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## PHARMACOGNOSTICAL INVESTIGATION AND EVALUATION OF *FICUS CARICA* FRUIT EXTRACT FOR ADAPTOGENIC ACTIVITY

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### Keywords:

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**ABSTRACT:** *Ficus carica* is the common fig fruit belonging to the family Moraceae, The Pharmacognostical studies of the half ripened fruits were carried out using the various micro-chemical tests. The dried fruits were subjected to extraction using 90 % ethanol and this extract was further evaluated for the adaptogenic activity. Swim Endurance test in mice and Cold Stress model in rats was used to screen the antistress potential. Biochemical parameters such as Blood Glucose level, Total Leukocyte count, Total Cholesterol, HDL, LDL, Triglyceride levels were estimated. The microscopic studies confirmed that the fruit of *Ficus* is a modified receptacle of the inflorescence; this contains the male and the female flowers which are situated in the interior of the receptacle. The ethanolic extract was found to increase the swim endurance time significantly. In case of cold stress model the ethanolic extract increased the depleted blood glucose and lowered the elevated total cholesterol as well as triglyceride levels. It was able to maintain the normal homeostasis as well as act as an anti-hyperlipidemic agent. It was also observed that the Leukocyte count too was lowered in case of the extract treated group. The results clearly indicate the potential of *Ficus carica* as an adaptogenic agent.

**INTRODUCTION:** Stress, as mentioned by Dr. Hans Selye<sup>1</sup> is the non-specific response of the body to any demand made upon it. Whenever our body gets excited, confused or otherwise feel unsafe or threatened, the body's physical, mental or chemical reaction is known as Stress. Stress is the outcome of people reacting to each other and to the day to day situations, when the daily demands are easy and well balanced an individual is fit and fine.

But when an individual perceives that the pressure is unreasonable or the situation too demanding, that leads to the stress, and the stress reactions begin in one's mind.

**Adaptogens:** Adaptogens<sup>2</sup> are the natural bioactive substances, which improve physical endurance in difficult environmental conditions and even in adverse circumstances and which appear to induce a state of non specific increase of resistance of the individual to diverse aversive assaults which threaten internal homeostasis<sup>3</sup>. These agents are basically preventive rather than curative in action and appear to function best when the resistance of the body is diminished, as seen in the case of prolonged illness, chronic stress and old age. They increase tolerance to change in environmental

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conditions and resistance to noxious stimuli such as exposure to cold, heat, pain, general stress and infectious organisms. Such agents have been claimed to arrest ageing process and age induced deterioration in physical and mental performance.

#### Introduction to *Ficus carica* (Figure 1)

Figs are infructescences of the *Ficus carica* L., a deciduous tree belonging to the Moraceae family. It is a native of Carica which present in Asia Minor. It is also found in Turkey, Portuguese, Iran, Iraq, China, and Japan and in southern parts of India. It is also seen in Punjab, Uttar Pradesh and Mysore region of India. In Ayurvedic literature it is known as Vatt kul, in Sanskrit named as Falgu and known as Anjeer in Marathi.

Figs are an excellent source of minerals, vitamins<sup>4</sup> and dietary fibre; they are fat and cholesterol-free and contain a high number of amino acids<sup>5</sup>. The milk contains resin, sugar and protein. *F. carica* has been reported to have numerous bioactive compounds<sup>6</sup> such as arabinose,  $\beta$ -amyrins,  $\beta$ -carotenes, glycosides,  $\beta$ -sitosterol and xanthotoxol<sup>7,8,9</sup>. Earlier chemical examination of this plant has shown the presence of Psoralen, Bergapten, Umbelliferone<sup>10,11</sup>, nematicidal coumarin<sup>12</sup>, Campesterol, Stigmasterol, Fucosterol, fatty acids, 6-(2-methoxy-Z-vinyl)-7-methyl-pyrano-coumarin and 9,19-cycloarlane triterpenoid as an anticancer<sup>14,15</sup>, and antiproliferative agent: 6-O-acyl- $\beta$ -Dglucosyl - $\beta$ -sitosterol<sup>16</sup>, calotropenyl acetate, and lupeol acetate<sup>17,18,19</sup>. The Hypoglycemic and antioxidant activities of fig & leaves are been reported<sup>20, 21</sup>. The laxative effect of *Ficus carica* has been also reported<sup>22</sup>.



FIGURE 1: *FICUS CARICA* FRUITS

#### Materials & Methods

##### Plant Material:-

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#### Collection and Authentication

Plant material was collected from the surroundings of Pune and authentication was done by Dr. J. Jayanathi Scientist 'C' from the Botanical survey of India, Pune. The herbarium was preserved with the Voucher specimen No: DAYFIC3.

#### Extraction

The plant material was size reduced and was further extracted by maceration with 95% Ethanol. The extract was concentrated using Rota evaporator and dried under vacuum to get reddish brown syrupy semisolid mass (32.2% w/w).

#### Pharmacognostic investigation

##### Preparation of specimens

The plant specimens for the proposed study were cleaned and care was taken to select healthy plants and normal organs. The required samples of the fruit were cut and fixed in FAA (Formalin-5ml+ Acetic acid-5ml + 70% Ethyl alcohol 90ml). After 24hrs of fixing, the specimens were dehydrated with graded series of tertiary – Butyl alcohol as per the schedule given by Sas<sup>23</sup>. Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58-60°C) until TBA solution attained super saturation. The specimens were cast into paraffin blocks.

##### Sectioning

The paraffin embedded specimens were sectioned with the help of Rotary Microtome. The thickness of section was 10-20  $\mu$ m. Dewaxing of section was customary procedure<sup>24</sup>. The sections were stained with Toluidine blue as per the method published by O'Brein<sup>25</sup> et al. Since Toluidine blue is polychromatic stain. The staining results were remarkably good; and some cytochemical reactions were also obtained. The dye rendered pink color to cellulose walls, blue to lignified cells, darkgreen to suberin, violet to mucilage, blue to protein bodies etc. wherever necessary sections were also stained with safranin and fast green and IKI (for Starch).

For studying the stomatal morphology, venation pattern and trichome distribution, paradermal sections (sections taken parallel to surface of leaf) as well as clearing of leaf with 5% sodium hydroxide or epidermal peeling by partial maceration employing Jeffery's maceration fluid<sup>23</sup> were prepared. Glycerin mounted temporary preparations were made for macerated/ cleared

materials. Powdered materials of different parts were cleared with NaOH and mounted in glycerin medium after staining. Different cell components were studied and measured.

### Microscopical Investigation

Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon labphoto 2 microscopic Unit. For normal observations bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have birefringent property, under polarized light they appear light against dark background. Magnifications of figures are indicated by scale-bars. Descriptive terms of anatomical features are given in standard anatomy books<sup>26</sup>.

### Determination of Physical Constants

The physical constants such as ethanol soluble extractive, water soluble extractive, ash value, acid insoluble ash, water soluble ash, were determined by the standard procedure<sup>27</sup>.

### Phytochemical Screening

The preliminary phytochemical investigation of the alcoholic extract was done and the tests for the presence of various phyto-constituents were carried out as per standard procedure<sup>27</sup>.

### Antistress activity<sup>28, 29</sup>

Prior approval of the Institutional animal ethics committee was obtained for the research work (ICP/IEAC/09-10/ P-11 & P-12).

### Animals

Healthy adult Swiss Albino mice of either sex weighing between 25 & 40 gm were used for swim endurance test. Healthy Wistar albino rats of either sex weighing between 150-200 gm were used for the evaluation of adaptogenic activity in cold stress model.

The animals were fed ad-libitum with commercial pellet diet (Lipton India Ltd., Mumbai) and had free access to water. The animals were divided into 4 groups for the swim endurance test and 5 groups for the cold stress model.

I- Normal (Vehicle – CMC 1%, p.o)

II- Standard (Gereforte tablets 43mg/kg BW, p.o).

III – FCE1 (*Ficus carica* Ethanolic Dose - 250 mg/kg BW, p.o)

IV – FCE2 (*Ficus carica* Ethanolic Dose - 500 mg/kg BW, p.o)

V- Negative Control (For cold stress - Vehicle – CMC 1%, p.o)

### Extract & Standard Drug

The Ethanolic extract FCE was used in all the models. The extract was formulated into an emulsion using gum CMC (1%). Gereforte Tablets (Himalaya drugs) 43 mg/kg was used as a standard Adaptogenic drug<sup>28</sup>.

### Swim Endurance Test

Greater Swimming endurance has been reported in mice when pretreated with anti- stress agents<sup>28</sup> and the test has been utilized to investigate the adaptogenic activity of different agents, based on the fact that swim endurance reflects physical endurance<sup>29</sup>.

Swiss albino mice of either sex weighing between 25-40 gm were used in this model. The animals were divided into 4 groups in two sets and were treated with respective extracts and the standard drug for 9 consecutive days, on the 10<sup>th</sup> day the test was performed. Individual Mice were allowed to swim in half filled Plastic buckets of 20 inches diameter. Two different temperatures were maintained - Normal (20°C), Cold (10°C). The mice were allowed to swim till they got exhausted and the moment they drowned was considered as the endpoint<sup>30</sup>. The time was noted and Swim endurance time was calculated. The data obtained was subjected to statistical analysis.

### Cold Stress Model

Wistar rats of either sex weighing between 150-200 gm were used in this model. The animals were divided into 5 groups of six animals each and were treated with respective extracts. They were administered orally a dose of 250 & 500 mg/kg b.w. of the extracts one hour prior to stress exposure daily. The animals were exposed to a temperature of 4 - 8 °C 4 hours daily for 10 days<sup>29, 30</sup>, by keeping them in a refrigerator. On the 10th day blood was withdrawn by retro-orbital puncture. Various biochemical parameters<sup>28</sup> such as Blood Glucose level, Total Leukocyte count (TLC), Lipid profile were estimated using different biochemical kits (Biolabs/ Span Kits).

### Statistical analysis

Comparison between control & drug treated groups were made by Student 't' test, P values of less than 0.05 were considered to be significant.

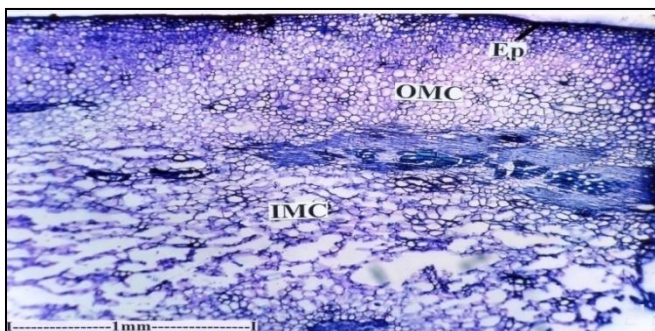
**RESULTS:**

**Pharmacognostic investigation**

**Anatomy of the Fruit**

The fruit of Ficus is a modified receptacle of the inflorescence. The receptacle becomes obvoid, fleshy hollow body with terminal opening. The opening is covered by membranous scales. All

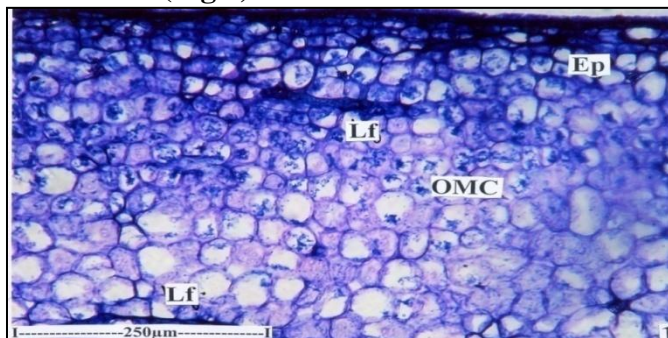
along the interior of the receptacle occur flowers. The male flowers are situated along the entry of the fruit and female ones are in the inner part. The receptacle is fleshy. It is smooth on the surface. The receptacle is thick. The outermost part of the receptacle consists of a narrow layer of thick walled epidermal cells. The epidermis is followed by 600 µm thick zone of circular or angular, thin walled, less compact parenchymatous outer mesocarp (**Fig. 2.**)



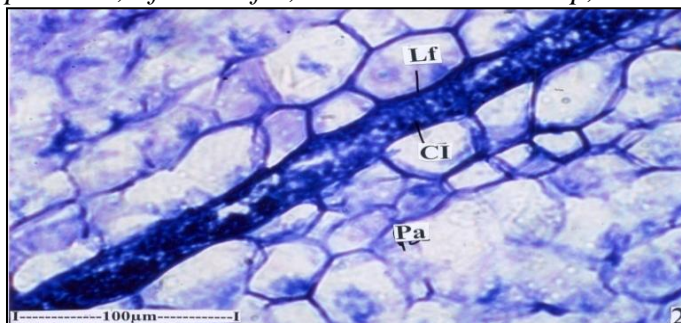
**FIGURE 2: T.S. OF FRUIT OUTER ZONE (EP: Epidermis, IMC: Inner Mesocarp, OMC: Outer Mesocarp, VB: vascular bundles)**

In the mesocarp region occurs the laticiferous canals. These canals secrete the latex in which starch grains are densely accumulated (**Fig.3**). The

laticiferous canals are unbranched and non septate. They are 20µm thick (**Fig.4**).



**FIGURE 3: OUTER ZONE OF THE FRUIT (OUTER MESOCARP) SHOWING LATICIFEROUS CANALS (CI: Cell inclusions, EP: Epidermis, Lf: Laticifer, OMC outer Mesocarp, Pa: Parenchymatous Tissue)**

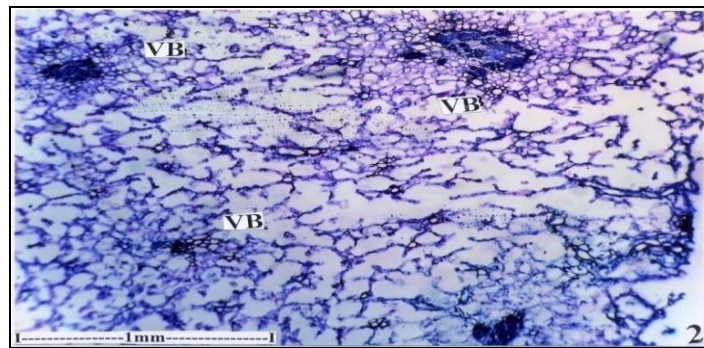


**FIGURE 4: A LATICIFER ENLARGED**

(CI: Cell inclusions, EP: Epidermis, Lf: Laticifer, OMC outer Mesocarp, Pa: Parenchymatous Tissue)

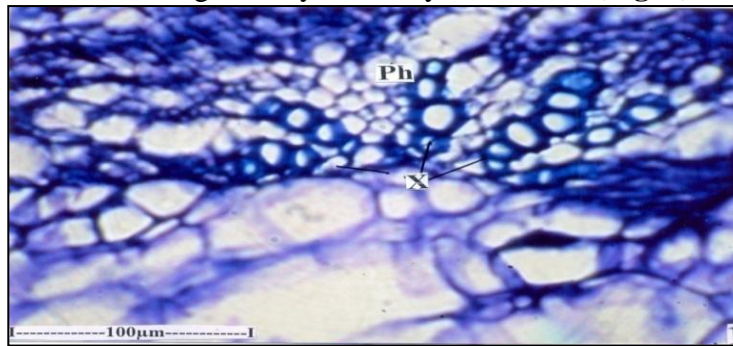
Inner and the outer mesocarp is wide zone of parenchymatous ground tissue. The cells in the inner mesocarp are loosely arranged forming

irregular air chambers. Small and large vascular strands are seen scattered in the ground tissue (**Fig. 5**).

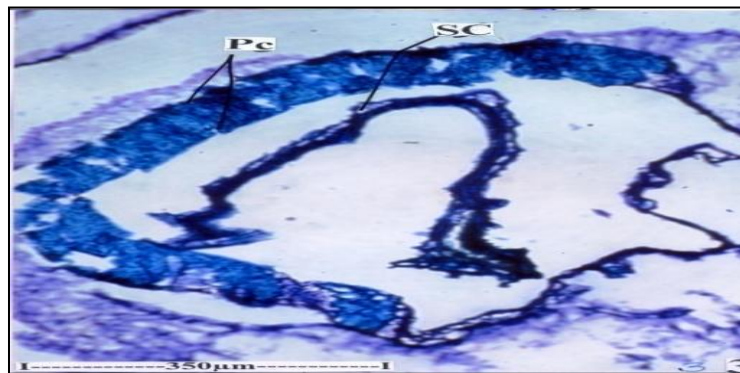


**FIGURE 5: T.S. OF FRUIT INNER ZONE** (EP: Epidermis, IMC: Inner Mesocarp, OMC: Outer Mesocarp, VB: vascular bundles)

The vascular strands include one to five, uniseriate elements. Phloem occurs in between and above the radiating lines of thick walled angular xylem xylem strands (**Fig. 6**).

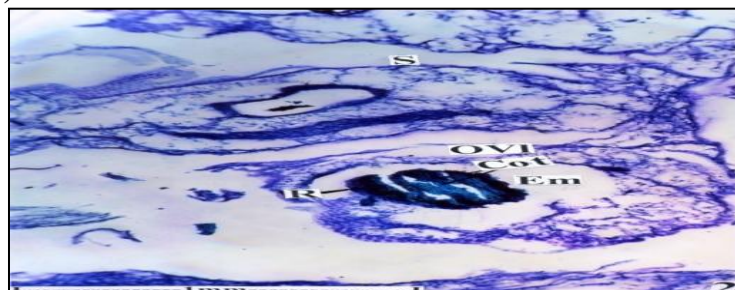


**FIGURE 6: VASCULAR STRAND OF INNER MESOCARP** (Em: Embryo, Ovl: Ovule, Pc: Pericarp, Ph: Phloem, X Xylem, S: Seed, Sc: Sclerotesta)



**FIGURE 7: A ACHENE (SEED) ENCLOSED WITHIN A SCLEROTIC PERICARP** (Em: Embryo, Ovl: Ovule, Pc: Pericarp, Ph: Phloem, S: Seed, Sc: Sclerotesta)

Seeds (**Fig.7**): the ovules are solitary in each female flower. They possess only one thick radical and two folded cotyledons (**Fig.8**).



**FIGURE 8: SEED SECTION SHOWING COTYLEDON** (Em: Embryo, Ovl: Ovule, Pc: Pericarp, Ph: Phloem, S: Seed, Sc: Sclerotesta)

**Physical Constants****TABLE 1: DETERMINATION OF PHYSICAL CONSTANTS**

Physical constants	<i>Ficus carica</i> %
Total ash value	5.73
Acid insoluble ash value	2.16
Water soluble ash value	1.41
Alcohol-soluble extractive value	9.62
Water-soluble extractive value	9.48

**Phytochemical Screening**

The preliminary phytochemical investigation of the alcoholic extract was done and Sterols, Triterpenoids, Flavonoids, Tannins, Proteins & carbohydrates were found to be present.

**TABLE 2: QUALITATIVE PHYTOCHEMICAL ANALYSIS**

Tests	FCE
Test For Sterol's & Triterpenoids	+ve
Test For Phenolic Compound	+ve
Test For Tannin's	+ve
Test For Flavonoid's	+ve
Test For Proteins & Amino Acid	+ve
Test for Carbohydrates	+ve
Test For Free Sugars	+ve

**Adaptogenic Activity****Effect of *Ficus carica* fruit extract on Swim Endurance**

The mice were forced to swim in water at 10<sup>0</sup>C as well as 20<sup>0</sup>C temperature till exhaustion and death. It was observed that all the FCE extract at both the doses increased the physical endurance as

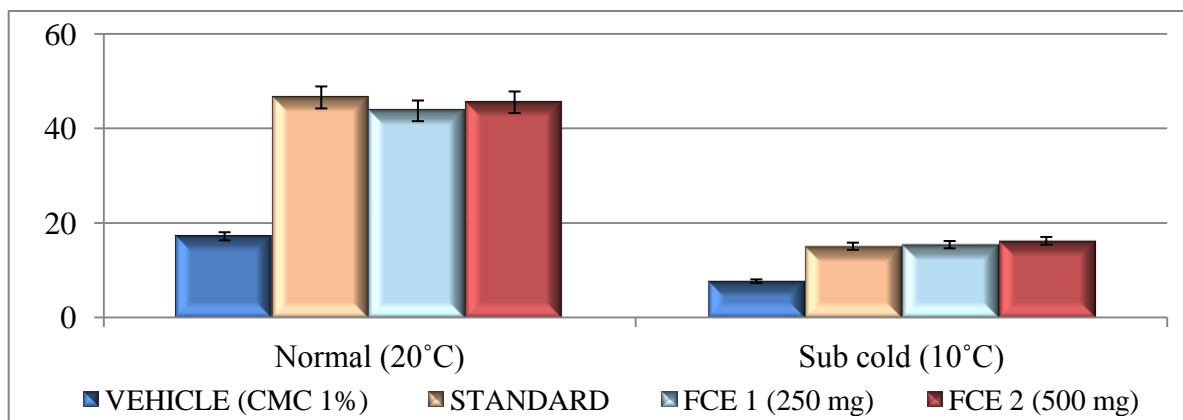
compared with untreated animals. The swimming time was significantly increased in both the treated groups and was at par with the standard treated group. The Ethanolic extract at a dose of 500 mg/kg effects were at par with that of the standard drug. (Table 3). Figure 9.

**TABLE 3: SWIM ENDURANCE TIME IN MINUTES**

	N(20 <sup>0</sup> C)	Sub(10 <sup>0</sup> C)
VEHICLE (CMC 1%)	17.19 ± 0.20	7.69 ± 0.89
FCE 1 (250 mg)	43.73 ± 2.70***	15.42 ± 0.30***
FCE 2 (500 mg)	45.55 ± 3.41***	16.24 ± 2.34***
STANDARD Gereforte (43mg/Kg)	46.57 ± 0.35***	15.09 ± 0.14***

Data represent Mean ± S.E.M. volume in ml (n=6).

\*\* P < 0.05 when compared to control. \*\*\* P < 0.001 when compared to control.

**FIGURE 9: SWIM ENDURANCE TIME IN MINUTES****Effect of *Ficus carica* fruit extracts on Blood Glucose level & on Total Leukocyte Count in rats exposed to cold stress.**

In the stress induced animals the blood glucose level was found to be below the baseline indicating hypoglycemia, (Table 4, Figure 10, 11) this has

already been reported earlier by Kannur D M et al<sup>28</sup>. The extract at both the doses exhibited a moderately significant activity in preventing the alterations in the blood glucose levels as compared to the untreated animals. It was notable that the extract at 250 mg dose was evidently effective in elevating the blood glucose level back to the normal and in comparison the 500mg dose the blood glucose level was found to be slightly

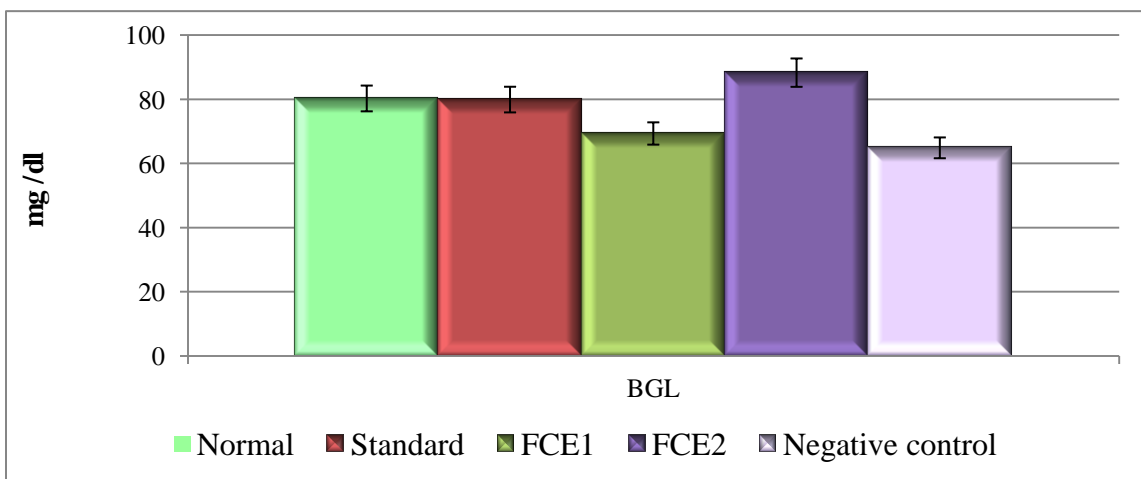
elevated than the normal levels. The Leukocyte count too was found to be significantly increased<sup>33,34</sup> in the stress induced animals in comparison to that of normal animals. The Standard drug as well as *Ficus carica* extracts exhibited strong potential to reduce the elevated leukocyte count. The results exhibited that the *Ficus carica* extract at 500 mg was more effective in controlling the pathogenic condition.

**TABLE 4: BLOOD GLUCOSE LEVEL AND TOTAL LEUKOCYTE COUNT**

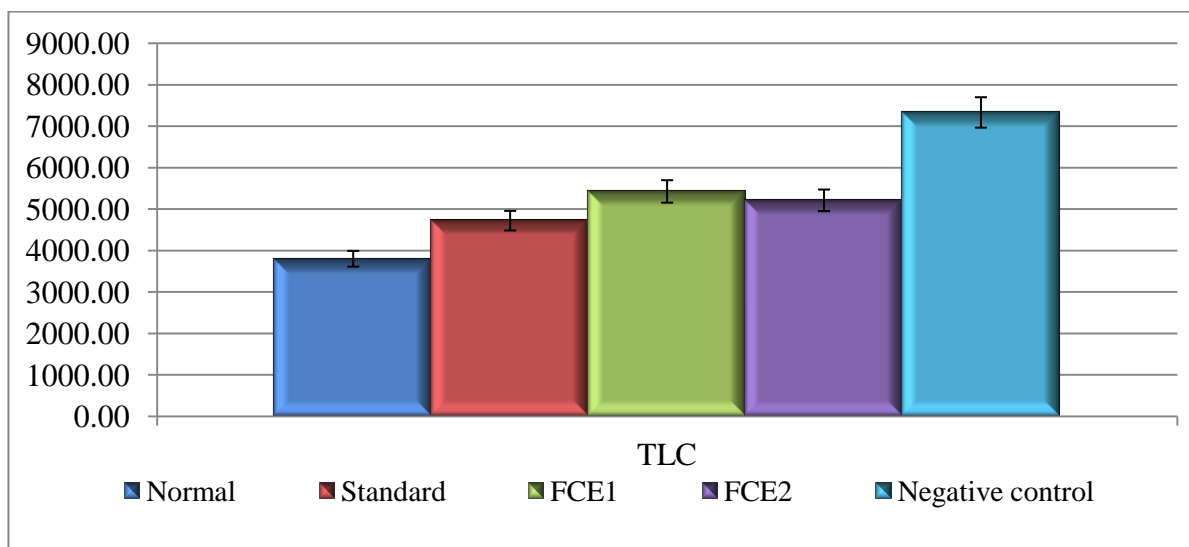
	BGL mg%	TLC
Normal	80.31 ± 2.09	3800.17 ± 230.5
STANDARD (43mg/Kg)	79.96 ± 4.29***	4719.00 ± 213.58 ***
FCE 1 (250 mg/kg)	69.39 ± 6.36 **	5424.67 ± 112.63**
FCE 2 (500 mg/kg)	88.35 ± 1.67 ***	5210.00 ± 53.80***
Negative control (CMC 1%)	64.91 ± 5.25	7331.17 ± 127.88

Data represent Mean ± S.E.M. volume in ml (n =6).

\*\* P < 0.05 when compared to control. \*\*\* P < 0.001 when compared to control.



**FIGURE 10: EFFECT OF *FICUS CARICA* FRUIT EXTRACTS ON BLOOD GLUCOSE LEVEL MG/DL**



**FIGURE 11: EFFECT OF *FICUS CARICA* FRUIT EXTRACTS ON TOTAL LEUKOCYTE COUNT****Effect of *Ficus carica* fruit extracts on Differential Leukocyte Count (DC) level in rats exposed to cold stress.**

In Differential count<sup>34</sup> the percentage of Neutrophils was increased significantly, where as the Eosinophils were slightly increased, the lymphocytes were decreased and the Monocytes were not affected much (Table.5) Both the extracts exhibited preventive action

against stress induced alterations in the TLC as well as DC. The FCE 2 extract was more effective in comparison to the FCE1 extract in controlling the increased level of Total Leukocyte count. In the differential the ethanolic extract at both the doses was significantly effective. It was observed that the Standard maintaining the DC level near to the normal.

**TABLE 5: EFFECT OF *FICUS CARICA* FRUIT EXTRACTS ON DIFFERENTIAL LEUKOCYTE COUNT**

	NORMAL	STANDARD	FCE1	FCE2	NEGATIVE CONTROL
NEUTRTOPHLS	24.2 ± 0.5	26.7 ± 1.3	31.6 ± 0.8***	30.4 ± 0.9***	35.3 ± 1.4
MONOCYTES	5.8 ± 0.3	5.9 ± 0.2	5.7 ± 0.3 <sup>NS</sup>	6.1 ± 0.6 <sup>NS</sup>	6.2 ± 0.5
LYMPHOCYTES	71.4 ± 1.2	72.6 ± 4.6	68.4 ± 2.5**	70.8 ± 2.6 <sup>NS</sup>	66.3 ± 2.9
EOSINOPHLS	2.2 ± 0.2	2.1 ± 0.4	2.8 ± 0.4**	2.4 ± 0.6**	3.7 ± 0.3

Data represent Mean ± S.E.M. volume in ml (n=6).

\*\* P < 0.05 when compared to control. \*\*\* P < 0.001 when compared to control.

**Effect of *Ficus carica* fruit extracts on Lipid profile in rats exposed to cold stress**

In the stress induced group the Triglyceride, Total cholesterol and the LDL levels were increased significantly, the % of HDL of total cholesterol was found to be decreased as compared to the normal. There was slightly significant action on the VLDL levels. Stress induced animals thus had a moderately hyperlipidemic condition as compared to the normal animals (Table 6, Figure 12)

The Standard drug and the *Ficus carica* ethanolic extract at both the doses were effective in controlling the variation in lipid levels due to the cold stress. The Standard was comparatively more effective than the FCE1 group but, the FCE2 group was highly significant in controlling the rise in Total cholesterol levels and the Triglyceride levels, in the comparison of FCE1 & FCE2 the % increase in the HDL levels to the total cholesterol was highest in the FCE 1 treated group

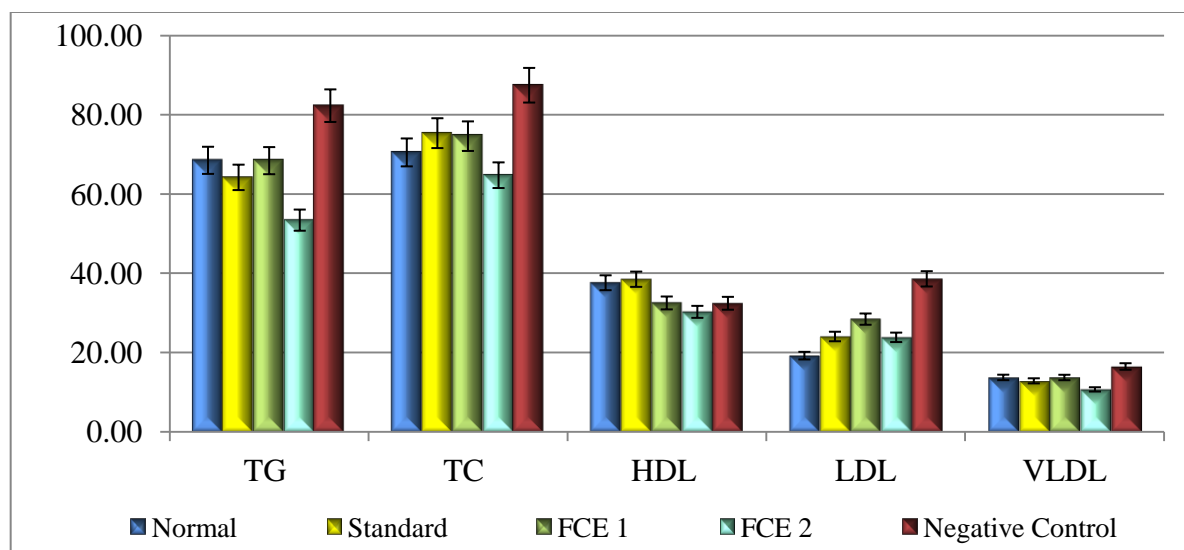
**TABLE 6: EFFECT OF *FICUS CARICA* FRUIT EXTRACTS ON LIPID PROFILE**

	TG	TC	HDL	LDL	VLDL
Normal	68.50 ± 6.97	70.50 ± 7.23	37.60 ± 6.74	19.20 ± 3.36	13.70 ± 3.03
Standard (43mg/Kg)	64.20 ± 8.61***	75.36 ± 11.25***	38.49 ± 5.32**	24.03 ± 6.72***	12.84 ± 2.8***
FCE 1 (250 mg/kg)	68.42 ± 8.16 **	74.60 ± 15.22 **	32.50 ± 7.69**	28.42 ± 5.37**	13.68 ± 2.1 **
FCE 2 (500 mg/kg)	53.40 ± 9.44 ***	64.75 ± 13.09***	30.25 ± 4.36**	23.82 ± 4.39***	10.68 ± 3.6 ***
Negative Control (CMC 1%)	82.30 ± 12.67	87.46 ± 12.15	32.42 ± 4.91	38.58 ± 3.41	16.46 ± 2.3

Data represent Mean ± S.E.M. volume in ml (n=6).

\*\* P < 0.05 when compared to control. \*\*\* P < 0.001 when compared to control.





**FIGURE 12: EFFECT OF *FICUS CARICA* FRUIT EXTRACTS ON THE LIPID PROFILE**

**DISCUSSION & CONCLUSIONS:** The swim endurance test result clearly indicates that the *Ficus carica* extract have the properties whereby they increase the physical endurance as well as the overall performance in rats. The enhanced swimming endurance in mice as compared to the normal animals may be attributed to the sterols and the Triterpenoids<sup>31</sup>.

The studies conducted so far indicate that the *Ficus carica* extract at both the doses had a protective action on the animals against the alterations inflicted due to cold stress, such as changes in the normal biochemical parameters<sup>32, 33, 34</sup> like Blood Glucose levels, Total Leukocyte count, Differential count as well as the Lipid Levels which has been reported in the literature<sup>35</sup>.

This study clearly reveals that when the animals are exposed to the cold stress, it leads to changes in the normal physiological conditions and the increase in the total leukocyte count may result in slight pathological condition. Though stress induced hypoglycaemia and the resultant mechanism is rarely reported<sup>28</sup>, here in our studies as the fruit being a rich source of carbohydrates may be able to overcome the depleted glucose levels.

Stress is a known causative agent leading to hyperlipidemic conditions<sup>35, 36</sup>, which very well correlates with our studies. Here we can conclusively comment that moderate hyperlipidemic conditions due to cold stress are controlled by the *Ficus carica* fruit extract at both

the doses, but at a higher dose it significantly lowers the elevated Triglyceride as well as Cholesterol levels. The Antihyperlipidemic result observed in this study could be attributed to the presence of  $\beta$  - Sitosterol<sup>37,38</sup> and few Triterpenoids such as Lupeol which is known to prevent hepatotoxicity as well<sup>39</sup>.

*Ficus carica* fruit extract at a dose of 250 mg/kg as well as 500 mg/kg significantly exhibit the ability to overcome stress related imbalance in the biochemical parameters. The above results are promising and substantiate the claim of adaptogenic action of this drug. *Ficus carica* thus can prove to be an effective nutraceutical as well. Thus we can surely conclude that the fruits of *Ficus carica* possess Adaptogenic properties.

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