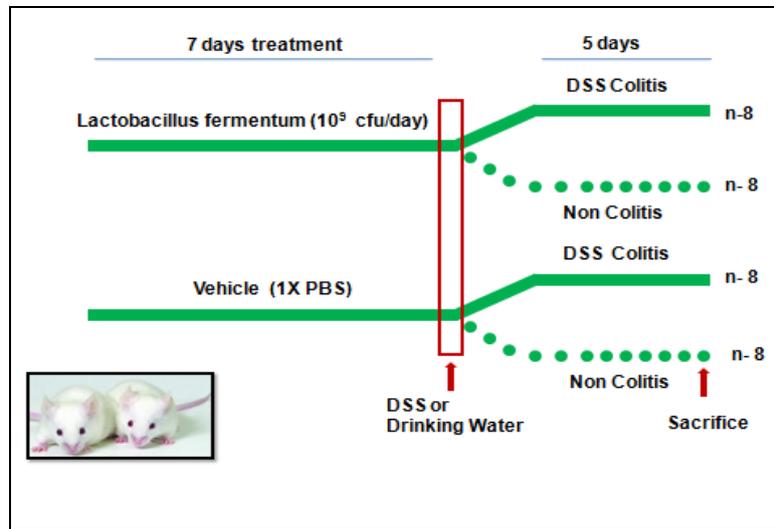
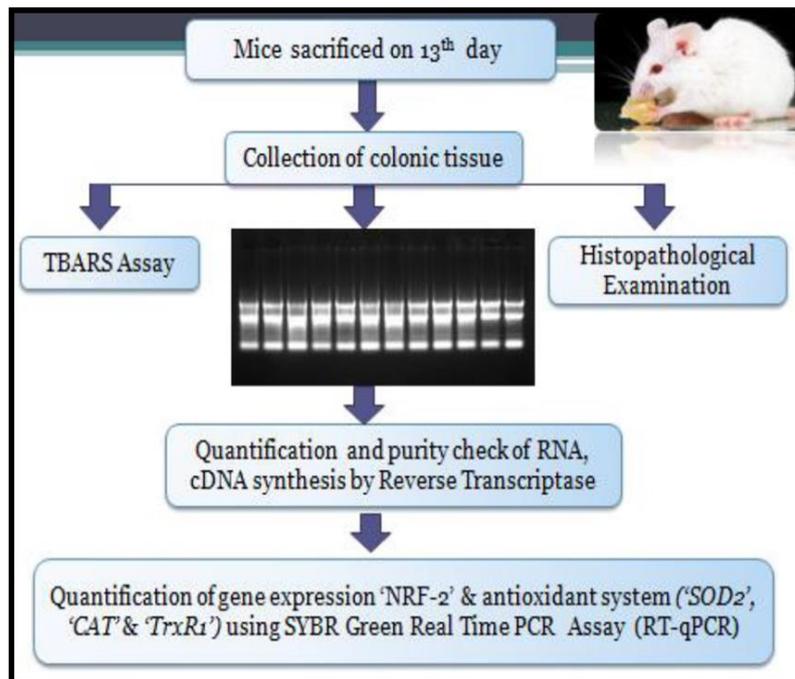


1 **Supplementary Data:**

2 **Fig. 1S Detailed description of *In vivo* experimental design**



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9 **Relative Expression Studies**

10 The primers for the house keeping genes (*ACTB* for mouse colon) and target genes
11 (*Nfr2*, *SOD2*, *CAT1* and *TrxR-1* from mouse colon) were either taken from the published
12 papers or designed from the genome sequence available at NCBI gene bank and using Primer
13 3 plus software (<http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi>). The
14 primers were got custom-synthesized from Imperial Biomed (Integrated DNA Technologies,
15 Inc.; www.idtdna.com) and Sigma, USA. While designing the primers for Real Time PCR,
16 the G-C content was kept within 40-60%, the amplified products size in the range of 100-200
17 bp and T_m in between 55-60°C. The specificity of the designed primer pair was ensured by
18 BLAST analysis. The amplified products were checked on 2% agarose gel followed by melt
19 curve analysis of the PCR amplified products to rule out the possibility of primer dimer
20 formation.

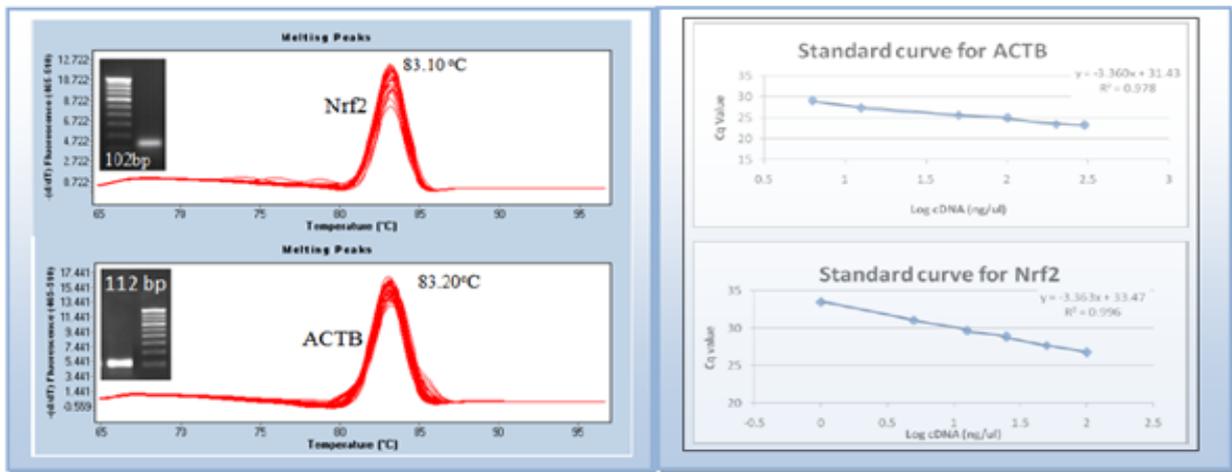
21 **i) Melt Curve Analysis and Amplification of *Nfr2***

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23 The amplification parameters for '*Nfr2*' included initial denaturation at 95°C for 5
24 min. followed by 40 cycles each of denaturation at 95°C for 30 sec, annealing at 53°C for 30
25 sec. and extension at 72°C for 45 sec. and for '*ACTB*' were kept same as for '*Nfr2*'.
26 Furthermore, in order to calculate RT-qPCR efficiencies and linearity, standard curves were
27 prepared for the target and reference gene by plotting C_q values versus log cDNA (reverse
28 transcribed total RNA) concentration inputs by preparing serial dilutions of cDNA i.e. 300,
29 200, 100, 50 and 12.5 and 6.25 ng/μl for '*ACTB*' and 100, 50, 25, 12.5, 5 and 1 ng/μl for
30 '*Nfr2*' respectively. As is quite evident from the **Fig. 2S (Supplementary Data)**, both the
31 curves showed high linearity (Pearson correlation coefficient $R^2 > 0.978$). The slopes of
32 '*Nfr2*' and '*ACTB*' curves were -3.363 and -3.360 respectively that indicated high real-time
33 PCR efficiencies of 1.983 and 1.984 for both the genes.

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36 **Figure: 2S Melt Curve Analysis and Amplification Curves of ‘Nfr2’ and ‘ACTB’**



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39 **ii) Melt Curve Analysis and Amplification curve of ‘SOD2’, Thioredoxin Reductase and**
 40 **Catalase**

41 ‘SOD1’, ‘TrxR-1’ and ‘CAT’ as well as ‘ACTB’ from the given slopes plotted with the help of
 42 LightCycler software. Cq values versus log cDNA (reverse transcribed total RNA)
 43 concentration inputs by preparing serial dilutions of cDNA i.e. 300, 200, 100, 50 and 12.5
 44 and 6.25 ng/μl for ‘ACTB’ and 100, 50, 25, 12.5 and 5 ng/μl target genes respectively were
 45 plotted to calculate the slope (mean ± SD; n = 3). The corresponding real-time PCR
 46 efficiency (E) of one cycle in the exponential phase was calculated according to the equation:
 47 $E = 10^{[-1/\text{slope}]}$. As is quite evident from the **Fig. 3Sa**, all the curves showed high linearity
 48 (Pearson correlation coefficient R2 > 0.978). The slopes of ‘SOD2’, ‘TrxR-1’, ‘CAT’ and
 49 ‘ACTB’ curves were -3.351, -3.363, -3.349 and -3.360 respectively that indicated high real-
 50 time PCR efficiencies of 1.988, 1.98, 1.989 and 1.984 in respect of the targeted genes.
 51 ‘SOD1’, ‘TrxR-1’ and ‘CAT’ as well as ‘ACTB’ from the given slopes plotted with the help of
 52 LightCycler software. Cq values versus log cDNA (reverse transcribed total RNA)
 53 concentration inputs by preparing serial dilutions of cDNA i.e. 300, 200, 100, 50 and 2.5 and
 54 6.25 ng/μl for ‘ACTB’ and 100, 50, 25, 12.5 and 5 ng/μl target genes respectively were
 55 plotted to calculate the slope (mean ± SD; n = 3). The corresponding real-time PCR
 56 efficiency (E) of one cycle in the exponential phase was calculated according to the equation:
 57 $E = 10^{[-1/\text{slope}]}$.

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60 **Fig. 3Sa : Standard curves for the target and reference genes**

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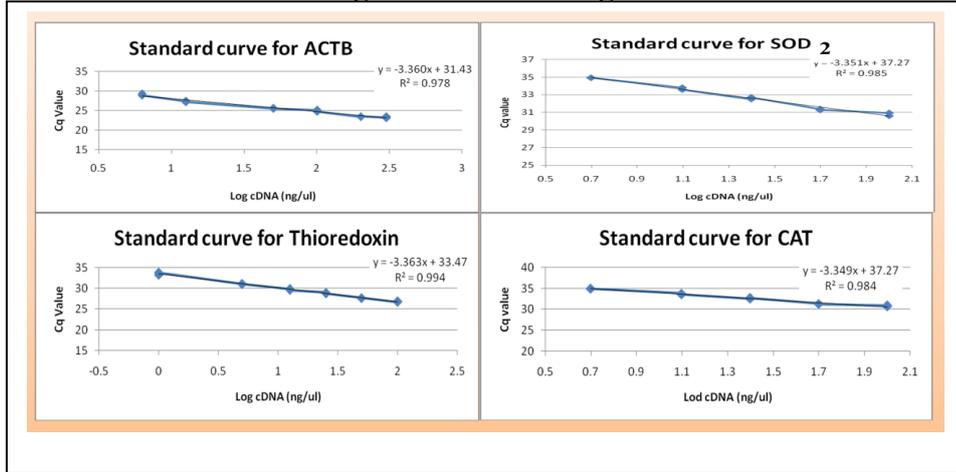
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68 The amplification parameters for ‘SOD2’ and ‘ACTB’ included initial denaturation at 95°C
69 for 5 min. followed by 40 cycles each of denaturation at 95°C for 30 sec, annealing at 53°C
70 for 30 sec. and extension at 72°C for 45 sec

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72 **Fig. 3Sb. Specificity of RTqPCR products on agarose gel and melt curve analysis of**
73 **‘SOD2’, Thioredoxin Reductase and Catalase in colitis mouse model**

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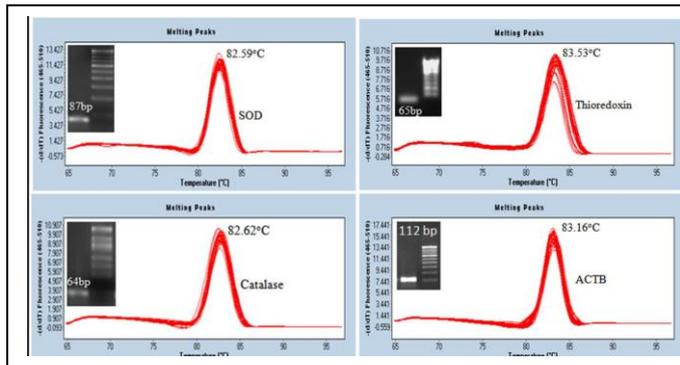
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98 **Table.1S – Disease activity index parameters**

Parameter	Change	Score
Weight loss	0	0
	1 to < 5%	1
	5 to < 10%	2
	10 to < 20%	3
	>20%	4
Stool Consistency	Negative	0
	Loose	2
	Diarrhoea	4
Blood in stool	Normal	0
	Blood traces	2
	Gross Bleeding	4

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101 **Table. 2Sa -Grade of Histological Changes**

Grade of Disease	Score
Crypt intact	0
Loss 1/3 crypt	1
Loss of 2/3 crypt	2
Loss of entire crypt with intact surface epithelium	3
Loss of entire crypt with erosion of surface epithelium	4

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105 **Table. 2S b – Extent of Histological damage recorded in experimental animals**

Extent of Damage (% involvement)	Score
1-25%	0
26-50%	1
51-75%	2
76-100%	3

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107 **Table. 2S c – Severity of Histological damage recorded in experimental animals**

Score for Severity	Score
Normal	0
Focal inflammatory cell infiltrate	1
Inflammatory cell infiltrate, gland drop out and crypt abscess	2
Mucosal ulceration	3

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Source: Meeryeld and Tyler, 2006