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Effect of Green Tea Extract on Behavioral Co-Morbidities In Experimentally Induced Ulcerative Colitis Rat Model

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ABSTRACT

The current study investigated the effect of green tea extract (GTE) on behavioral comorbidities related to ulcerative colitis (UC)using experimentally induced colitisrat model. UC was experimentallyinduced by acetic acid (10%(v/v), 0.2 ml/rat) given by rectal route. Rats were divided into five groups (n=5-6): in which all rats were given 0.2 ml acetic acid rectally so as to induce ulcerative colitis, except the first group that was given 0.2 ml distilled water and it served a normal control group. Group 2 served as experimental control.The third group represented the standard group that was given Sulfasalazine, whereas fourth and fifth groups were the treatment groups which were administered an extract of green tea in two different doses. The result of the experiment revealed a beneficial effect ofGTE in reducing the severity of mucosal damage and improvement in behavioral parameters also. It may be reported thatGTE is effective in treatment of ulcerative colitis and possesses a potential to treat behavioral co-morbidities manifesting in ulcerative colitis by modulating the neuroendocrine mechanism of gut brain axis.

INTRODUCTION

UC manifests aschronic inflammation and sores in the colon or rectum part of large intestine. Inflammation is restricted to the innermost mucosal layer which is made up of simple column like epithelial cells and secretes mucus from the glands which helps in the lubrication process. In UC, the inflammation generally starts from rectum and it affects colon in the uninterrupted pattern.The alteration in the levels of pro-and anti-inflammatory cytokines isconsidered a general cause of UC.Raised levels of pro-inflammatory cytokines and increased levels of malondialdehyde as well as myeloperoxidase are significant markers of UC[1].The exact

ISSN: 0971-1260
Vol-22-NO-30 Special Issue-December-2019

pathophysiology of UC is still not fully understood.There is no curative treatment till date. Moreover,available drugs have lower efficacy and recurrence is common after their use. And long-term use of anti-inflammatory drugs can lead to various unwanted effects[2-4].Thus,plant-based products because of their comparatively less toxic potential are being investigated nowadays.

Green tea is a beverage obtained from the leaf buds of *Camellia sinensis*belonging to family Theaceae. It is consumed worldwide as a beverage and also used as a home remedy[5].Green tea shows antioxidant and anti-inflammatory activity because of its polyphenols. Green tea polyphenols include epigallocatechin-3-gallate, epicatechin, epigallocatechin and epicatechin-3-gallate[6].They help in the reduction of several inflammatory markers such as myeloperoxidase enzyme, TNF-α, nuclear factor κ B and increasingantioxidant levels[7, 8]. Various studies have concluded that green tea has anti-inflammatory propertiessimilar tosulphasalazine and the antioxidants ofgreen tea reduce the severity ofUC[9].Similarly, benefits of green tea in the behavioral disorders have also been reported.

Various studies have reported a correlation between psychological disorders and the incidence of UC.Gastrointestinal-brain axis is a signal transduction pathway that occurs between the gastrointestinal tract and central nervous system. Because of the presence of this axis, there is a correlation between the gastrointestinal and psychological disorders [10]. Psychological stress alters gut motility and secretion due to hormonal imbalances[11].But there is a lack of evidence for the fact that inflammation in the gastrointestinal tract or UCmay be responsible for the psychological disorders. As ulcerative colitis may be a pathogenic factor for psychological disorders like depression or anxiety, the modulatory effect of GTE was investigated in behavioral co-morbidities manifesting in UC.

MATERIALS &METHODS

The present study was carried out in Department of Pharmacology, LFAMS, Lovely Professional University, Phagwara.

ISSN: 0971-1260
Vol-22-NO-30 Special Issue-December-2019

Experimental animals

The investigation was endorsed by Institutional Animal Ethics Committee (IAEC) (ATRC/11/2013). Female adult Wistar rats weighing between 200-300 gm were used in the study. Free access todiet and water was given to animals.Prior to directing experiments, animals were acclimatized to research facility conditions for ten days.

Drugs/chemicals

GTE was purchased from NutrijaLifesciences and sulphasalazine from Jackson labs, Amritsar.

Experimental design

Total 27 animals were used in the study. Animals were assorted into total5 groups (n= 5-6). Group I, II, III had five animals and group IV and V had six animals individually. After 10 days of adaptation period, all the animals wereassorted into five groups as follows:

S. No.	Groups	Treatment	No. of animals	Dosage and route of administration
I	Normal Control	Distilled water + 0.5% CMC	5	0.2 ml/rat distilled water, intracolonic per rectal administration + 0.5ml/100g of rat of distilled water, p.o.
II	Experimental Control	Acetic acid + 0.5% CMC	5	Acetic acid (10% v/v) 0.2ml/rat, intracolonic per rectal administration + 0.5ml/100g of rat ofdistilled water, p.o.
III	Standard	Acetic acid + Sulfasalazine (360mg/kg)	5	Acetic acid (10% v/v) 0.2ml/rat, intracolonic per rectal administration + 0.5 ml/100g of rat of 360mg/kg sulphasalazine in 0.5% w/v CMC, p.o.
IV	Treatment group (low dose)	Acetic acid + GTE (35 mg/kg)	6	Acetic acid (10% v/v) 0.2ml/rat, intracolonic per rectal administration + 0.5ml/100g of rat of 35mg/kg GTEsuspended in distilled wáter , p.o.
V	Treatment group (high dose)	Acetic acid + Green tea extract (70mg/kg)	6	Acetic acid (10% v/v) 0.2ml/rat, intracolonic per rectal administration + 0.5ml/100g of rat of 70mg/kg GTE suspended in distilled wáter , p.o.

ISSN: 0971-1260
Vol-22-NO-30 Special Issue-December-2019

Behavioral assessmentprior to conduct of experimental study

Vaginal smear was performed in all the groups before giving treatment to determine the estrous phases in the individual animals of all the groups[12].After grouping of animals into their respective groups, animals were subjected to behavioral assessment tests so as to check the depression and anxiety like status of the animals. Three behavioural tests were performed for all the animals of their respective groups. Sucrose preference test (SFT) was performed to check the depression like behavior[13].Elevated plus maze test (EPM) and pen field (OFT) testwere carried out so as to investigate the behavioral parameters like anxiety[14, 15].After checking the behavioral status of all the animals, they were subjected to their respective treatment as per their respective groups.

Induction of colitis

UC was induced in the group II, III, 1V, V by a single intracolonic per rectal application of freshly prepared 10%v/v acetic acid in a volume of 0.2ml per rat into the rectum. Rats were anaesthetized and a rubber catheter was used to administer the acetic acid into the rectum at a distance of 8cm from the anorectal junction[16]. After administration of 0.2ml (10%v/v acetic acid), the animals were placed into head down position for few minutes to prevent the removal of acetic acid from rectum. After the period of 4 hours the animals were subjected to their respective treatment[8].

Behavioral assessment after the completion of treatment period

After completion of the treatment period (14 days) of the respective groups, again vaginal smear was taken in individual animals of each group using the same procedure[12]. Then all the female rats of the respective groups were subjected to the behavioral assessment tests. Assessment of the body weight, food intake, stool consistency, bleeding in the stool was checked before, during and after the experimentation period. After completion of the behavioral studies,the rats were anaesthetized and blood was collected. Then, rats were sacrificed and colon as well as brain were harvested for assessment of various parameters.

ISSN: 0971-1260
Vol-22-NO-30 Special Issue-December-2019

Macroscopic assessment of colitis

Disease activity index (DAI) was determined before, during and after the treatment to evaluate the macroscopic assessment in individual animals.The severity levels were quantified by a clinical scoring system by two investigators in a blinded fashion[17].

Scoring of DAI

Score	Percentage weight loss	Stool consistency	Rectal bleeding/ Melena
0	None	Normal	-vehemoccult
1	1-5	Soft, well formed	+ vehemoccult
2	5-10	Very soft	Traces of blood in the stool/melena
3	10-20	Liquid stool	Gross bleeding
4	>20	_	_

Assessment of inflammation

Gross morphological assessment was done for isolated colon of individual animals to examine inflammation by using following scoring system[18].

Gross morphology scoring

Score	Findings
0	Absence of damage in the mucosal lining
1	Mucosal lining with mild erythema and oedema
2	Moderate erythema, oedema, superficial mucosal ulceration or erosion in the mucosal lining

ISSN: 0971-1260
Vol-22-NO-30 Special Issue-December-2019

3 Significant erythema, oedema and ulcers in the mucosal lining

Histological analysis

Colonic and brain tissue of different groups were subjected to histological investigation. Histological grading of colon tissue was done by a qualified pathologist in a blinded fashion[19].

Biochemical estimations

After the macroscopic assessment, levels of myeloperoxidase (MPO) and malondialdehyde (MDA)were estimated in colonic tissue[20, 21].As cortisol is a major stress hormone, cortisol levels were estimated (Gargi diagnostic labs, Jalandhar).

Statistical Analysis

Data was analyzed by data base program (Sigma Stat software) using ANOVA followed by appropriate holm sidak method. Data was expressed as mean ± SEM. p value <0.05, <0.01,<0.001, was considered for statistical significance.

RESULTS

Macroscopic assessment of colitis

Table 1indicates the DAI score. The acetic acid experimental group had liquid stools or diarrhea along with fecal occult or gross rectal bleeding which significantly increased the DAI score ($p<0.01$) with respect to the normal control group. All test groups showed a significant ($p<0.05$) decrease in DAI scores when compared with the experimental group. Administration of GTE (70mg/kg) was found to be a most effective intervention in alleviating the severity of acetic acid-induced ulcerative colitis ($p<0.01$) and results were compared with the sulfasalazine used as standard drug.Bothhigh and low doses of GTE were effective in decreasing the symptoms of ulcerative colitis in rats and decreasing DAI.

ISSN: 0971-1260
Vol-22-NO-30 Special Issue-December-2019

Table 1: DAI of different experimental groups.

Group No.	Experimental groups	Score			DAI
		%Weight loss	Stool Consistency	Rectal Bleeding	
I	Normal control	1	0	0	$0.33\pm0^{\#\#}$
II	Experimental control	3	3	3	$3\pm0^{*,\dagger\dagger,\alpha,\varphi\varphi}$
III	Standard	1	0	0	$0.33\pm0^{\#\#}$
IV	Treatment group low dose	2	0	0	$0.47\pm0.16^{\#}$
V	Treatment group high dose	1	0	0	$0.38\pm0.16^{\#\#}$

The values were communicated as Mean ±SEM. as Mean ±SEM. Data were analyzed by One way ANOVA followed by the Holm Sidak method. (*, #, †, α, φ = p<0.05; **, ##, ††, αα, φφ = p<0.01; ***, ###, ††† ααα, φφφ = p<0.001). *=with respect to healthy control, # = experimental control,† = standard group,α=treatment group low dose, φ =treatment group high dose.

Assessment of inflammation

GTE showed its anti-inflammatory effect at both low and high doses (Table 2). The higher dose of GTE (70mg/kg) was found to be the most significant in reducing the severity of mucosal damage ($p<0.01$) similar to the standard sulphasalazine drug (360mg/kg). The lowest dose of GTE (35mg/kg) was moderately effective in reducing the ulceration, edema, erosion and tissue necrosis in the colon as compared to the standard sulphasalazine drug. There was a significant difference found between the normal control group when compared with the experimental control group ($p<0.05$). The experimental control group has shown alteration in the gross morphology when compared with the standard ($p<0.01$), treatment group low dose ($p<0.05$), treatment group high dose ($p<0.01$).

Table 2:Mean enteritis gross morphology score of different experimental groups.

Sr.No	Experimental groups	Score (Mean ±SEM)
1	Group I (normal control)	$0^{\#}$
2	Group II (experimental control)	$3.83\pm0.16^{**,\dagger\dagger,\alpha,\varphi\varphi}$
3	Group III (standard group)	$0.39\pm0.21^{\#\#}$
4	Group IV (treatment group low dose)	$1.12\pm0.21^{**,\#}$
5	Group V (treatment group high dose)	$0.74\pm0.30^{\#\#}$

ISSN: 0971-1260
Vol-22-NO-30 Special Issue-December-2019

The values were communicated as Mean ±SEM. as Mean ±SEM. Data were analyzed by One way ANOVA followed by the Holm Sidak method. (*, #, †, α, φ = p<0.05; **, ##, ††, αα, φφ = p<0.01; ***, ###, ††† ααα, φφφ = p<0.001). *=with respect to healthy control, # = experimental control,† = standard group,α=treatment group low dose, φ =treatment group high dose.

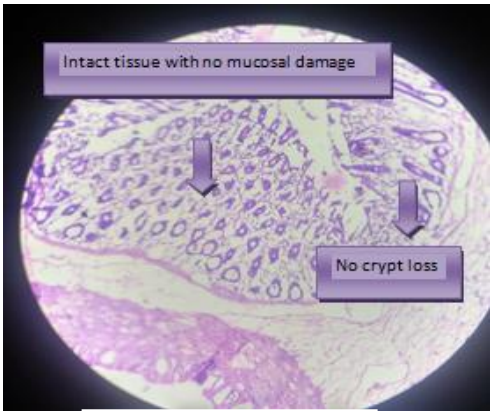
Histopathological analysis

Colitis was confirmed on the basis of histological damage, cell infiltration and loss of crypt cell as shown in table 3. The crypt loss in the acetic acid experimental group was found to be more than 70% (approx.) means the complete destruction of tissue. Animals ofthe experimental control group showed marked inflammation. Histopathological scores appeared to be reversed in standard as well as in both the treatment groups. Sulfasalazine was helpful in maintaining the normal architecture of the cell crypt.GTE at high dose 70mg/kg showed its complete effectiveness in the proper recovery of cell damage, ulceration, cell infiltration and maintaining the crypt architecture, whereas GTE low dose 35mg/kg was not completely effective in reversing the cell damage, ulceration, and cell infiltration (Figure 1-2).

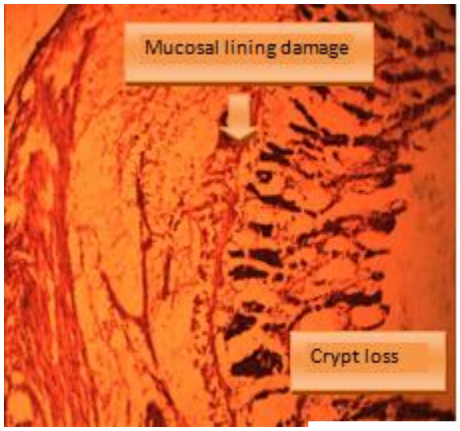
Table 3:Histopathological analysis of colon tissue in different experimental groups.

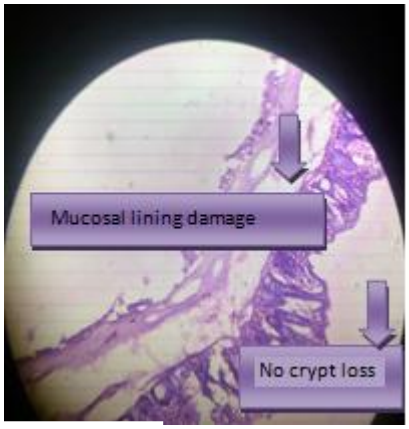
Sr. No	**Experimental groups**	**Score 0**	**Score 1**	**Score 2**	**Score 3**	**Score 4**	**Total**
1	Group I (normal control)	5 (100%)	-	-	-	-	5
2	Group II (experimental control)	-	-	-	-	5 (100%)	5
3	Group III (standard group)	1 (20%)	3 (60%)	1 (20%)	-	-	5
4	Group IV (treatment group low dose)	-	3 (49.8%)	2 (33.2%)	1 (16.6%)	-	6
5	Group V (treatment group high dose)	2 (33.2%)	4 (66.4%)	-	-	-	6
Total		**8 (29.6%)**	**10 (37.0%)**	**3 (11.1%)**	**1 (3.7%)**	**5 (18.5%)**	**27 animals**

ISSN: 0971-1260
Vol-22-NO-30 Special Issue-December-2019

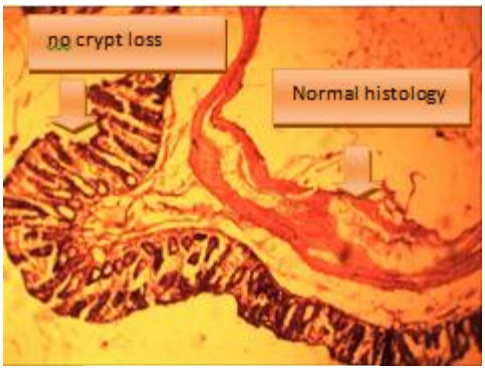


a) Control group

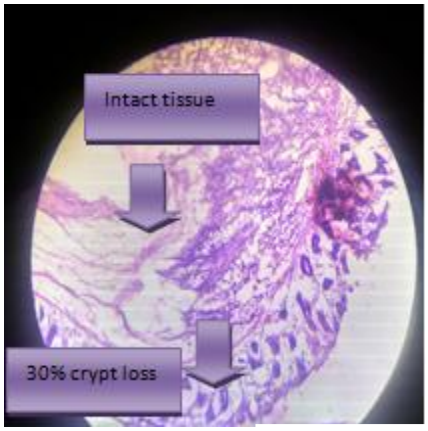


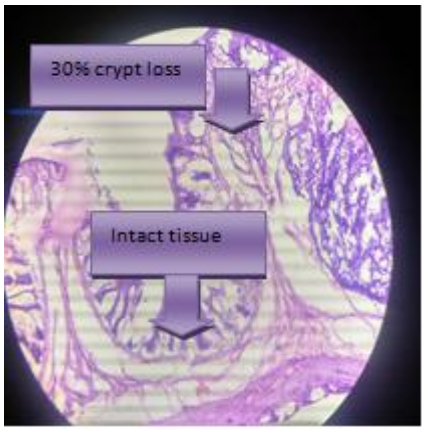


b) experimental group

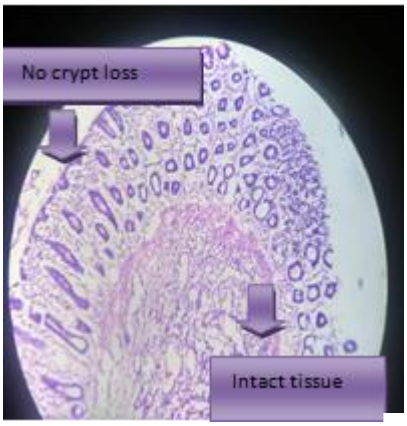


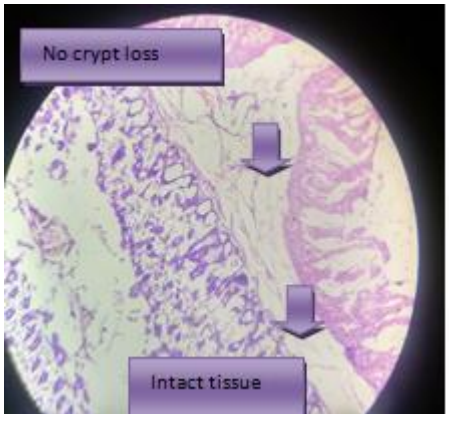
c) Standard group





d) Treatment group low dose

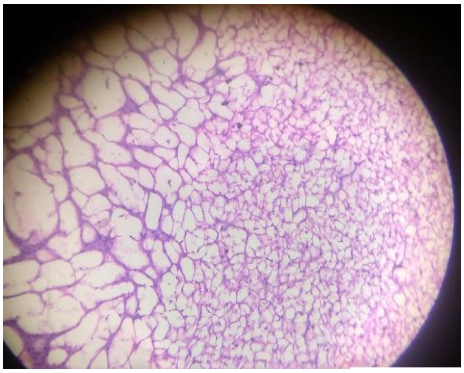
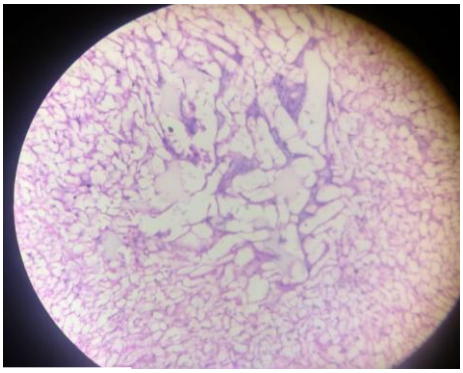




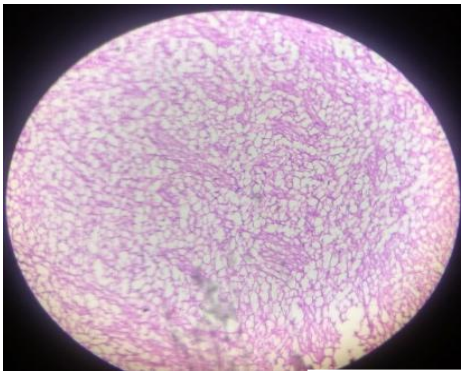
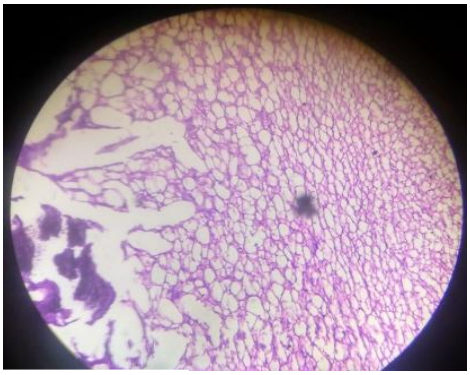
e) Treatment group high dose

Figure (5) Photomicrograph of the colon tissue of: a) Normal control: showing the normal histology of colon tissue, b) Experimental group: showing the cell infiltration, severe inflammation and 70% crypt loss, c) Standard group: showing normal histology of colon tissue total recovery from ulceration, d) Treatment 1 low dose: Less inflammatory changes as compared to the acetic acid group. Crypt loss more than 20%, decreased cell infiltration, e) Treatment 2 high dose: epithelial lining maintains their integrity. No crypt loss and cell infiltration was there. Normal morphological structure (HE, 40x)

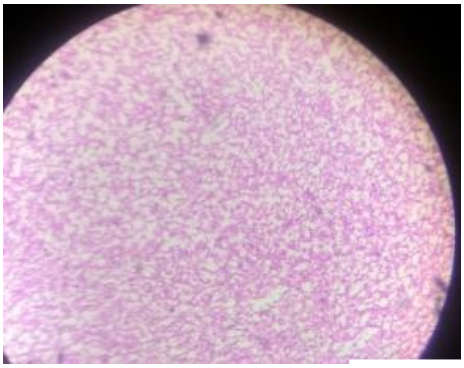
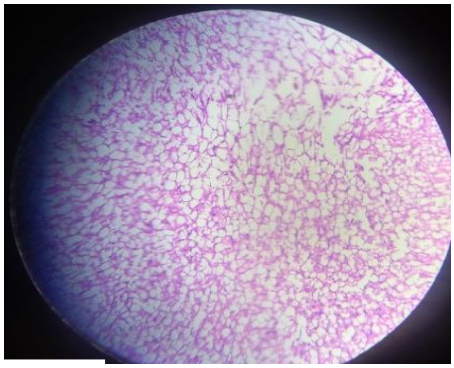
ISSN: 0971-1260
Vol-22-NO-30 Special Issue-December-2019

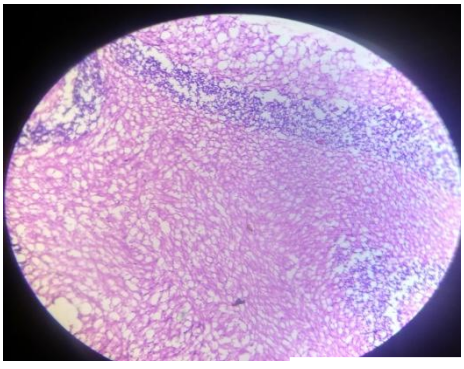
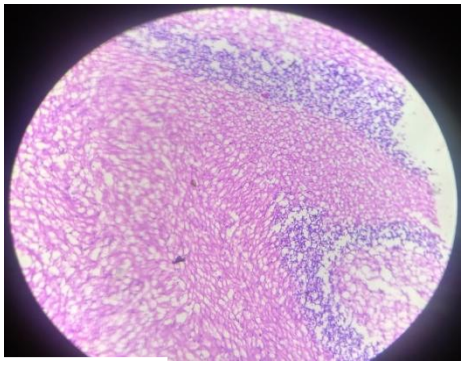
a) Normal control

b) Experimental control

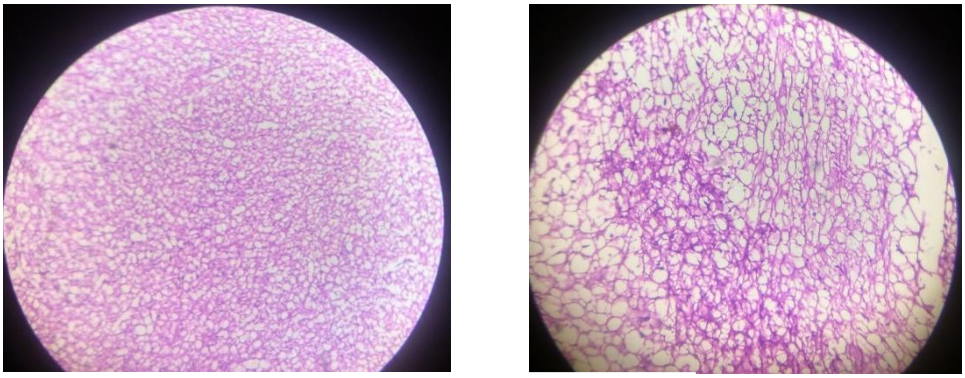



c) Standard group

d) Treatment group low dose

ISSN: 0971-1260
Vol-22-NO-30 Special Issue-December-2019



e) Treatment group high dose

Figure 2: Photomicrograph of the brain tissue. Transverse section of brain composed of both cerebral cerebella tissues. The cerebral tissues were composed of neurons disposed within abundant glial tissue foci of nuclei composed of giant cells, cells were properly intact, no change in the white matter. Histological findings shown that there was no destruction in the layers and cells of the brain due to acetic acid induced ulcerative colitis. There was no change observed in the brain cells during histological findings in all the animal groups (HE 40x)

Biochemical Estimations

The level of MPO in experimental control rats (0.867±0.2274) was significantly higher ($p<0.05$) than in the normal control rats (0.113±0.38).The GTE administration at (35mg/kg) and (70mg/kg) demonstrateda significant difference ($p<0.05$, $p<0.01$) in MPO levels as compared to normal control and standard control groups. The level of MDA in experimental control (0.8395±0.1463) was significantly higher ($p<0.05$) than the normal control rats (0.1766±0.0636). The GTE administration at (35mg/kg) was moderately effective in reversing the rise in the MDA level. The higher dose of GTE (70mg/kg) was significant ($p<0.001$) compared to the experimental control group, standard, treatment group low dose (Table 4).The GTE administration at 35mg/kg was moderately effective in reversing the rise in the level of cortisol as compared to normal control and experimental control. The higher dose of GTE (70mg/kg) was significant compared to an acetic acid group.

ISSN: 0971-1260
Vol-22-NO-30 Special Issue-December-2019

Table 4: Different biochemical parameters evaluated in the study

Sr.No.	Treatment	Colon		Serum
		MPO(µmol/g of tissue)	MDA (nmol/g of tissue)	Cortisol level (µg/dl)
1	Normal control	0.113±0.38	0.1766±0.0636	0.39±0.032
2	Experimental control	0.867±0.2274**	0.8395±0.1463**	1.37±0.067 **
3	Standard group	0.223±0.00713##	0.2576±0.14480 *,##	0.25±0.039 **,##
4	Treatment group(low dose)	0.391±0.09616 ##	0.163±0.11375##	0.49±0.041 **,##,††
5	Treatment group (high dose)	0.21±0.02587 ##	0.093±0.0136##,††	0.1345±0.023 **,##,††

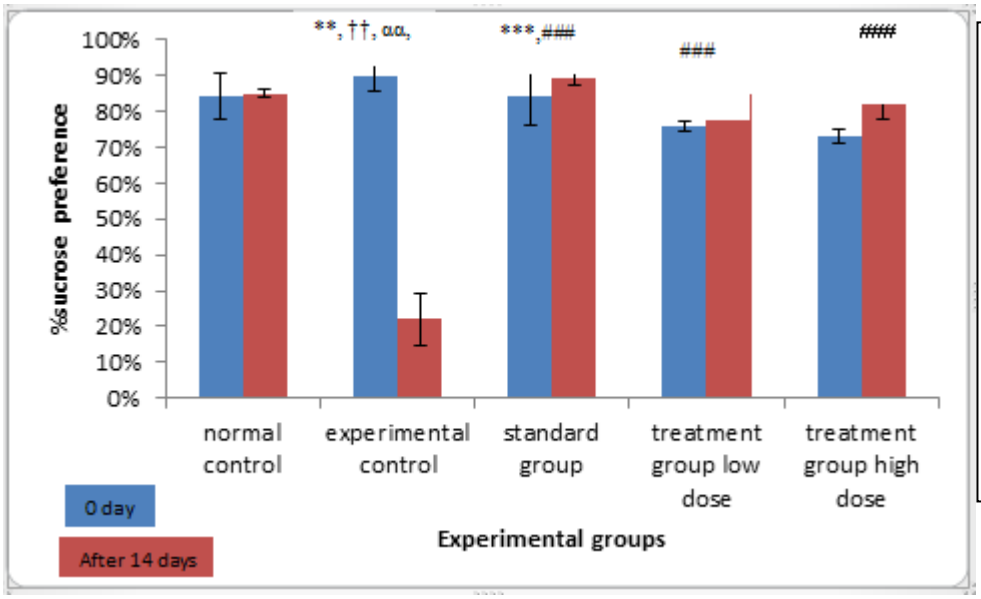
The values were communicated as Mean ±SEM. as Mean ±SEM. Data were analyzed by One way ANOVA followed by the Holm Sidak method. (*, #, †, α, φ = p<0.05; **, ##, ††, αα, φφ = p<0.01; ***, ###, ††† ααα, φφφ = p<0.001). *=with respect to healthy control, # = experimental control,† = standard group,α=treatment group low dose, φ =treatment group high dose.

Behavioral Studies

Sucrose preference was calculated before and after treatment to check the anhedonic state of animals. Results of the study (Fig. 3) had shown that in control group the %age of sucrose preference was high before and after treatment (84%±0.064) and (85%±0.01) with respect to the acetic acid control groups (90%±0.044) and (22%±0.072).Hence significant difference was observed both the groups. The increase in %age sucrose preference with GTE(35mg/kg) was found to be (64%±0.014) and (80%±0.02). Similarly, with GTE (70mg/kg) the values found to be (73%±0.021) and (82%±0.044) as compared to acetic acid group (p<0.001), but there was no large difference between the low and high dose of GTE. Improvement in the sucrose preference after treatment showed the efficacy of the treatment in their respective groups. Fig. 4-7 illustrate the findings of elevated plus maze test open field test.

ISSN: 0971-1260
Vol-22-NO-30 Special Issue-December-2019

Figure 3: Percentage sucrose preference on day 0 and after 14 days of treatment.



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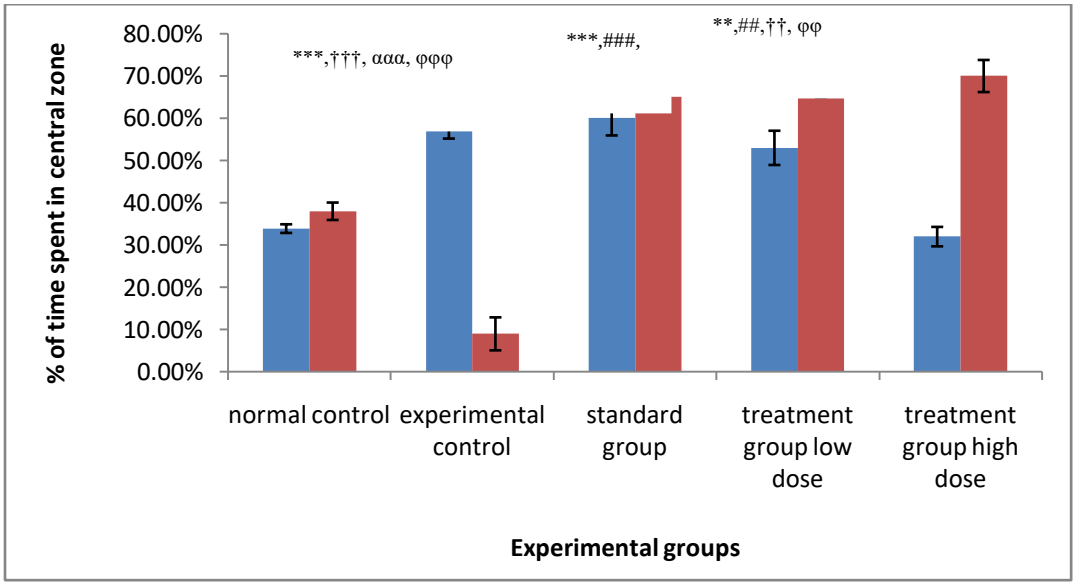

Figure 4: Percentage time spent in central zone at day 0 and after 14 days of treatment (Open field test).

ISSN: 0971-1260
Vol-22-NO-30 Special Issue-December-2019

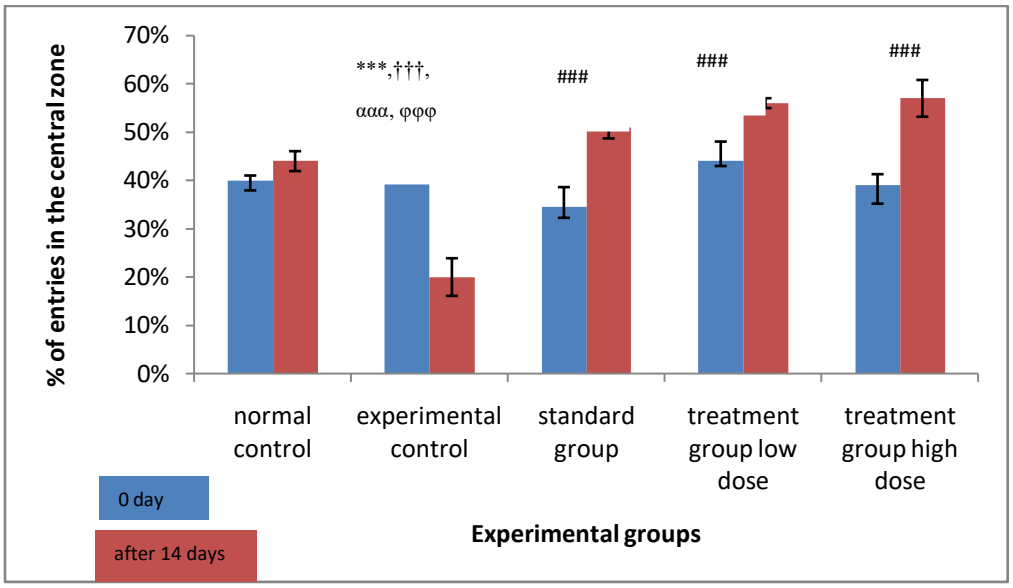

Figure 5:Percentage of entries in central zone at day 0 and after 14 days of treatment (Open field test).

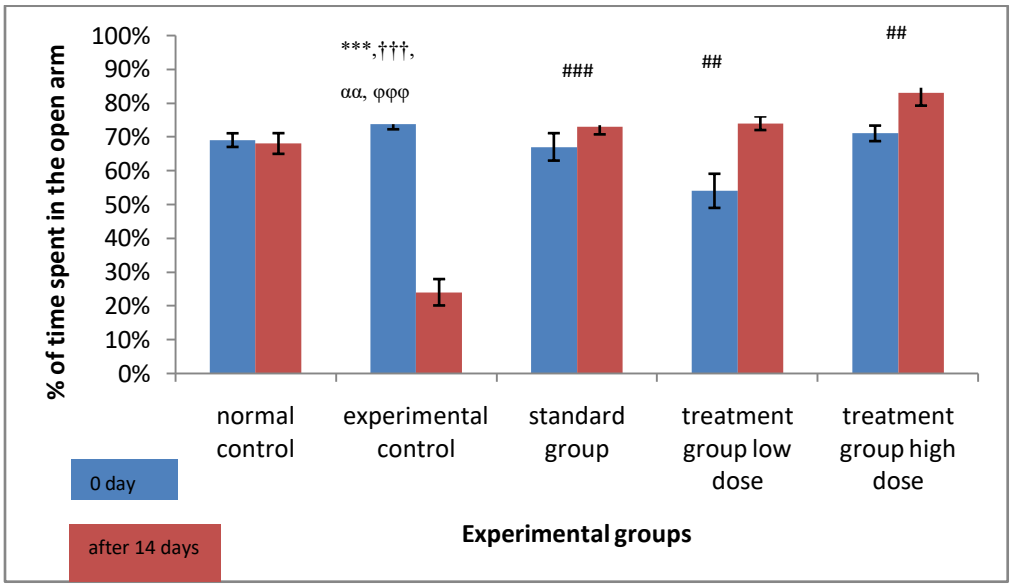

Figure 6: Percentage time spent in open arms at day 0 and after 14 days of treatment (Elevated plus maze test).

ISSN: 0971-1260
Vol-22-NO-30 Special Issue-December-2019

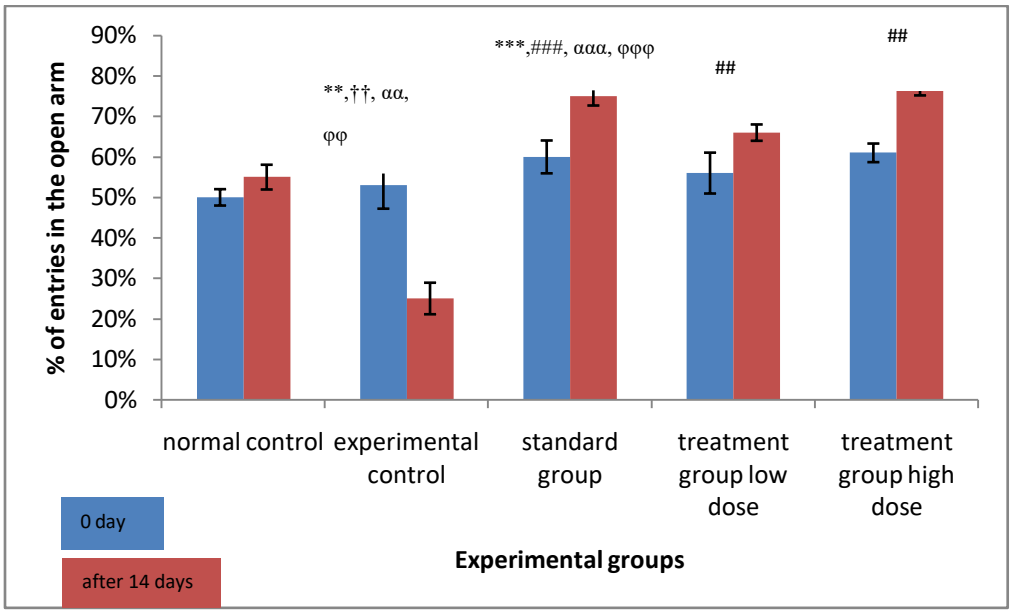

Figure 6: Percentage of entries in open arms at day 0 and after 14 days of treatment (Elevated plus maze test).

DISCUSSION

UC is characterized by chronic and recurrent inflammation that occurs in the colon and rectum part of the large intestine. It mainly affects the mucosal layer of the large intestine. Histological analysis of the colon tissue showed that changes occurred at the cellular level in the mucosal layer in animals of different groups. Necrosis, denudation of surface lining epithelium, degenerative changes in the crypt epithelium alongwith the neutrophils infiltration in the lamina propria, mucosal and submucosal dilated vessels were observed in the experimental control group. Less inflammatory changes as compared to the acetic acid group were observed in both the low and high dose GTE groups. But more significant results were observed in high dose treatment group. Results of MPO and MDA level estimation provided evidence that level of MPO was significantly less in treatment groups as compared with the experimental control group. Further, it was also observed that level of MPO and MDA was significantly less in animals treated with the high dose of GTE as compared to the low dose GTE. So, it was concluded that high dose of green tea was more effective in reducing the oxidative stress in the colon tissue.Various behavioral studies were performed at day zero and after 14 days treatment period to demonstrate the effect of GTE in behavioral comorbidities such as anxiety depression

ISSN: 0971-1260
Vol-22-NO-30 Special Issue-December-2019

occurred in the ulcerative colitis. Results of these tests support the significant correlation between psychological and gastrointestinal tract disorders. Further it was also observed that high dose of GTE (70mg/kg) was more effective than the low dose treatment (35mg/kg) in effecting the stress and depression occur in the UC. Further, the level of cortisol in blood was estimated that provided evidence regarding the role of neuroendocrine pathway of gut brain axis in the pathogenesis of psychological and gastrointestinal tract disorders. In the present study, increased level of cortisol in the experimental control group provided marked evidence about the hyperactivation of HPA axis in the acetic acid induced UC. High dose treatment group of GTE (70mg/kg) was found to be more effective in reducing the cortisol level than the other groups. Results of the present study showed that both low dose and high dose treatment groups of GTE were effective in treating the behavioral co-morbidities induced by UC. But it was found that high dose of green tea was significantly more effective in treating the depression and anxiety induced by UC.

CONCLUSION

GTE was effective in the treatment of UC due to its potent antioxidant anti-inflammatory properties. It possesses a potential to treat behavioral co-morbidities manifesting in UC by modulating the neuroendocrine mechanism of gut brain axis. The positive results of GTE in behavioral studies confirms the neuroendocrine mechanism of gut brain axis.

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Vol-22-NO-30 Special Issue-December-2019

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