inflammatory, antioxidant, anticancer, antibacterial, antiseptic and astringent and hepatoprotective properties (N. Nagpal et.al, 2010).

Liver is the most important organ of the human body involved in metabolism, detoxification and excretion of various endogenous and exogenous substances (Parmaret.al, 2010). In India the percentage of liver disorder is more as compared to developed countries. The available synthetic drugs to treat liver disorders in this condition also cause further damage to the liver (Garima et.al, 2015). Hence, Herbal drugs have become increasingly popular and their use is wide spread. Keeping in view the above facts, the current study focuses to elucidate the possible Hepatoprotective activity of *Eucalyptus globulus* against Pesticide i.e. Glufosinate ammonium induced hepatotoxicity rat model.

MATERIAL AND METHODS

Animals: In this study, Ten animals were taken in each group (5 males and 5 females) with an average body weight of 160-200g were used. Prior to starting the experiment, necessary approvals were taken from IAEC (Institutional animal ethics committee) for conducting the study.

The animals were housed (3 rats each cage) in an air conditioned room (12-15 air changes per hour) at the temperature 22 ± 3° C and 50-60 % relative humidity with a 12 hour light/ dark cycle. They were provided with standard laboratory animal diet (Amrut feed Ltd., Pune) and filtered water *ad-libitum*. The animals were acclimatized for five days prior to the initiation of dosing. The animals were obtained from the animal house facility, ShriramInstitute of Industrial Research, Delhi.(OECD 408, 1998).

Assessment of Hepatoprotective activity

Experimental Procedure: 28 days repeated exposure (7 days/week)

Liver damage was induced in animals using toxicant at the dose rate of 500 mg/kg body weight through oral route for 28 consecutive days. The Liver damage was confirmed by performing liver function test. These animals were then treated with *Eucalyptus globulus* extract for a period of 28 days to assess its Hepatoprotective effect. The plan of treatment was as given below:

Observations and Evaluations: During this study the animals were carefully observed for general appearance and behavior. At the end of 28 days, all the animals were sacrificed by light CO_2 anesthesia to collect the blood for biochemical estimations. Following parameters were analyzed on serum samples collected on Day 29 of the experiment: Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), alkaline phosphatase (ALP) and Total Bilirubin.

Histopathology

Liver tissues collected after completion of the study were preserved in 10% neutral buffered formalin, Tissues collected for histopathology were processed and then embedded in paraffin wax and sectioned at 3-5 microns and stained with haematoxylin and eosin method, subjected to histopathological examination (Luna, 1968).

Table -1: Animal Groups and Dose Levels

Group	Dose Level (mg/kg B.wt. per day)
Group I Pesticide control	500 mg / kg
Group II Vehicle Control (distilled water)	0 mg / kg
Group III *Eucalyptus globulus* extract (Pesticide pretreated)	50 mg / kg
Group IV *Eucalyptus globulus* extract (Pesticide pretreated)	250 mg / kg
Group V *Eucalyptus globulus* extract (Pesticide pretreated)	500 mg / kg
GroupVI Silymarin (Pesticide pretreated)	100 mg / kg
Group VII *Eucalyptus globulus* extract	500 mg / kg

Statistical Analysis

The data are expressed as Mean ±S.D.The differences among control and experimental groups were determined with statistical software IBM-SPSS 22.

The criteria for significance at 95% confidence are dependent on the p value.

If p value <0.05 = Significant

If p value >0.05 = Non significant

RESULTS

Clinical Signs

No toxic signs were observed in the animals of treatment groups when compared with their control counterparts.

Biochemical Investigations

The activities of AST, ALT, Total bilirubin and ALP were estimated inserum samples as the liver function biomarkers (Table-2) and Fig.5.

ALT: After the *Eucalyptus globulus* treatment i.e. at the end of the study a dose dependent decrease in the serum ALT level was observed. On Day 29 serum ALT level was significantly low in all the Herbal treated induced groups as compared to pesticide control group. In Silymarin treated induced group also, the serum ALT level decreased and the level was significantly low as compared to pesticide control group. In Herbal treated normal group serum ALT level was comparable to that in vehicle control group.

AST: At the end of the study, in *Eucalyptus globulus* treated induced groups, a decreasing trend in serum AST level was observed. In *Eucalyptus globulus* highest dose (500 mg/kg b.wt) treated induced group there was significant decrease in serum AST level as compared to pesticide control group. In Silymarin treated induced group also a decreasing trend in serum AST level was observed. In *Eucalyptus globulus* treated normal group serum AST level was comparable to that in vehicle control group.

Total bilirubin: *Eucalyptus globulus* treatment group caused a dose dependent decrease in the serum bilirubin level. However in herbal extract treated induced group at the dose level of 500 mg/kg b.wt and silymarin treated induced group serum bilirubin level was significantly low as compared to that in pesticide control group. In herbal extract treated normal group serum bilirubin level was comparable to that in vehicle control group.

ALP: Serum ALP level in herbal extract treated induced group (500 mg/kg b.wt) and silymarin treated induced group was significantly low as compared to pesticide control group. Inherbal extract treated normal group serum ALP level was comparable to that in vehicle control group.

Histopathological Findings

Histological evaluation of liver sections of vehicle control group showed normal liver parenchyma with perfect arrangement of hepatocytes and normal portal triad. (Figure 1A & 1B). Evaluation of liver sections of pesticide control group (500mg/kg b.wt) showed sinusoidal space engorgement, fatty infiltration and degeneration of hepatocytes. Complete liver parenchyma was haemorrhagic. (Figure 2A & 2B).

Histological evaluation of liver tissues from animals treated with silymarin (100mg/kgb.wt) which were pretreated with pesticide showed healthy anatomy when compared to pesticide control group. (Figure 3A & 3B). Evaluation of liver sections from animals treated with *Eucalyptus globulus*(500mg/kgb.wt) which were pretreated with pesticide also showed normal architecture of liver parenchyma and no significant alterations were observed (Figure 4A & 4B).

Evaluation and comparison of liver tissues of all the animals revealed hepatoprotective effect of *Eucalyptus globulus* against pesticide when administered orally to the animals at the dose rate of 500mg/kg body weight.

The histopathological alterations in the present study could be summarized as follows:

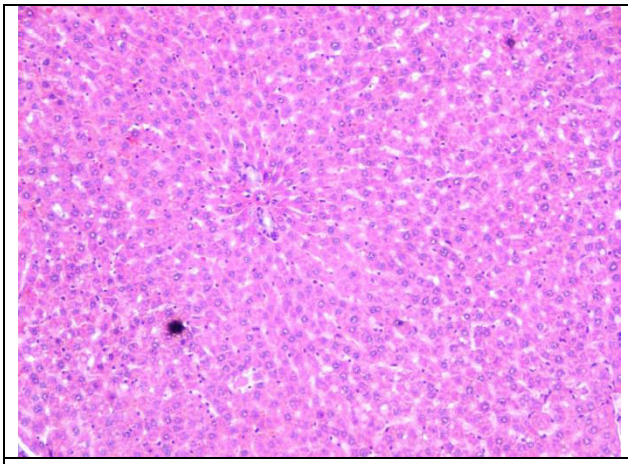

Figure 1A: Low power photomicrograph of liver showing normal architecture of liver parenchyma, portal triad and hepatocytes. (Vehicle control)(H & E, 10X).

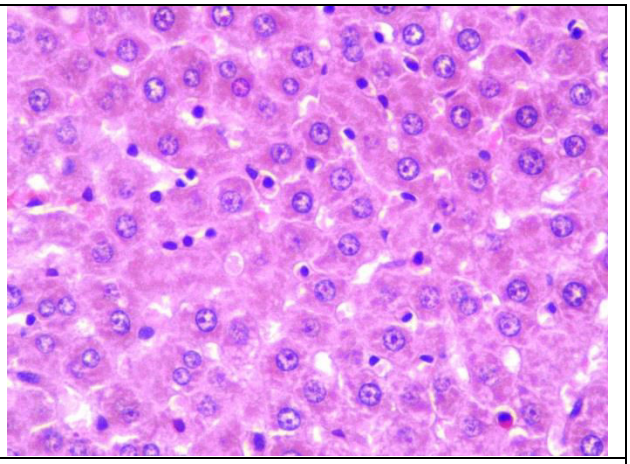

Figure 1B: High power photomicrograph of liver showing normal architecture of hepatocytes. (Vehicle control) (H & E, 40X).

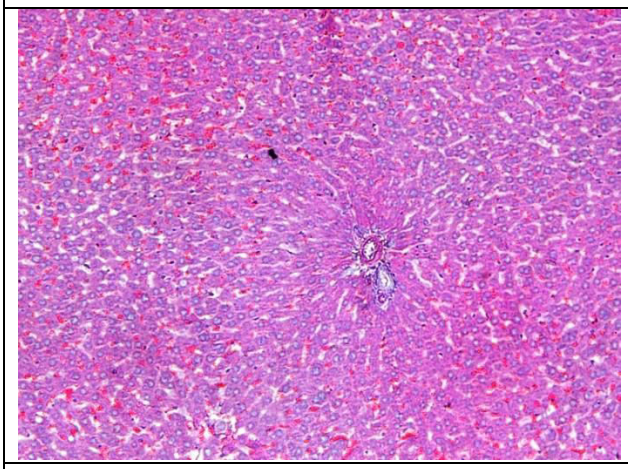

Figure 2A: Low power photomicrograph of liver showing enlarged sinusoidal space and mild haemorrhage. (Pesticide control) (H & E, 10X)

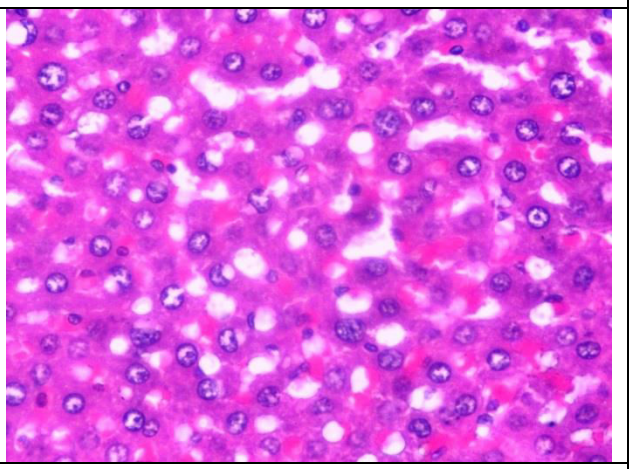

Figure 2B: High power photomicrograph of liver showing severe fatty infiltration, haemorrhage and mild degeneration of hepatocytes. (Pesticide control) (H & E, 40X)

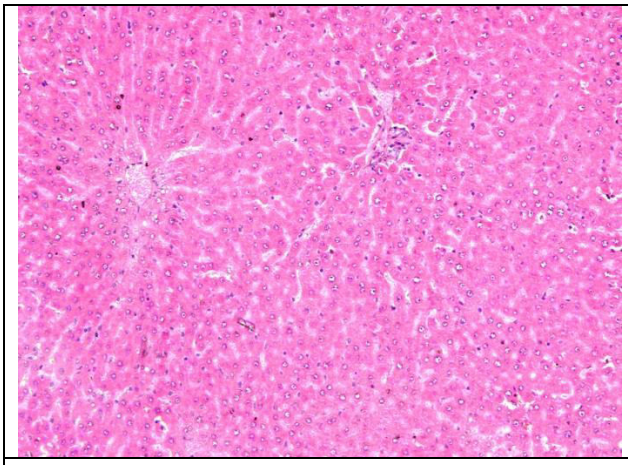	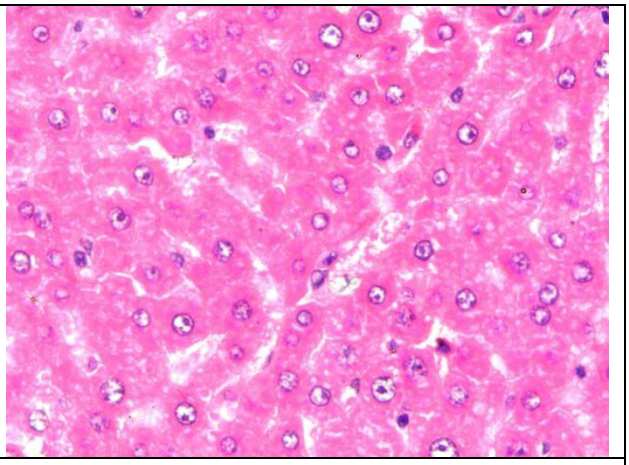
Figure 3A: Low power photomicrograph of liver showing comparatively normal architecture of liver parenchyma and hepatocytes. Silymarin (pesticide pretreated) 100mg/kg. (H & E, 10X)	**Figure 3B: High power photomicrograph of liver showing comparatively normal architecture of hepatocytes. Silymarin (Pesticide pretreated) 100mg/kg. (H & E, 40X)**
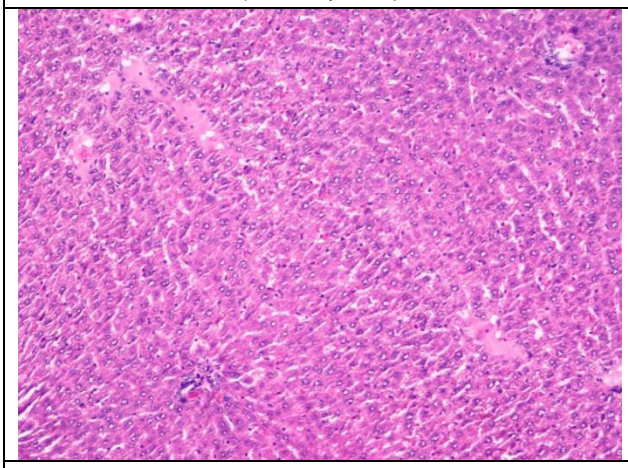	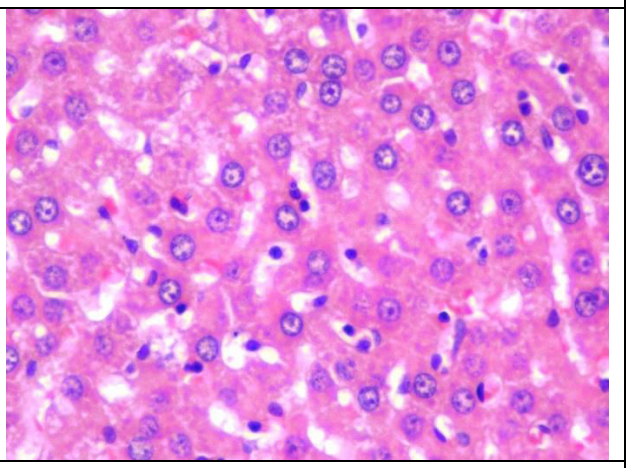
Figure 4A: Low power photomicrograph of liver showing comparatively normal architecture of parenchyma with no significant pathological alteration. *Eucalyptus globulus* (Pesticide pretreated) 500mg/kg. (H & E, 10X)	**Figure 4B: High power photomicrograph of liver showing comparatively normal hepatocytes. *Eucalyptus globulus* (Pesticide pretreated) 500mg/kg. (H & E, 40X)**

Table-2: Effect of *Eucalyptus globulus* extracts on ALT, AST, ALP and Bilirubin

Group	Dose Level (mg/kg B.wt. per day)	SGOT (U/I) (Mean±S.D)	SGPT (U/I) (Mean±S.D)	ALP (U/I) (Mean±S.D)	T-BIL (mg/dl) (Mean±S.D)
Group I Pesticide control	500 mg / kg	190.10± 3.37	115.30±3.40	180.50±3.30	1.35± 0.1
Group II Vehicle Control (distilled water)	0 mg / kg	93.24±1.90	57.56±3.21	86.20±1.10	0.27±0.04
Group III *Eucalyptus globulus* extract (Pesticide pretreated)	50 mg / kg	134.25±3.56	85.20± 1.90	165.30±3.30	1.06±0.04
Group IV *Eucalyptus globulus* extract (Pesticide pretreated)	250 mg / kg	115.20±5.25	60.10±3.12	89.10±2.21	0.71±0.04
Group V *Eucalyptus globulus* extract (Pesticide pretreated)	500 mg / kg	108.20±1.25	57.20±4.21	92.40±1.21	0.51±0.02
GroupVI Silymarin (Pesticide pretreated)	100 mg / kg	105.10±3.50	56.20±1.32	90.40±3.11	0.41±0.02
Group VII *Eucalyptus globulus* extract	500 mg / kg	95.10±3.25	49.10±2.28	86.30±2.21	0.31±0.03

The criteria for significance at 95% confidence are dependent on the p value.
If p value <0.05 = Significant
If p value >0.05 = Non significant

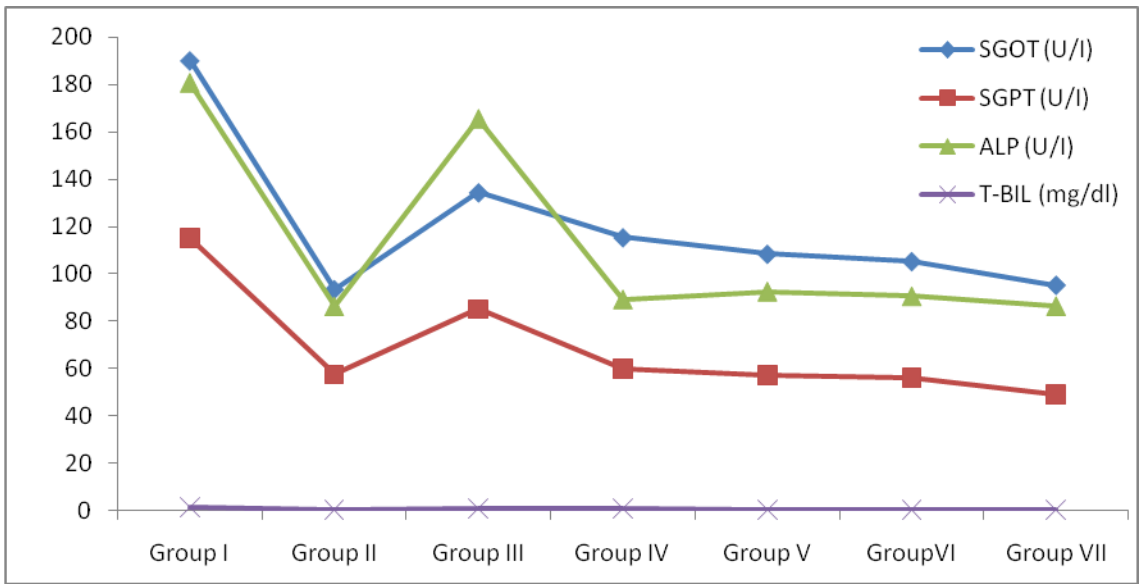

Fig.5: Effect of *Eucalyptus globules* extracts on ALT, AST, ALP and Bilirubin, graph representsthe activities of Liver markers in serum sample.

DISCUSSION

Liver is a target organ of toxicity and plays a major role in detoxification and excretion of many endogenous and exogenous compounds (Thyagarajan S et.al, 2002).In this study, we investigated the hepatotoxic effects of the agrochemical i.e. Glufosinate ammonium using biochemical evaluation histopathological changes in the liver of rats. However, glutamine synthetase inhibition expected as a result of glufosinate ammonium metabolism formed by in vivo deacetylation, as the liver has two distinct systems for dealing with ammonia. A high-capacity, low-affinity system exists in the periportalhepatocyte which is based on carbamoyl phosphate synthetase and the urea cycle.

In central vein hepatocytes, a low-capacity, high-affinity system exists which is based on glutamine synthetase and ornithine aminotransferase, showed that doses of glufosinate-ammonium increase ammonia concentrations in liver(Peltzer PM, et al,2013). Strengthening the above mechanisms involved in the generation of hepatotoxicity by pesticide, significant increased levels of biochemical parameters i.e. AST, ALT, total bilirubin and ALP; noticed. Hayes et al.reported that one of the indicators for liver damage and function is increase in the activities of transaminases (AST and ALT) in the serum. They play a role in amino acids catabolism and biosynthesis. ALP mainly reaches the liver from bone, excreted into the bile; therefore its elevation in serum can be associated with hepatobiliarydisease (Patrick-Iwuanyanwu.et.al., 2007). This increase may be indicative of initial cell injury occurring in advance of gross hepatic pathology.

Silymarin is a polyphenolic flavonoid isolated from the fruit and seeds of the milk thistle (*Silybummarianum*).Various studies indicate that silymarin exhibits strong antioxidant activity and shows protective effects against hepatic toxicity induced by a wide variety of agents by inhibiting lipid peroxidation (Valenzuela A.et.al., 1994).

However, coadministration of *Eucalyptus globulus* to pesticide intoxicated rats decrease ALT, AST, total bilirubin and ALP activity to within normal levels.Similar protective effects were also observed in rats receiving silymarin, which was used as a positive control, although the mechanism of action for these effects may not be the same.

These results indicated the ability of *Eucalyptus globulus* to protect against pesticide-induced hepatocyte toxicity, the effect was more pronounced at the dose of 500 mg/kg b.wt. A possible mechanism of the *Eucalyptus globulus* extract as hepatoprotective may be due to its anti-oxidant effect, cytochrome p450 enzyme inhibitor and hepatoprotective properties. The main chemical constituent (1-8 cineole) are responsible for various activities, also known as eucalyptol (Hardelet.al, 2011).

This might be due to the higher contents of flavonoids present in the extract which could have reduced the accumulation of pesticideaccumulated metabolites.

Histopathological examination also supported the evidence of biochemical analysis. Histological examination of rat liver treated with pesticide shows showed enlarged sinusoidal space and fatty degeneration of hepatocytes. However, in animals treated with *Eucalyptus globulus* extract at the dose of 500 mg/kg b.wt showed sign of protection against pesticide to considerable extent as evident from formation of normal hepatic cards and absence of necrosis and vacuoles, which further indicated its significant hepatoprotective effect.

CONCLUSION

In view of the data of the present study, it can deduce that Glufosinate Ammonium caused biochemical and histopathological liver damage in Wistar rats. According to these results, it is suggested that systemic pesticides exposure might cause hazardous effects; especially at high doses to non-target organisms, including humans. However, precautionary measures should be put in place during field application to circumvent possible adverse effects on consumers, who are increasingly being exposed to contamination from food and drinking water. The coadministration of *Eucalyptus globulus* extract attenuated the toxic effect of pesticide. Therefore, administration of *Eucalyptus globulus* extract may be useful, easy, and economical to protect humans exposed to Glufosinate Ammonium against their toxic effects. Thus it may act even in humans as important constituent in potent liver tonic. Further studies should be conducted to identify theactive components responsible for these activities anddetermination of synergistic effects among variouscompounds present in the extract.

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