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Ameliorative effect of herbal extracts on lipid profile in albino rats, *Rattus norvegicus* exposed to metanil yellow

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ABSTRACT

Synthetic food colours are used as key component by food manufacturers to increase the consumer acceptance towards food items and beverages as well as for having certain properties like low cost, high colour intensity and more colour stability. These food items and beverages may have more than recommended amount of permitted food colours or some non-permitted synthetic food colours, which may lead to several health problems like disturbances in biochemical parameters, allergic reaction, cancer, mutations etc. Some herbs are having active chemical components and could be used regularly to ameliorate the toxic effect of synthetic food colours. The aim of this study was to analyse the effect of garlic and turmeric extract as a herbal antihyperlipidemic agent in albino rats fed on an azo dye, metanil yellow. The albino rats were divided into four groups (6 rats in each group). Group I (Negative control) fed on normal pellet diet, Group II (Positive control) fed on metanil yellow (MY), Group III fed on MY+ garlic extract and Group IV fed on MY+ turmeric extract. All experimental group fed on normal pellet diet and water ad libitum. Total cholesterol (TC), LDL, HDL and triglycerides (TG) were observed in serum of albino rats from all the groups. The results showed that administration of garlic and turmeric extract raise the level of HDL and lowered the level of LDL, TC and TG in blood serum of albino rats exposed to metanil yellow for 12 and 24 weeks of exposure periods. Garlic was found to be more potent in correcting the lipid profile of metanil yellow fed albino rats in comparison to turmeric extract. However, it has been concluded that both the herbs could be used as antihyperlipidemic agent to avoid health risk in human beings caused by chronic consumption of food colours in different food types consumed daily.

Introduction

Synthetic food colours are being used from last few decades for aesthetic appearance and new trends of food technology. The overall worldwide turnover of food colouring agents is nearly 8000 tons per year and India accounts for only 2% of this output (Das and Mukherjee, 2004). Azo dyes are widely used as colorants in food which can trigger toxic effects such as allergic reactions, biochemical disturbances, tumour formation, and endocrine disruptions. The long-term use of any synthetic colour can cause serious damage to human health. Among the azo dyes, metanil yellow is used in many food processing industry for increasing visual appearance of different kinds of food stuffs like juices, ice creams, sweets, carbonated drinks, etc. It is basically used as yellow colouring factor in food stuffs. Limited amount of azo dyes can be consumed and recommended amounts are mentioned as acceptable daily intakes (ADI) (Das and Mukherjee 2004). The acceptable daily intake (ADI) of metanil yellow given by food and agricultural organization (FAO) was 0-0.3mg/kg b.w and according to Food

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Adulteration act 1954, it was declared as illegal colouring agent in India (Khan *et al.,* 2020). Metanil yellow is found to cause damage to internal tissues (Rahman *et al.,* 2019) and induce changes in biochemical parameters like lipid profile, enzyme activity and oxidative stress, if consumed in more amount than ADI. Herbal extracts are being tested during the last decades to reduce toxic effects induced by food colours as well as other chemicals used in food stuffs. *Allium sativum*, commonly known as garlic, is small perennial herb and could be used to reduce abnormal cholesterol and blood pressure, and as antioxidant to neutralize free radicals (Mahdi *et al.,* 2019). Turmeric has many bio-functional properties including anti-inflammatory, anticancer, antitumour and antilipidemic effects (El-Hack *et al.,* 2021). So, in present study ameliorative effect of garlic and turmeric extract have been studied on the serum lipid profile in young male albino rats exposed to chronic administration of metanil yellow.

Material and Methods

Preparation of Garlic and Turmeric extract

Aqueous extract of garlic was prepared using method of Iwalokun *et al.* (2004) with some modification. Fresh bulbs of garlic (*Allium sativum* L.) were purchased from local markets of Bareilly. 50 gms of cloves were separated, peeled, chopped and homogenized in 200 mL of autoclaved water and the homogenate was then filtered by muslin cloth to give a crude aqueous extract of 250 mg of garlic/mL. Similarly, 50 gms of Rhizome of turmeric have been homogenized in 200 ml of autoclaved water and placed on muslin cloth and squeezed to give a crude aqueous extract of 250 mg of turmeric/mL. The extracts were collected in a sterile vial and stored at 4°C until used.

Experimental animal

Twenty-four males *Rattus norvegicus* with 2 months of age and ± 200g in body weight were used as experimental animals. They were acclimatized for at least 2 weeks before starting the experiment. The animals were maintained under control condition of temperature (22 $\pm 1^0$C), humidity (50±10%) and normal photoperiod (12-hour light /dark cycle). Rats were provided standard dried pellet diet and water ad libitum (Ebrahimi *et al.,* 2015).

Experimental design

Rats were randomly divided into four groups (6 rats in each group). Group I (Negative control) served as untreated control group. Group II (Positive control) received metanil yellow @ 250 mg/kg/day body weight (bw). Group III (MY+GE) were given metanil yellow (at the same rate as in group II) followed by garlic extract @250mg/kg/day bw. Group IV (MY+TE) were given metanil yellow (at the same rate as in group II) followed by turmeric extract @250mg/kg/day body weight. All doses corresponding to 1ml/kg/day bw and injected orally for 12 and 24 weeks. Normal pellet diet and water was given ad libitum to all groups.

Blood collection and assay of lipid profile:

After 12- and 24-weeks blood was drawn from the retro-orbital sinus of rats from each group to obtain serum. The blood was transferred into non heparinized glass centrifuged tube and permitted to clot at room temperature followed by centrifugation at 3500 rpm for 15 minutes (El-Desoky *et al.,* 2017). Blood serum was used for measurement of lipid profile. Analysis of lipid profile was performed by use of biochemical analyser with commercially available Beacon laboratory kit.

Statistical analysis

The statistical analysis was done using SPSS software. Mean and standard deviation were descriptive measures of quantitative data using ANOVA, followed by the post hoc Tukey test for multiple comparisons of mean. P values <0.05 were considered significant.

Results and Discussion

Table 1 and 2 illustrates the changes in serum lipid profile for the control and experimental group of albino rats. Rats administered with metanil yellow (group II) showed significant increased TC, TG and LDL and decreased HDL after 12 and 24 weeks of exposure as compared to control. Concentration of serum TC, TG and LDL of rat exposed to MY (group II) were significantly higher by 18.59%, 24.60 % and 40.60% after 12 weeks of exposure period and 43.18%, 47.81% and 62.14% higher after 24 weeks of exposure period respectively as compared to control (group I). The concentration of HDL decreased by 27.63% after 12 weeks of exposure period and 34.44% after 24 weeks of exposure period as compared to control. The altered lipid profile is significantly different in comparison to control ($p<0.05$).

Table 1: Effect of garlic and turmeric extract on lipid profile of albino rats exposed to metanil yellow for 12 weeks.

Groups	Total Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Group I (Negative control)	133.36±0.98[a]	117.87±5.36[a]	49.01±1.62[c]	65.29±0.96[a]
Group II (Positive control)	163.83±1.12[d] (+18.59%)	156.34±7.31[c] (+24.60%)	38.40±1.66[a] (-27.63%)	109.92±1.72[d] (+40.60%)
Group III (MY+GE)	140.41±1.13[b] (+5.02%)	132.16±1.47[b] (+10.81%)	44.19±0.95[b] (-20.09%)	78.34±0.74[b] (+16.65%)
Group IV (MY+TE)	151.27±0.54[c] (+11.82%)	138.56±0.88[b] (+14.93%)	40.81±1.02[a] (-10.90%)	83.35±3.05[c] (+21.66%)

Table 2: Effect of garlic and turmeric extract on lipid profile of albino rats exposed to metanil yellow for 24 weeks

Groups	Total Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Group I (Negative control)	125.20±1.57[a]	112.64±1.60[a]	44.07±1.00[b]	60.95±1.14[a]
Group II (Positive control)	220.36±0.60[d] (+43.18%)	215.86±1.16[d] (+47.81%)	32.78±1.48[a] (-34.44%)	161.02±1.41[d] (+62.14%)
Group III (MY+GE)	170.25±0.68[b] (+26.46%)	180.75±2.05[c] (+37.68%)	42.44±1.37[b] (-3.84%)	108.78±1.00[b] (+43.96%)
Group IV (MY+TE)	191.17±0.99[c] (+33.98%)	162.07±0.89[b] (+30.49%)	41.86±1.17[b] (-4.74%)	122.99±1.66[c] (+50.44%)

Values are expressed as Mean±SD, Data were analysed by one way ANOVA followed by Tukey's test, P values <0.05 were considered significantly different from control. Value in parentheses represent percent change with respect to control.

Ameliorative effect of garlic extract on lipid profile

When albino rats were supplemented with garlic extract @250mg/kg bw after administration of metanil yellow (group III) then significantly lesser concentration of TC, TG and LDL by 16.68 %, 18.29 % and 40.31% were reported after 12 weeks of exposure period and 29.43%, 19.42% and 48.02% after 24 weeks of exposure period as compared to rats exposed to MY (group II). However, these concentrations of TC, TG and LDL showed recovery and remained only 5.02%, 10.81% and 16.65 % higher after 12 weeks (Figure 1) and 26.46%, 37.68% and 43.96% higher after 24 weeks exposure period as compared to control (Figure 2). The mean conc. of HDL in rats fed MY along with GE showed higher conc. (42.44±1.37) than that in rats fed metanil yellow (32.78±1.48) and became nearly equivalent to that of control (44.07±1.00) after 24 weeks of exposure (Figure 2). All values were found to be significant at $p<0.05$.

Ameliorative effect of turmeric extract on lipid profile

When albino rats were supplemented with turmeric extract @250 mg/kg bw after administration of metanil yellow (group IV) then significantly ($p<0.05$) lesser concentration of TC, TG and LDL by 8.31%, 12.83% and 31.87% were reported in serum after 12 weeks of exposure period as compared to rats exposed to MY (group II). These concentrations in serum were decreased by 15.26 %, 33.18% and 33.92 % in comparison to rats exposed to MY (group II) after 24 weeks of exposure period. However, treatment with turmeric extract showed lesser recovery and concentration of studied parameters remained only 11.82 %, 14.93% and 21.66 % higher after 12 weeks and 33.98 %, 30.94 % and 50.44% higher after 24 weeks exposure period as compared to control (group 1) (Figure1 &2). The mean conc. of HDL in rats fed MY along with TE showed higher conc. than that in rats fed metanil yellow (Group II) i.e., 40.81±1.02 and 41.86±4.74 after 12- and 24-weeks exposure period respectively.

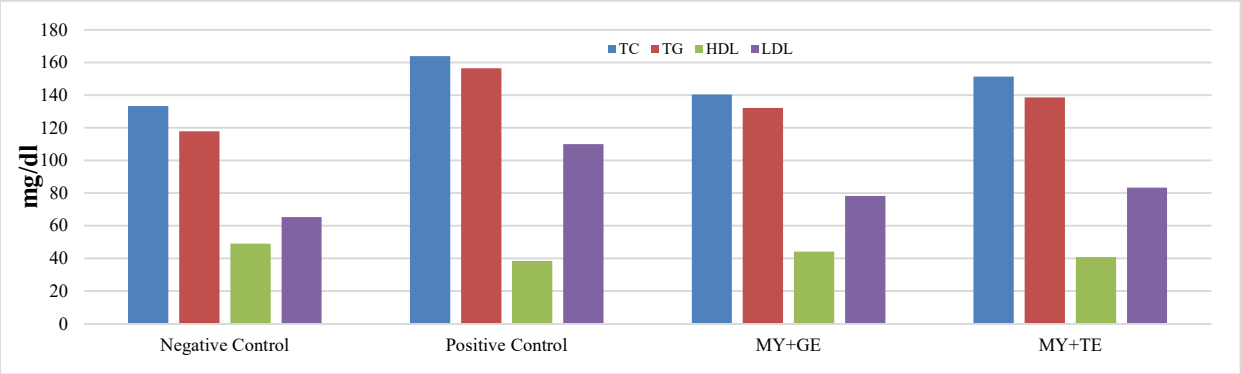

Figure 1: Effect of garlic and turmeric extract (250mg/kg bw) on lipid profile in comparison with negative control and positive control group of albino rats after 12 weeks of exposure.

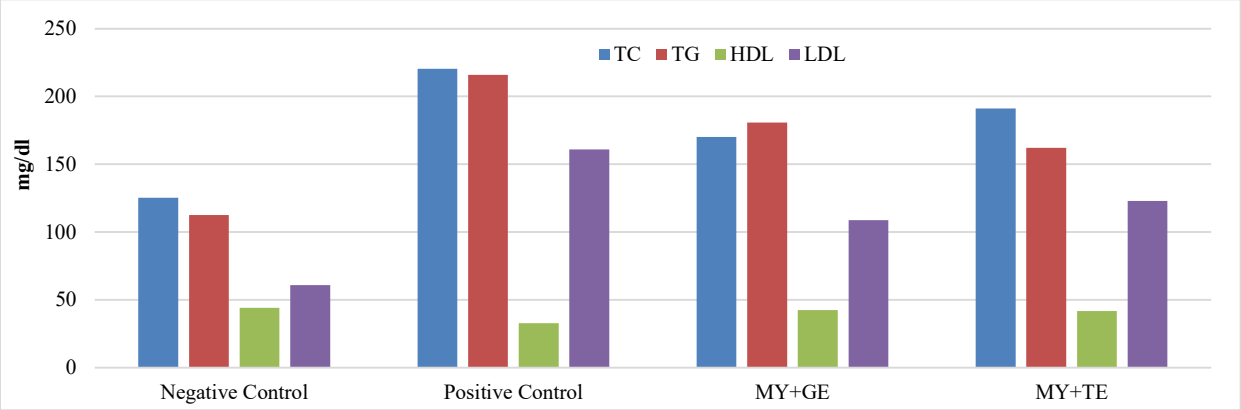

Figure 2: Effect of garlic and turmeric extract (250mg/kg bw) on lipid profile in comparison with negative control and positive control group of albino rats after 24 weeks of exposure.

Altered lipid profile in blood serum observed in rats fed with common azo dye during this study also agree with other studies (Saxena and Sharma, 2015; El-Desoky *et al.,* 2017; Reza *et al.,*2019). Sharma *et al.* (2009) and Bahnasy and Ragheb (2020) reported changes in lipid profile of rats fed with other food colours also viz., sunset yellow and chocolate brown. Significantly greater concentration of TC and TG has also been reported in rats when administered to fast green orally (Turley 2004; Ashour and Abdelaz, 2009). So, changes in lipid profile on exposure to azo dye in albino rats were observed by other workers, which favours the present study. In current study, when aquatic extract of garlic and turmeric (@ 250 mg/kg bw) was given to rats fed with metanil yellow, it successfully normalized concentration of lipid which indicates that these plant extracts were able to reduce toxicity caused by food colours. Other researchers also studied that plant extracts could be effective means for reducing toxicity caused by food colours. Bahnasy and Ragheb (2020) studied ameliorative effect of *Chenopodium quinoa* for toxicity caused by chocolate brown and sunset yellow and observed significant decrease in TC, TG and LDL and significant increase in HDL treated with its extract along with food colour. Yanam *et al.,* (2020) also reported that quinoa could ameliorate the hyperlipidaemic condition induced by high fat diet. Ebrahimi *et al.* (2015) studied that dietary supplementation with garlic extract could be used as remedy for hypercholesterolemia. These data are in the line of present study where turmeric and garlic extract both showed ameliorative effect on lipid profile of albino rats fed on metanil yellow.

Conclusion

In current study, albino rats fed on metanil yellow dye in the diet for 12 and 24 weeks showed increase in the serum TC, LDL, TG and decrease in serum HDL. Administration of garlic and turmeric extract

in metanil yellow fed albino rats caused significant improvement in their serum lipid profile. Garlic extract is found to be more potent than turmeric in rescuing concentration of serum total cholesterol, low density lipoprotein, high density lipoprotein and triglycerides to values near to those of control. Artificial food colours are consumed by children and adults without realising its bad effect on health. So, it is necessary to evaluate natural herbal products which can be used in diet once daily to get rid of from chronic toxicity caused by food colours.

Conflict of interest

The authors declare that they have no conflict of interest.

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