Evaluation of hepatoprotective activity of ethanolic extract of *Stereospermum colais* Linn. leaves against paracetamol-induced hepatotoxicity

Gunjan Rani*, S.S. Sisodia

Bhupal Nobles' College of Pharmacy, Bhupal Nobles' University, Udaipur, Rajasthan

gdchaudhary.pharma@gmail.com

Abstract

The present study was aimed to assess the hepatoprotective activity of ethanolic extract of *Stereospermum colais* Linn. (*S. colais*) leaves against paracetamol-induced liver damage in Wistar rats. The liver damage was induced by the administration of paracetamol (2000mg/kg/day, p.o) on 7th day. Other groups of rats were pretreated with two doses of *S. colais* (200mg/kg and 400mg/kg) and silymarin (25mg/kg, bw, p.o.) 30 min prior to paracetamol administration. The various biochemical parameters like SGOT, SGPT, ALP, total bilirubin and total protein were estimated in blood serum along with antioxidants enzymes in liver homogenates. There was a significant increase in serum enzymatic levels of SGOT, SGPT, ALP and total bilirubin with a decrease in total protein, in paracetamol treated animals, reflecting liver injury. Pretreatment with two different doses (200mg/kg and 400mg/kg) of *S. colais* produced significant reversal in level of liver homogenates like LPO, SOD, CAT, GSH, GST and GPx. The results of the study indicate that the extract of *S. colais* possesses significant protection against paracetamol-induced liver damage.

Keywords: *Stereospermum colais*, paracetamol, hepatic, biochemical, silymarin

DOI: 10.48047/ecb/2022.11.12.020

Introduction

A normal working liver is a crucial for survival because of its important role in plasma protein synthesis, drug metabolism, bile production, glycogen storage and a host of other functions. Because of its large size and strategic location, together with its various functions make it unsafe to constant attack by toxic agents which can cause harm or disease **(**Chiang, 2014). If an injury or disease progresses beyond the ability of liver tissue to regenerate new cells, an imbalance occurs which leading to hepatic injury and subsequently liver failure (Espinoza, 2013, Esrefoglu *et al*., 2018). Herbal drugs have existed worldwide with long last history and they were used in Indian, Chinese, Greek and Egyptian from ancient time for various treatments of diseases. WHO estimated

approximately 80% of the word's peoples still remain on traditional medicines for their health care needs (Parasuraman *et al*., 2009). Several medicinal plants reported to possess hepatoprotection activity have been scientifically investigated to identify the phytochemical constituents responsible for this activity and ascertain their effectiveness.

2. Material and Method

2.1 Plant material

The fresh leaves of *Stereospermum colais* were collected from Mandya, Mysore, Karnataka and authenticated by Dr. Y.S. Sarangdevot, Professor, B.N. College of Pharmacy, Udaipur. Air-dried leaves of *Stereospermum colais* were ground into course powder. About 250 g of powder was transferred in glass jar containing 600 ml of ethanol, kept at room temperature for 7 days with regularly shaking. The extract was filtered through a cotton plug, finally with a Whatman No. 1 filter paper. The extract was concentrated by rotary evaporator (RE-2 Aditya Scientific, India) and allow to air dry for complete evaporation of solvent. The dried ethanolic extract was transferred to air tight container and kept in desiccators for further uses (Jahan et al., 2014, Dey et al., 2012).

2.2 Animals

The young healthy Albino Wistar rats of either sex, weighing between 170 to 200g were used for the experiments. They were housed in the cages under the standard laboratory condition at a temperature of 23 ± 2°C, humidity 60-70% and 12 hr light/dark cycles. The rats were feed with standard pellet diet and water was given *ad libitum*. The animals were maintained as per the CPCSEA regulations and the study was approved by the Institutional Animal Ethical Committee at Bhupal Nobles' College of Pharmacy, Bhupal Novels' University, Udaipur (Rajasthan).

2.3 Acute toxicity study

The acute toxicity study of ethanolic extract of *Stereospermum colais* leaves were performed using female Albino Wistar rats. The animals were fasted overnight prior to the experiment and maintained under standard conditions. The ethanolic extract of leaves were administered orally in increasing dose and found safe up to dose of 2000 mg/kg.

2.4 Study design and experimental protocol

Healthy Wistar rats of either sex weighing between 170 to 200 g were randomly divided into five groups of 6 animals each. The animals from group I served as the control and received the vehicle (0.5% CMC) at a dose of 1 ml/kg/day, p.o. for 7 days. Group II animals were similarly treated as group I. Group III animals received silymarin (Microlab) at a dose of 25 mg/kg/day, p.o. for 7

days. Group IV-V animals were treated with two different doses (200 mg/kg and 400 mg/kg) of ethanolic extract of *S. colais* leaves for 7 days respectively. On the 7th day paracetamol (Baxil Pharma Private Limited, India) suspension in a dose of 2g/kg p.o. was administrated to all rats except rats of group I, 30 min after the administration of silymarin and extract. Animals were sacrificed 48 h after the last treatment. Blood was collected, allowed to clot and serum was separated at 2500 rpm for 15 min for carrying out further biochemical investigations. Liver was dissected out and used for biochemical and histopathological studies.

2.5 Biochemical studies

The Serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP), total protein and total bilirubin were estimated using the assay kit (Lab Care Diagnostics (India) Pvt. Ltd). Malondialdehyde (MDA) and catalase (CAT) (Henry et al., 1974), Superoxide dismutase (SOD) (Spitz and Oberley, 1989), glutathione (GSH) (Henry et al., 1974), Glutathione Peroxidase (GPx) and Glutathione S transferase (GPx) (Rotruck et al., 1973) were measured.

2.6 Statistical analysis

The data were expressed as mean ± S.E.M. Statistical differences at $P < 0.05$ between the groups were analyzed by one-way ANOVA followed by Dunnett's multiple comparison test using Graphpad Prism 5.0 software.

3. Results

3.1 Acute toxicity studies

The plant *S. colais* did not show any sign and symptoms of toxicity and mortality with oral administration of dose up to 2000 mg/kg dose.

3.2 Effect of *S. colais on* biochemical parameters

Administration of paracetamol induced a marked increased significantly in the serum enzymes levels and decreased in total protein when compared to normal group The animals pre-treated with two different doses (200mg/kg and 400mg/kg) of *S. colais* extract showed a significantly reduction in the level of SGPT, SGOT, ALP and total bilirubin, whereas increased in total protein as presented in Table 1. Group II animals showed increased in blood serum levels of SGPT (175.46 ± 19.83 IU/L, $P < 0.001$), SGOT (156.50 ± 14.31 IU/L, $P < 0.001$), ALP (127.25 ± 13.19 IU/L, $P < 0.001$) and total bilirubin (2.64 ± 0.28 mg/dl, $P < 0.001$) while decreased in total protein level

to (5.08 ± 0.74 g/dl, $P < 0.001$) as compared to Group I animals of SGPT 80.52 ± 10.78 IU/L, SGOT 69.36 ± 7.77 IU/L, ALP 51.60 ± 3.62 IU/L, total bilirubin 0.66 ± 0.09 mg/dl and total protein 7.01 ± 0.26 g/dl in that order. The extract of *S. colais* in Groups IV as well as in Group V animals decreased significantly in serum levels of SGPT to (147.60 ± 13.52 IU/L, $P < 0.05$ and 139.10 ± 9.68 IU/L, $P < 0.01$), SGOT to (133.12 ± 8.30 IU/L, $P < 0.05$ and 124.79 ± 12.45 IU/L, $P < 0.01$), ALP to (108.30 ± 9.48 IU/L, $P < 0.5$ and 105.56 ± 6.54 IU/L, $P < 0.05$) and total bilirubin to (1.21 ± 0.24, $P < 0.05$ and 1.17 ± 0.15 mg/dl, $P < 0.05$), while increased in total protein to (5.85 ± 0.23 and 6.08 ± 0.32 g/dl, $P < 0.05$) compared to group-II animals where the levels were elevated (or decreased in protein) due to paracetamol administration. Silymarin used as standard drug in Group III animals decreased significantly ($P < 0.001$) the SGPT, SGOT, ALP and total bilirubin levels to 116.85 ± 7.56 IU/L, 97.29 ± 5.84 IU/L, 67.84 ± 4.37 IU/L and 0.85 ± 0.08 mg/dl respectively, while increased significantly ($P < 0.001$) the level of total protein 6.41 ± 0.41 g/dl in that order compared to animals in group II.

Table 1: Effect of *S. colais* on biochemical parameters in paracetamol intoxicated rats.

Group	Treatment	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	TB (mg/dl)	TP (mg/dl)
I	Control 1ml/kg	69.36 ± 7.77	80.52 ± 10.78	51.60 ± 3.62	0.66 ± 0.09	7.01 ± 0.26
II	PCM treated 2000 mg/kg	156.50 ± 14.31^{+++}	175.46 ± 19.83^{+++}	127.25 ± 13.19^{+++}	2.64 ± 0.28^{+++}	5.08 ± 0.74^{+++}
III	Silymarin 25mg/kg	97.29 ± 5.84***	116.85 ± 7.56***	67.84 ± 4.37***	0.85 ± 0.08***	6.41 ± 0.41***

IV	*S. colais* 200mg/kg	133.12 ± 8.30*	147.60 ± 13.52*	108.30 ± 9.48*	1.21 ± 0.24*	5.85 ± 0.23
V	*S. colais* 400mg/kg	124.79 ± 12.45**	139.10 ± 9.68**	105.56 ± 6.54*	1.17 ± 0.15*	6.08 ± 0.32*

All values represent mean ± SEM (n = 6)

P Value: +++ p<0.001 significantly different groups when comparing with control.

*** P < 0.001, ** P < 0.01, * P < 0.05 significantly different groups when comparing with Group-II (PCM treated).

3.3 Effect of *S. colais* on oxidative stress parameters

The effects of *S. colias* extract on the levels of LPO, SOD, CAT, GSH, GPx and GST in hepatic homogenate is presented in Table 2. Group-II animals received paracetamol showed significant decrease hepatic SOD (45.72 ± 8.30 U/mg protein, $P < 0.001$), CAT (14.83 ± 5.03 U/mg protein, $P < 0.001$), GSH (2.46 ± 0.72 U/mg protein, $P < 0.01$), GPx (2.68 ± 0.59 U/mg protein, $P < 0.001$) and GST (0.59 ± 0.22 nmol/mg protein, $P < 0.001$), while enhancement in the level of hepatic LPO (0.49 ± 0.08 nmole MDA/mg protein, $P < 0.001$) as compared to group-I rats of SOD (92.86 ± 6.72), CAT (30.67 ± 2.78), GSH (3.69 ± 0.29), GPx (4.26 ± 0.28), GST (1.28 ± 0.12) and LPO (0.49 ± 0.08). The extract SCE in groups IV and V animals increased significantly the levels of SOD to (63.81 ± 9.64, $P < 0.05$ and 70.11 ± 6.01, $P < 0.01$), CAT to (22.89 ± 2.51, $P < 0.05$ and 23.63 ± 3.11, $P < 0.05$), GSH to (2.75 ± 0.52 and 2.83 ± 0.28), GPx to (3.48 ± 0.31, $P < 0.05$ and 3.69 ± 0.27, $P < 0.01$) and GST to (0.90 ± 0.11, $P < 0.05$ and 0.93 ± 0.14, $P < 0.05$), while decrease in the level of hepatic LPO to (1.29 ± 0.20 and 1.08 ± 0.15, $P < 0.05$) as compared to group-II animals where the levels were altered due to paracetamol administration. Silymarin in group-III rats significantly ($P < 0.001$) increased the levels of SOD, CAT, GSH, GPx and GST to 82.98 ± 5.27, 27.39 ± 1.80, 3.48 ± 0.37, 4.43 ± 0.37 and 1.37 ± 0.09 respectively, while significantly ($P < 0.001$) decreased the level of MDA to 0.56 ± 0.11 as compared to group-II animals. Extract SCE in groups IV as well as in group V rats showed significant activity in the levels of SOD, CAT, GSH, GPx, GST and MDA compared to animals in group II (Paracetamol treated). *S. Colias* at a dose of 400 mg/kg body weight showed more protection to liver than that of 200 mg/kg body weight but showed less protection than that of silymarin 25 mg/kg body weight.

Table 2: Effect of *S. colais* on oxidative stress parameters in paracetamol intoxicated rats

Group	Treatment	LPO (nmol/mg protein)	SOD (U/mg protein)	CAT (U/mg protein)	GSH (U/mg protein)	GPX (U/mg protein)	GST (nmol/mg protein/min)
I	Normal 1ml/kg	0.49 ± 0.08	92.86 ± 6.72	30.67 ± 2.78	3.69 ± 0.29	4.26 ± 0.28	1.28 ± 0.12
II	PCM treated 2000 mg/kg	1.69 ± 0.55^{+++}	45.72 ± 8.30^{+++}	14.83 ± 5.03^{+++}	2.46 ± 0.72^{+++}	2.68 ± 0.59^{+++}	0.59 ± 0.22^{+++}
III	Silymarin 25mg/kg	0.56 ± 0.11***	82.98 ± 5.27***	27.39 ± 1.80***	3.48 ± 0.37**	4.43 ± 0.37***	1.37 ± 0.09***
IV	*S. colais* 200mg/kg	1.29 ± 0.20	63.81 ± 9.64*	22.89 ± 2.51*	2.75 ± 0.52	3.48 ± 0.31*	0.90 ± 0.11*
V	*S. colais* 400mg/kg	1.08 ± 0.15**	70.11 ± 6.01**	23.63 ± 3.11*	2.83 ± 0.28	3.69 ± 0.27**	0.93 ± 0.14*

All values represent mean ± SEM (n = 6)

P Value: +++ p<0.001 significantly different groups when comparing with control.

*** P < 0.001, ** P < 0.01, * P < 0.05 significantly different groups when comparing with Group-II (PCM treated).

4. Discussion

Herbal plants play an important role for the treatment of various liver diseases. Some have been analysed and validated scientifically for their potential uses (Ali et al., 2017). Here, we designed studies to examine the hepatoprotective activity of ethanolic extract of *S. colais* leaves. Paracetamol also known as acetaminophen is widely consumed as an analgesic and antipyretic over-the-counter drug that is safe at therapeutic doses but can result in hepatic damage (Rotundo and Pyrsopoulos, 2020). Paracetamol is metabolized by cytochrome P450 enzymes to highly reactive and toxic metabolite N-acetyl-*p*-benzoquinone imine (NAPQ1). NAPQ1 is normally conjugated with glutathione and excreted in urine (Hamza and Al-Harbi, 2015). Overdose of PCM will result in deposition of NAPQ1 (Giri et al., 2020). The free NAPOI will binds covalently to cellular mitochondrial proteins, produce dysfunction and suppresses mitochondrial fatty acid-oxidation and results in development of acute hepatic necrosis (Yahya et al., 2013).

A clear sign of hepatic injury is the leaking of cellular enzymes such as SGPT, SGOT and ALP into plasma due to the dyfunctions of hepatocytes transport system (Karwani and Sisodia, 2015).

SGOT mainly present in mitochondria of hepatocytes. SGPT is more specific to liver and thus is a good marker to detect liver injury. Serum ALP and bilirubin is also connected with hepatocytes damage. The SGOT, SGPT ALP and serum bilirubin level are largely used as most common biochemical parameters to evaluate liver injury (Islam et al., 2021). Administration high dose of PCM caused a significant increases of enzymes level such as SGOT, SGPT, ALP and bilirubin level has been attributed to the damage structural integrity of hepatic cells, because they are found inside of liver cell cytoplasm and released into the blood circulation after hepatic cellular damages indicating development of hepatotoxicity (Chaitanya et al., 2012). Despite that, total protein levels are lowered in the presence of hepatic damage.

The enzymatic antioxidant such as SOD, CAT and GPx are very vital defence system to protect organisms from reacting oxygen species. SOD is an enzyme, which converts superoxide free radicals to hydrogen peroxide molecule (Bhattacharyya et al., 2014). Catalase is involved in conversion of hydrogen peroxide molecule in to water and oxygen (Chance et al., 1951). GPx is very important enzyme which provide protect against chemically induced oxidative destruction process of lipid and proteins. The GST is multifunctional enzyme which involved in the metabolism of a broad variety of xenobiotics and endogenous compounds and provide protection against toxic compound by metabolised them (Sivakumar et al., 2018). The reduction of GSH level in paracetamol treated group may be due to the conjugation of GSH with reactive metabolite NAPQ1 to form mercapturic acid (Parmar et al., 2010).

Lipid peroxidation (LPO) has been occurred due to the liver injury after paracetamol administration. The rise in malondialdehyde (MDA) level of liver indicate boost in lipid peroxidation leading to tissue damage and failure or depression of antioxidant defence mechanisms of body which prevent excessive formation of free radicals (Rotundo and Pyrsopoulos, 2020). The decrease of SOD, CAT, GSH, GPx, and GST enzymes activity may indicate the toxic effects of reactive free radicals of oxygen species which are produced by toxicants (Shields et al., 2021).

The administration of 200 mg/kg and 400 mg/kg of ethanolic extract S. colais leaves showed capability to reduced significantly liver markers (SGOT, SGPT, ALP and total bilirubin) level in blood, whereas increases in protein level. Simultaneously, significantly increases in the level of enzymatic antioxidants like SOD, CAT, GSH, GPx and GST and whereas reduction in LPO were also showed by S. colais in dose dependent manner.

The hepatoprotection activity of *S. colais* may be due to their antioxidant potential. This suggests that *S. colais* can reduce the reactive oxygen species that may reduce the oxidative damage to the liver cells and enhance the activities of the liver enzymatic antioxidants, thus protecting the liver from paracetamol induced damage. The stimulation of hepatic regeneration through improved protein synthesis or accelerated detoxification and excretion could be another possible mechanism. Similar findings also reported by the Chavan *et al*., (2021) demonstrated the hepatoprotective effects of *Allophylus cobbe* against paracetamol induced hepatotoxicity in rats.

Conclusion

The results of this present study have demonstrated that ethanolic extract of *S. colais* has a potent hepatoprotective effect against paracetamol-induced hepatic damage in rats. It also recommends that *S. colais* warrants future detailed investigation as a promising hepatoprotective agent. However, the exact mechanism and the active compounds involved in these effects need to be clarified in future studies.

Acknowledgment

The authors are thankful to Dean, Faculty of Pharmacy, Bhupal Nobles' University, Udaipur, Rajasthan for providing all necessary facilities to carry out research work.

References

1. Agbaje, E. O. & Ogunleye, O. Hepatoprotective activity of aqueous leaf extract of *Calotropis procera* (*Asclepladaceae*) in paracetamol-induced liver injury. *J. Pharm. Sci. & Res.*, 8(9): 988-994.
2. Ali, M., Khan, T., Fatima, K., Ali, Q. ul A., Ovais, M., Khalil, A. T., Ullah, I., Raza, A., Shinwari, Z. K. & Idrees, M. (2017). Selected hepatoprotective herbal medicines: Evidence from ethnomedicinal applications, animal models, and possible mechanism of actions. *Phytotherapy Research*, 32(2):199-215.
3. Aneja, S., Vats, M., Aggarwal, S. & Sardana, S. (2013). Phytochemistry and hepatoprotective activity of aqueous extract of *Amaranthus tricolor* Linn. roots. *Journal of Ayurveda and Integrative Medicine*, 4(4): 211-215.
4. Chaitanya, A. K. D., Challa, S. R. & Reddy, M. A. (2012). Hepatoprotective effect of biherbal ethanolic extract against paracetamol-induced hepatic damage in Albino rats. *Journal of Ayurveda and Integrative Medicine*, 3(4): 1-9.

5. Chavan, S., Dias, R. & Magdum, C. (2021). Hepatoprotective effect of ethanolic extracts of *Allophylus cobbe (L.) Raeusch* leaves against paracetamol induced hepatotoxicity in rats. *Journal of Applied Pharmacy*, 13(7): 1-5.
6. Chiang, J. (2014). Liver physiology: Metabolism and detoxification. *Pathobiology of Human Disease*, 1770-1782.
7. Cordero-Espinoza, L. & Huch, M. (2018). The balancing act of the liver: Tissue regeneration versus fibrosis. *Journal of Clinical Investigation*, 128(1):85-96.
8. Dey, T. K., Emran, T. B., Saha, T. D., Rahman, A. M., Hosen, S. M. Z. & Chowdhury, N. (2012). Antioxidant activity of ethanol extract of *Cassia hirsuta* (L.) leaves. *Bulletin of Pharmaceutical Research*, 2(2): 78-82
9. Donkor, S., Larbie, C., Gustav, K. & Benjamin, O. E. (2020). Evaluation of the acute hepatoprotective potential of hydroethanolic extract of *Duranta erecta L.* Parts. *Journal of Toxicology*,1-10.
10. Giri, A., Rao, V., Singhal, S., Biswas, S., Nandakumar, K., Chamllamudi, M. R., Dave, J., et. al., (2020). Evaluation of hepatoprotective effect of a polyherbal megakutki against paracetamol induced hepatotoxicity. *Indian Journal of Pharmaceutical Education and Research, 54*(4): 1080-1088.
11. Hamza, R. Z & Al-Harbi, M. S. (2015). Amelioration of paracetamol hepatotoxicity and oxidative stress on mice liver with silymarin and Nigella sativa extract supplements. *Asian Pacific Journal of Tropical Biomedicine*, 5(7): 521-531.
12. Henry, R. J., Cannon, D. C. & Winkelman, J. W. (1975). Clinical chemistry: Principles and technics. *Clinica Chimica Acta*, 65:249-250.
13. Islam, M. T., Quispe, C., Islam, M. A., Ali, E. S., Saha, S., Asha, U. H., Mondal, M. et al., (2021). Effects of Nerol on paracetamol-induced liver damage in Wistar albino rats. *Biomedicine and Pharmacotherapy*, 140: 1-10.
14. Jahan, N., Parvin, Mst. S., Das, N., Islam, M. S. & Islam, Md. E. (2014). Studies on the antioxidant activity of ethanol extract and its fractions from *Pterygota alata* leaves. *Journal of Acute Medicine*, *4*(3):103-108.
15. Karole, S., Jain, D. K., Abbas, S., Abbas, N., & Kare, P. (2022). Antioxidant and hepatoprotective effects of *Ougeinia dalbergioides benth* against paracetamol and CCL_4

induced liver damage in rats. *Indian Journal of Pharmacy and Pharmacology,* 9(1): 67-74.

16. Karwani, G. & Sisodia, S. S. (2015). Hepatoprotective activity of *Tagetes erecta* Linn. in ethanol induced hepatotoxicity. *Sch. Acad. J. Pharm.,* 4(3): 181-189.
17. Kiran, P. M., Raju, A. V. & Rao, B. G. (2012). Investigation of hepatoprotective activity of *Cyathea gigantea* (Wall. ex. Hook.) leaves against paracetamol induced hepatotoxicity in rats. *Asian Pacific Journal of Tropical Biomedicine*, 2(5): 352-356.
18. Koirala, S. & Kumar, S. J. (2019) Evaluation of hepatoprotective activity of ethanolic extract of root of *Prunus persica* on paracetamol induced hepatotoxic in rats. *Journal of Natural & Ayurvedic Medicine*, 3(2): 1-8.
19. Parmar, S. R., Vashrambhai, P. H. & Kalia, K. (2010). Hepatoprotective activity of some plants extract against paracetamol induced hepatotoxicity in rats. *Journal of Herbal Medicine and Toxicology*, 4(2): 101-106.
20. Rotruck, J. T., Pope, A. L., Ganther, H. E., Swanson, A. B., Hafeman, D. G., & Hoekstra, W. G. (1973). Selenium: Biochemical role as a component of glutathione peroxidase. *Science*, *179*(4073): 588-590.
21. Rotundo, L. & Pyrsopoulos, N. (2020). Liver injury induced by paracetamol and challenges associated with intentional and unintentional use. *World Journal of Hepatology*, *12*(4): 125-136.
22. Saidurrahman, M., Mujahid, M., Siddiqui, M. A., Alsuwayt, B. & Rahman, M. A. (2022). Evaluation of hepatoprotective activity of ethanolic extract of *Pterocarpus marsupium Roxb*. leaves against paracetamol induced liver damage via reduction of oxidative stress. *Phytomedicine Plus*, 2(3): 1-6.
23. Shields, H. J., Traa, A. & Raamsdonk, J. M. V. (2021). Beneficial and detrimental effects of reactive oxygen species on lifespan: A comprehensive review of comparative and experimental studies. *Frontiers in Cell and Developmental Biology*, 9: 1-27.
24. Spitz, D. R. & Oberley, L. W. (1989). An assay for superoxide dismutase activity in mammalian tissue homogenates. *Analytical Biochemistry*, *179*(1): 8-18.
25. Tripathi, A., Yadav, A. K., Mishra, P. K. & Rao, C. V. (2017). Hepatoprotective activity of *Ficus carica Linn* stems extract against carbon tetrachloride liver damage in Wistar rats. *World Journal of Pharmacy and Pharmaceutical Sciences*, 6(5): 766-778.

26. Yahya, F., Mamat, S. S., Kamarolzaman, M. F. F., Seyedan, A. A., Jakius, K. F., Mahmood, N. D., Shahril, M. S., et. al., (2013). Hepatoprotective activity of methanolic extract of *Bauhinia purpurea* leaves against paracetamol induced hepatic damage in rats. *Evidence Based Complementary and Alternative Medicine*, 2013: 1-11.