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Hepatoprotective Effect of *Carica papaya* Leaves and *Andrographis paniculata* Plant Material in Cyclophosphamide Induced Male Wistar Rats

Pavan Goyal, D.B. Mondal, S. Dey, K.P. Singh, S.K. Dixit, S. Mehrotra, S.K. Maiti, A.G. Telang

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ABSTRACT

Cyclophosphamide is used for the treatment of cancer cells and for suppressing the immune system during implantation. It adversely affects the cancer cells as well as healthy cells and increases the oxidative stress in these cells so cellular proteins, lipids and nucleic acids get denatured. It also produces liver, spleen and bone marrow toxicity. Medicinal plants such as *Carica papaya* and *Andrographis paniculata* possess hemopoietic, thrombopoietic and hepato-renal protective properties. The current study aimed to study the phytoconstituents in the hydro-ethanolic extracts of *Carica papaya* leaves and *Andrographis paniculata* plant material and their hepatoprotective effect in cyclophosphamide induced male Wistar rats.The collected plant materials were washed, dried, pulverized into fine powder, stored in air-tight containers and subjected to quantitative estimation of phytoconstituents present in the hydro-ethanolic extracts of *Carica papaya* leaves and *Andrographis paniculata* plant material. The hepato-protective properties of hydro-ethanolic extracts of these plants were evaluated on the basis of serum biochemistry analyses. Total phenolic content, alkaloids, flavonoids, saponin and cardiac glycosides were present in higher amount in hydroethanolic extract of *Carica papaya* leaves and was found more hepatoprotective @ 200 mg/kg b. wt. compared to *Andrographis paniculata*.

Key words: *Andrographis paniculata*, *Carica papaya*, Hepatoprotective, Hydroethanolic extract, Phytoconstituents.

The medicinal plants are widely used to treat livestock, human and pet animals and this idea is not new. Most of the active principles of medicines or drugs are originally extracted from plant materials. After extraction and purification, these medicinal plants serve as source of drugs (Mir *et al.,* 2016).

Carica papaya (*Family: Caricaceae*) is the well-known plant in the world (Ong *et al.,* 2011). It is a soft wooded single-stemmed perennial tree, 2-10 m in height, with a crown of large palmate from the apex of the trunk (Jiao *et al.,* 2010). Its leaves contain secondary metabolites like flavonoids, alkaloids, saponins, tannins, β-carotene, glycosides and steroids having immunomodulatory, antitumor and antipathogenic activities (Anibijuwon and Udeze, 2009). Papaya leaves also having bioactive compounds such as chymopapain and papain that help in the process of digestion and also inhibit the growth of pathogenic micro-organisms (Unaeze and Brikwa, 1986; Yismaw *et al.,* 2008). Papaya leaves are rich in protease and amylase. These enzymes have high anti-inflammatory properties which reduce the inflammation of stomach and colon (Alabi *et al.,* 2012). Papaya leaves extract heals peptic ulcers by killing bacteria *Helicobacter pylori* owing to their antimicrobial properties. Papaya leaves contain carpain, acetogenin and phenolic compounds. Carpain is a chemical compound or a substance with ability to kill micro-organisms that often intervene in food digestion processes (Anibijuwon and Udeze, 2009; Calzada *et al.,* 2007) and also stops the excess growth of skin flora by sanitizing skin from the toxins and provides protection against skin problems like pimples, freckles and acne (Udoh *et al.,* 2005).

Division of Medicine, Indian Veterinary Research Institute, Izatnagar, Bareilly-243 122, Uttar Pradesh, India.

Corresponding Author: Pavan Goyal, Division of Medicine, Indian Veterinary Research Institute, Izatnagar, Bareilly-243 122, Uttar Pradesh, India. Email: drpawangoyal880@gmail.com

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Andrographis paniculata is commonly called as "Kalmegh" (*Family: Acanthaceae*) and referred to as a medicinal plant (Saranya *et al.,* 2010). It possesses various medicinal properties such as anti-malarial, anticancer, anti-inflammatory, antiulcer, anti-hyperglycemic, antiviral, anti-angiogenic, hepatoprotective, antimicrobial, immunomodulatory, anti-parasitic and antioxidant properties (Chandrasekaran *et al.,* 2009; Akbar, 2011; Ojha *et al.,* 2012). Its plant extract contains diterpenes, flavonoids, xanthones, noriridoides *etc.* (Singha *et al.,* 2010). It has anti-microbial/ protozoan, anti-inflammatory, anti-oxidant, immuno-stimulant, anti-diabetic, hepato-renal protective and liver enzymes modulator properties (Okhuarobo *et al.,* 2014). Kalmegh is used for liver diseases, blood purifier and dermatological diseases

(Prathanturarug *et al.,* 2007). Keeping these aspects in mind, the proposed study has been designed for determination of phytoconstituents in *Carica papaya* leaves and *Andrographis paniculata* plant material extracts and their hepatoprotective effect in cyclophosphamide induced male Wistar rats.

The present study was conducted at Indian Veterinary Research Institute, Izatnagar, Bareilly during the year 2021-2022. The plant materials were collected (around 5 kg each as per requirement) from Bareilly district of Uttar Pradesh province of India and were washed three times using distilled water to clean and remove the waste materials and shade dried until dryness. The dried plant materials were pulverized into fine powder and stored in air-tight containers to avoid contact with moisture. The required plant materials were defatted using petroleum ether as solvent, sonicated for 3 minutes and a pause of 30 seconds for 6 times using 50% ethanol prepared by 50 mL ethanol and 50 mL distilled water and centrifuged at 4000 rpm and 4°C for 12 minutes. The total phenolic content and total tannins in the extracts were measured by the method described by (Makkar *et al.,* 1993) with little modifications. The total alkaloids, flavonoids, saponin and cardiac glycosides were estimated by the method described by the (Ajanal *et al.,* 2018; Dwivedi *et al.,* 2020; Mir *et al.,* 2016 and Onyema *et al.,* 2016), respectively. In this experiment, a total of thirty-six male Wistar rats were utilized by prior permission from *Institutional Animal Ethics Committee* and they were categorized in six groups having six rats in each (Table 1). Blood samples were collected aseptically in vacutainers without anticoagulant on day 14 and serum biochemistry was performed according to the procedures. The data obtained from various treatments under study were analysed by using student's "t" test and repeated measures "ANOVA".

In the present study, the total phenolic content, total tannins, total alkaloids, total flavonoids, total saponin and total cardiac glycosides in hydro-ethanolic extract of *Carica papaya* leaves were 44.27±1.50 mg TAE/g of dry weight, 17.76±5.13%, 29.80±1.55 mg/g, 6.63±0.20 mg GAE/g of dry weight, 1.31±0.01% and 3.08±0.01%, respectively whereas in hydro-ethanolic extract of *Andrographis paniculata* plant material were 33.51±1.66 mg TAE/g of dry weight, 11.84±2.96%, 15.71±1.02 mg/g, 3.93±0.53 mg GAE/g of dry weight, 1.01±0.01% and 2.15±0.09%, respectively (Table 2).

There was significant ($P<0.05$) increased in aspartate aminotransferase (AST) level in group-II but no significant difference ($P>0.05$) in group-III, group-IV, group-V and group-VI; significant ($P<0.05$) increased in alanine aminotransferase (ALT) level in group-II and group-V but no significant difference ($P>0.05$) in group-III, group-IV and group-VI and significant ($P<0.05$) increased in alkaline

Table 1: Experiment protocol.

Group	Procedure	Treatment period
Group-I: Healthy control	Animals were fed and managed as per Institutional animal ethics committee guidelines	-
Group-II: Disease control	Thrombocytopenia was induced by the cyclophosphamide with the dose rate of 75 mg/kg body weight subcutaneously.	-
Group-III: *Carica papaya* leaf extract (CPLE)-Low dose.	Thrombocytopenia was induced+CPLE with the dose rate of 100 mg/kg body weight orally.	Started from fourth day daily for ten days
Group-IV: CPLE-High dose	Thrombocytopenia was induced+CPLE with the dose rate of 200 mg/kg body weight orally.	Started from fourth day daily for ten days
Group-V: *Andrographis paniculata* plant extract (APPE)-Low dose	Thrombocytopenia was induced+*Andrographis paniculata* plant extract (APPE) with the dose rate of 100 mg/kg body weight orally.	Started from fourth day daily for ten days
Group-VI: APPE-High dose	Thrombocytopenia was induced+*Andrographis paniculata* plant extract (APPE) with the dose rate of 200 mg/kg body weight orally.	Started from fourth day daily for ten days

Table 2: Mean±SE values of phytoconstituents present in H-E extracts.

Phytoconstituents	H-E extract of *C. papaya* leaves (Mean±SE)	H-E extract of *A. paniculata* plant material (Mean±SE)
Total phenolic content **(mg TAE/g)	44.27b±1.50	33.51a±1.66
Total tannins (%) NS	17.76a±5.13	11.84a±2.96
Total alkaloids (mg/g)**	29.80b±1.55	15.71a±1.02
Total flavonoids**(mg GAE/g)	6.63b±0.20	3.93a±0.53
Total saponins** (%)	1.31b±0.01	1.01a±0.01
Total cardiac glycosides **(%)	3.08b±0.01	2.15a±0.09

** Significant (P≤0.05), NS: Non significant (P>0.05), H-E: Hydro-ethanolic.

Table 3: Serum biochemistry of different groups of male Wistar rats (Mean±S.E.).

Parameters	Group I	Group II	Group III	Group IV	Group V	Group VI
AST (IU/L)*	110.50^{bc}±10.1	219.96^{a}±13.80	103.40^{bc}±10.75	99.69^{c}±9.94	153.29^{b}±16.0	133.04^{bc}±10.93
ALT (IU/L)*	21.18^{c}±4.77	137.26^{a}±17.99	50.17^{bc}±13.22	29.26^{bc}±3.99	70.01^{b}±6.25	46.35^{bc}±2.70
TP (g/dl)*	7.04^{ab}±0.31	6.09^{ab}±0.26	6.01^{ab}±0.14	7.54^{a}±0.18	6.43^{ab}±0.75	5.63^{b}±0.26
Albumin (g/dl)*	3.93^{a}±0.12	2.64^{cd}±0.15	4.44^{a}±0.26	3.72^{ab}±0.32	2.99^{bc}±0.24	1.97^{d}±0.14
Globulin (g/dl)*	3.11^{ab}±0.33	3.45^{a}±0.27	1.58^{b}±0.34	3.82^{a}±0.46	3.44^{a}±0.56	3.67^{a}±0.27
A:G ratio*	1.35^{b}±0.17	0.80^{b}±0.11	3.77^{a}±0.92	1.09^{b}±0.20	0.95^{b}±0.12	0.56^{b}±0.07
AlKP (IU/L)*	98.64^{c}±15.23	218.18^{a}±12.92	149.38^{bc}±9.57	108.12^{c}±6.9	184.80^{ab}±15.95	123.94^{c}±4.89

Note: Values bearing different superscripts in the same row differ significantly. *Significant (P≤0.05).

phosphatase level in group-II and group-V but no significant difference (*P>0.05*) in group-III, group-IV and group-VI compared to group-I (Table 3). Zhang *et al.* (2021) reported that the ALT level was significantly greater (*P<0.01*), but the AST level was somewhat reduced. The leaching out of enzymes of liver damage (ALT, AST and alkaline phosphatase) from the hepatocytes, resulting in enhanced enzyme activity in the systemic circulation, is one of the effects of cyclophosphamide-induced hepatic injury (Senthilkumar *et al.,* 2006).

Mohammed *et al.* (2011) reported that on administration of the aqueous *Carica papaya* leaf extract in a dose-dependent manner in male Wistar rats, there was a significant (*P<0.05*) restoration of AST, ALT and alkaline phosphatase enzymes. Serum transaminase levels revert to normal as the hepatic parenchyma heals and hepatocytes regenerate (Thabrew and Joice, 1987). The aqueous extract (Vetriselvan *et al.*, 2011) and ethanolic extract (Sheeja and Kuttan (2006) of *Andrographis paniculata* lowered the level of alkaline phosphatase which was increased during cyclophosphamide administration, indicated the hepatoprotective effect of the plant extract.

There was no significant difference (*P>0.05*) in total protein and globulin in group-II, group-III, group-IV, group-V and group-VI; significant (*P<0.05*) decreased in albumin in group-II, group-V and group-VI but no significant difference (*P>0.05*) in group-III and group-IV; no significant difference (*P>0.05*) in A:G ratio in group-II, group-IV, group-V and group-VI but significant (*P<0.05*) increased in group-III compared to group-I (Table 3). Olukole *et al.* (2020) reported that cyclophosphamide caused a significant drop in serum protein levels (albumin, globulin and total protein) compared to control group. Alterations in serum proteins are the clinical markers of assessing the toxicity and health state of humans and animals (Sacher and Mcpherson, 2000). A decrease in total protein and albumin levels implies acute or chronic liver injury, which could be caused by a loss of liver biosynthetic ability (Singh *et al.,* 2011). Hepatoprotective effect of plants is due to presence of flavonoids (Wilma *et al.,* 2011).

CONCLUSION

The hydro-ethanolic extract of *Carica papaya* leaves is found better and more hepatoprotective as compared to hydro-ethanolic extract of *Andrographis paniculata* plant material in quantitative estimation of phytoconstituents and serum biochemical analyses.

Conflict of interest: None.

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